

FASCICULE DE BREVET D'INVENTION

21 Numéro de dépôt : 1202000464
PCT/US2019/038188

22 Date de dépôt : 20/06/2019

30 Priorité(s) :
US n° 62/688,611 du 22/06/2018

24 Délivré le : 07/06/2021

45 Publié le : 31/08/2021

73 Titulaire(s) :

OMEROS CORPORATION,
201 Elliott Avenue West,
SEATTLE,
WA 98119 (US)

72 Inventeur(s) :

DUDLER, Thomas (US)
DEMOPULOS, Gregory, A. (US)
NILSSON, Bo (SE)

74 Mandataire : S.C.P AKKUM, AKKUM & Associates,
Quartier Mballa II, Dragages,
B.P. 4966, YAOUNDE (CM).

54 Titre : Compositions and methods of inhibiting MASP-2 for the treatment of various thrombotic diseases and disorders.

57 Abrégé :

In one aspect, the invention provides compositions and methods for preventing, reducing, and/or treating a disease, disorder or condition associated with fibrin-induced activation of the complement system and the associated activation of the coagulation and/or contact systems comprising administering a therapeutic amount of a MASP-2 inhibitory antibody to a subject in need thereof. In some embodiments, the methods of the invention provide anticoagulation and/or antithrombosis and/or antithrombogenesis without affecting hemostasis. In one embodiment of this aspect of the invention, the compositions and methods are useful for treating a subject is suffering from, or at risk of developing, a disease, disorder or condition associated with complement-related inflammation, excessive coagulation or contact system activation initiated by fibrin or activated platelets.

MASP-2 mAb inhibition of MASP-2-ATIII
in plasma stimulated by fibrin

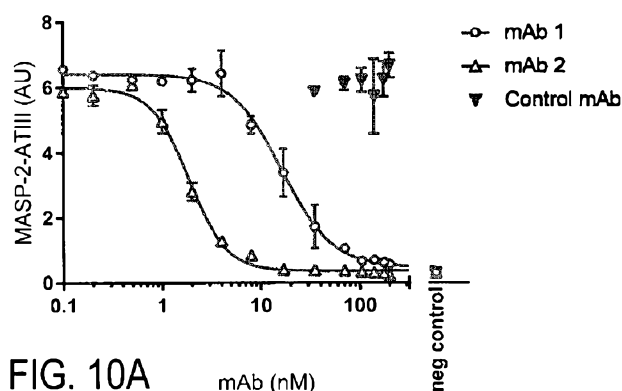


FIG. 10A

Fig. 10A

COMPOSITIONS AND METHODS OF INHIBITING MASP-2 FOR THE
TREATMENT OF VARIOUS THROMBOTIC DISEASES AND DISORDERS

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims the benefit of U.S. Provisional Application Number
62/688,611 filed June 22, 2018, which is hereby incorporated by reference in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

10 The sequence listing associated with this application is provided in text format in
lieu of a paper copy and is hereby incorporated by reference into the specification. The
name of the text file containing the sequence listing is
MP_1_0252_PCT_Sequence_Listing_20190620.txt; the text file is 26 KB, was created
on June 20, 2019; and is being submitted via EFS-Web with the filing of the
specification.

15

BACKGROUND

 The complement system provides an early acting mechanism to initiate, amplify
and orchestrate the immune response to microbial infection and other acute insults
(M.K. Liszewski and J.P. Atkinson, 1993, in *Fundamental Immunology*, Third Edition,
20 edited by W.E. Paul, Raven Press, Ltd., New York), in humans and other vertebrates.
While complement activation provides a valuable first-line defense against potential
pathogens, the activities of complement that promote a protective immune response can
also represent a potential threat to the host (K.R. Kalli, et al., *Springer Semin.*
Immunopathol. 15:417-431, 1994; B.P. Morgan, *Eur. J. Clinical Investig.* 24:219-228,
25 1994). For example, C3 and C5 proteolytic products recruit and activate neutrophils.
While indispensable for host defense, activated neutrophils are indiscriminate in their
release of destructive enzymes and may cause organ damage. In addition, complement
activation may cause the deposition of lytic complement components on nearby host cells
as well as on microbial targets, resulting in host cell lysis.

Currently, it is widely accepted that the complement system can be activated through three distinct pathways: the classical pathway, the lectin pathway, and the alternative pathway. The classical pathway is usually triggered by a complex composed of host antibodies bound to a foreign particle (*i.e.*, an antigen) and thus requires prior exposure to an antigen for the generation of a specific antibody response. Since activation of the classical pathway depends on a prior adaptive immune response by the host, the classical pathway is part of the acquired immune system. In contrast, both the lectin and alternative pathways are independent of adaptive immunity and are part of the innate immune system.

The lectin pathway is widely thought to have a major role in host defense against infection in the naïve host. Strong evidence for the involvement of MBL in host defense comes from analysis of patients with decreased serum levels of functional MBL (Kilpatrick, *Biochim. Biophys. Acta* 1572:401-413, (2002)). Such patients display susceptibility to recurrent bacterial and fungal infections. These symptoms are usually evident early in life, during an apparent window of vulnerability as maternally derived antibody titer wanes, but before a full repertoire of antibody responses develops. This syndrome often results from mutations at several sites in the collagenous portion of MBL, which interfere with proper formation of MBL oligomers. However, since MBL can function as an opsonin independent of complement, it is not known to what extent the increased susceptibility to infection is due to impaired complement activation.

In addition to its essential role in immune defense, the complement system contributes to tissue damage in many clinical conditions. Thus, there is a pressing need to develop therapeutically effective complement inhibitors to prevent these adverse effects.

SUMMARY

In one aspect, the present invention provides a method of preventing, reducing and/or treating a disease, disorder or condition associated with fibrin-induced activation of the complement system and the associated activation of the coagulation and/or contact systems comprising administering a therapeutic amount of a MASP-2 inhibitory antibody

to a subject in need thereof. In some embodiments, the methods of the invention provide anticoagulation and/or antithrombosis and/or antithrombogenesis without affecting hemostasis. In one embodiment of this aspect of the invention, the methods are useful for treating a subject suffering from, or at risk of developing, a disease, disorder or condition associated with complement-related inflammation, excessive coagulation or contact system activation initiated by fibrin or activated platelets. In one embodiment, the methods of the invention are useful for treating a subject suffering from a disease, disorder or condition selected from the group consisting of arterial thrombosis, venous thrombosis, deep vein thrombosis, post-surgical thrombosis, restenosis following coronary artery bypass graft and/or an interventional cardiovascular procedure (e.g., angioplasty or stent placement), atherosclerosis, plaque rupture, plaque instability, restenosis, hypotension, acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome (SIRS), disseminated intravascular coagulation (DIC), veno-occlusive disease (VOD), sickle cell disease, thrombotic microangiopathy, lupus nephritis, superficial thrombophlebitis, Factor V Leiden mutation, ischemic/reperfusion injury, human immunodeficiency virus (HIV) infection, undergoing hormone-replacement therapy (HRT), Alzheimer's disease and/or suffering from a hypercoagulable state, such as wherein the subject is suffering from, or at risk for developing an acquired hypercoagulable state due to at least one or more of the following: undergoing therapy with a drug selected from the group consisting of 5-FU, GM-CSF, cisplatin, heparin, COX-2 inhibitor, contrast media, corticosteroids and antipsychotics, venous stasis (due to immobilization, surgery, etc.), antiphospholipid syndrome, cancer (promyelocytic leukemia, lung, breast, prostate, pancreas, stomach and colon tumors), tissue injury due to trauma or surgery, presence of a catheter in a central vein, acquired deficiency of a protein involved in clot formation (e.g., protein C), paroxysmal nocturnal hemoglobinuria (PNH), elevated levels of homocysteine, heart failure, presence of a mechanical valve, pulmonary hypertension with in-situ thrombosis, atrial fibrillation, heparin-induced

thrombocytopenia (HIT), heparin-induced thrombocytopenia and thrombosis (HITT), Kawasaki disease with in-situ thrombus, Takayasu arteritis with in-situ thrombus, thrombophilia of metastatic cancer, elevated Factor VIII levels, pregnancy and inflammatory bowel disease (IBD).

5 In one embodiment, the methods of the invention are useful for treating a subject suffering from, or at risk for developing, a disease, disorder or condition that is amenable to treatment with a kallikrein inhibitor, such as, for example, wherein the disease or disorder amenable to treatment with a kallikrein inhibitor is selected from the group consisting of hereditary angioedema, diabetic macular edema, and bleeding during
10 cardiopulmonary bypass. In one embodiment, the methods of the invention are useful for treating a subject suffering from, or at risk for developing, a disease, disorder or condition that is amenable to treatment with a thrombin inhibitor, such as, for example, wherein the disease or disorder amenable to treatment with a thrombin inhibitor is selected from the group consisting of arterial thrombosis, venous thrombosis, pulmonary embolism, atrial
15 fibrillation, heparin-induced thrombocytopenia, conversion from one anticoagulant to another, and off-label use for extracorporeal circuit patency of continuous renal replacement therapy (CRRT) in critically ill patients with HIT (maintenance). In one embodiment, the method comprises identifying a subject that has previously experienced, is currently suffering from, or is at risk for developing atrial fibrillation and administering
20 a MASP-2 inhibitory antibody in an amount sufficient to reduce the risk of stroke in said subject.

 In one embodiment, the methods of the invention are useful for treating a subject suffering from, or at risk for developing, a disease, disorder or condition that is amenable to treatment with a factor XII inhibitor, such as, for example, wherein the disease or
25 disorder amenable to treatment with a factor XII inhibitor is selected from the group consisting of deep vein thrombosis (both primary prophylaxis and extended therapy), pulmonary embolism, nonvalvular atrial fibrillation, prevention of recurrent ischemia

after acute coronary syndrome in subjects with or without atrial fibrillation, end-stage renal disease, cerebral ischemia, angina, reduce or prevent clotting associated with implanted medical devices (e.g., valves, small caliber grafts, etc) and/or extracorporeal circuits. In one embodiment, the method comprises identifying a subject that has
5 previously experienced, is currently suffering from, or is at risk for developing nonvalvular atrial fibrillation and administering a MASP-2 inhibitory antibody in an amount sufficient to reduce the risk of stroke and/or embolism in said subject.

In one embodiment, the methods of the invention are useful for treating a subject determined to have a genetic defect that causes or increases the risk of developing a
10 hypercoagulable state, such as, for example, wherein the genetic defect is selected from the group consisting of a Prothrombin 20210 gene mutation, an MTHFR mutation, a deficiency of protein C, a deficiency of protein S, a deficiency of protein A, a deficiency of protein Z, an antithrombin deficiency and a genetic disorder producing thrombophilia.

In one embodiment, the methods of the invention are useful for treating a subject
15 that has an acquired disease, disorder or condition that increases the propensity for thromboembolism, such as, for example, wherein the acquired disease or disorder that increases the propensity for thromboembolism is selected from the group consisting of atherosclerosis, antiphospholipid antibodies, cancer (e.g., promyelocytic leukemia, lung, breast, prostate, pancreatic, stomach or colon cancer), hyperhomocysteinemia, infection,
20 tissue injury, venous stasis (such as due to surgery, orthopedic or paralytic immobilization, heart failure, pregnancy, or obesity) and a subject taking oral contraceptives that contain estrogen.

In one embodiment, the methods of the invention are useful for providing a replacement for standard anticoagulant therapy, such as Warfarin, in a subject in need of
25 anticoagulant therapy, such as wherein the subject has a condition that normally prohibits standard anticoagulant therapy (e.g., CNS amyloid angiopathy), or wherein the MASP-2

inhibitory antibody is administered as a bridging agent perioperatively in a subject otherwise on standard anticoagulation therapy.

In accordance with any of the embodiments of the methods of the invention described herein, the MASP-2 inhibitory antibody is preferably a MASP-2 monoclonal antibody, or fragment thereof that specifically binds to a portion of SEQ ID NO:5. In some embodiments, the MASP-2 antibody is a chimeric, humanized or human antibody. In some embodiments, the MASP-2 inhibitory antibody is an antibody fragment selected from the group consisting of Fv, Fab, Fab', F(ab)₂ and F(ab')₂. In some embodiments, the MASP-2 inhibitory antibody is a single-chain molecule. In some embodiments, the MASP-2 inhibitory antibody is selected from the group consisting of an IgG1 molecule, an IgG2 and an IgG4 molecule. In some embodiments, the MASP-2 inhibitory antibody is an IgG4 molecule comprising a S228P mutation. In some embodiments, the MASP-2 inhibitory antibody does not substantially inhibit the classical pathway. In some embodiments, the MASP-2 inhibitory monoclonal antibody, or antigen-binding fragment thereof, comprises: (a) a heavy-chain variable region comprising: i) a heavy chain CDR-H1 comprising the amino acid sequence from 31-35 of SEQ ID NO:6; and ii) a heavy-chain CDR-H2 comprising the amino acid sequence from 50-65 of SEQ ID NO:6; and iii) a heavy-chain CDR-H3 comprising the amino acid sequence from 95-107 of SEQ ID NO:6 and (b) a light-chain variable region comprising: i) a light-chain CDR-L1 comprising the amino acid sequence from 24-34 of SEQ ID NO:7; and ii) a light-chain CDR-L2 comprising the amino acid sequence from 50-56 of SEQ ID NO:7; and iii) a light-chain CDR-L3 comprising the amino acid sequence from 89-97 of SEQ ID NO:7. In some embodiments, the MASP-2 inhibitory monoclonal antibody comprises a heavy-chain variable region set forth as SEQ ID NO:6 and a light-chain variable region set forth as SEQ ID NO:7. In some embodiments, the MASP-2 inhibitory antibody or antigen binding-fragment thereof specifically recognizes at least part of an epitope recognized by a reference antibody comprising a heavy chain variable region as set forth in SEQ ID NO:6 and a light-chain variable region as set forth in SEQ ID NO:7.

These and other aspects and embodiments of the herein described invention will be evident upon reference to the following detailed description and drawings. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign

patents, foreign patent applications and non-patent publications referred to in this specification are incorporated herein by reference in their entirety, as if each was incorporated individually.

5

DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

10

FIGURE 1 is a schematic diagram adapted from Schwaebler et al., *Immunobiol* 205:455-466 (2002), as modified by Yongqing et al., *BBA* 1824:253 (2012), illustrating the MASP-2 and MAP19 protein domains and the exons encoding the same;

15

FIGURE 2A graphically illustrates lectin pathway specific C4b deposition on a zymosan-coated microtiter plate, measured *ex vivo* in undiluted serum samples taken from mice (n=3 mice/group) at various time points after subcutaneous dosing of either 0.3 mg/kg or 1.0 mg/kg of the mouse MASP-2 MoAb, as described in Example 6;

20

FIGURE 2B graphically illustrates the time course of lectin pathway recovery for three weeks following a single intraperitoneal administration of mouse MASP-2 MoAb at 0.6 mg/kg in mice, as described in Example 6;

FIGURES 3A and 3B present dose response curves for the inhibition of C4b deposition (FIG. 3A) and the inhibition of thrombin activation following the administration of a MASP-2 Fab2 antibody (H1) in normal rat serum, as described in Example 8;

25

FIGURES 4A and 4B present measured platelet aggregation (expressed as aggregate area) in MASP-2 (-/-) mice (FIG. 4B) as compared to platelet aggregation in untreated wild-type mice and wild-type mice in which the complement pathway is inhibited by depletory agent cobra venom factor (CVF) and a terminal pathway inhibitor (C5aR antagonist) (FIGURE 4A) in a localized Schwartzman reaction model of disseminated intravascular coagulation, as described in Example 9;

FIGURE 5 illustrates the results of a Western blot analysis showing activation of human C3, shown by the presence of the α' chain, by thrombin substrates FXIa and FXa, as described in Example 10;

FIGURE 6 graphically illustrates the time to onset of microvascular occlusion following LPS injection in MASP-2 $-/-$ and WT mice, showing the percentage of mice with thrombus formation measured over 60 minutes, demonstrating that thrombus formation is detected after 15 minutes in WT mice, with up to 80% of the WT mice demonstrating thrombus formation at 60 minutes; in contrast, none of the MASP-2 $-/-$ mice showed any thrombus formation during the 60-minute period (log rank: $p=0.0005$), as described in Example 11;

FIGURE 7 graphically illustrates, as a function of time after injury induction, the percentage of mice with microvascular occlusion in the FITC/Dextran UV model after treatment with isotype control, or human MASP-2 antibody mAbH6 (10mg/kg) dosed at 16 hours and 1 hour prior to injection of FITC/Dextran, as described in Example 12;

FIGURE 8 graphically illustrates the occlusion time in minutes for mice treated with the human MASP-2 antibody (mAbH6) and the isotype control antibody, wherein the data are reported as scatter-dots with mean values (horizontal bars) and standard error bars (vertical bars). The statistical test used for analysis was the unpaired t test; wherein the symbol “*” indicates $p=0.0129$, as described in Example 12;

FIGURE 9 graphically illustrates the time until occlusion in minutes for wild-type mice, MASP-2 KO mice, and wild-type mice pre-treated with human MASP-2 antibody (mAbH6) administered i.p. at 10mg/kg 16 hours before, and again 1 hour prior to the induction of thrombosis in the FITC-dextran/light induced endothelial cell injury model of thrombosis with low light intensity (800-1500), as described in Example 12;

FIGURE 10A graphically illustrates MASP-2 activation, as determined by MASP-2-antithrombin complex formation (MASP-2-ATIII), shown in Absorbance Units (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies mAb#1 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin,

demonstrating that MASP-2 inhibitory antibodies block MASP-2 activation in a dose-dependent manner, whereas the control antibody had no effect, as described in Example 14;

FIGURE 10B graphically illustrates MASP-2 activation, as determined by MASP-2-serpin complex formation (MASP-2-C1-INH), shown in Absorbance Units (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies mAb#1 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin, demonstrating that MASP-2 inhibitory antibodies block MASP-2 activation in a dose-dependent manner, whereas the control antibody had no effect, as described in Example 14;

FIGURE 11A graphically illustrates Kallikrein activation, as determined by Kallikrein-antithrombin complex formation (KK-ATIII), shown in Absorbance Units (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies mAb#1 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin, demonstrating that MASP-2 inhibitory antibodies block Kallikrein activation in a dose-dependent manner, whereas the vehicle control had no effect, as described in Example 14;

FIGURE 11B graphically illustrates Kallikrein activation, as determined by Kallikrein-serpin complex formation (KK-C1-INH), shown in Absorbance Units (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies mAb#1 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin, demonstrating that MASP-2 inhibitory antibodies block Kallikrein activation in a dose-dependent manner, whereas the vehicle control had no effect, as described in Example 14;

FIGURE 12A graphically illustrates Factor XII activation, as determined by Factor XII-antithrombin complex formation (FXIIa-ATIII), shown in Absorbance Units (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies

mAb#1 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin, demonstrating that MASP-2 inhibitory antibodies block Factor XII activation in a dose-dependent manner, whereas the control antibody had no effect, as described in Example 14; and

5 FIGURE 12B graphically illustrates Factor XII activation, as determined by Factor XII-serpin complex formation (FXIIa-C1-INH), shown in Absorbance Units (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies mAb#1 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin, demonstrating that MASP-2 inhibitory antibodies block Factor XII activation in a dose-
10 dependent manner, whereas the control antibody had no effect, as described in Example 14.

DESCRIPTION OF SEQUENCE LISTING

SEQ ID NO:1 human MASP-2 cDNA
15 SEQ ID NO:2 human MASP-2 protein (with leader)
SEQ ID NO:3 human MASP-2 protein (mature)
SEQ ID NO:4 rat MASP-2 cDNA
SEQ ID NO:5 rat MASP-2 protein (with leader)
SEQ ID NO:6 17D20_dc35VH21N11VL (OMS646) heavy chain variable region (VH)
20 polypeptide
SEQ ID NO:7 17D20_dc21N11VL (OMS646) light chain variable region (VL)
polypeptide

DETAILED DESCRIPTION

25

I. DEFINITIONS

Unless specifically defined herein, all terms used herein have the same meaning as would be understood by those of ordinary skill in the art of the present invention. The

following definitions are provided in order to provide clarity with respect to the terms as they are used in the specification and claims to describe the present invention.

As used herein, the term "MASP-2-dependent complement activation", comprises MASP-2 lectin-dependent activation, which occurs in the presence of Ca^{++} , leading to the
5 formation of the lectin pathway C3 convertase C4b2a and upon accumulation of the C3 cleavage product C3b subsequently to the C5 convertase C4b2a(C3b)_n , which has been determined to cause opsonization and/or lysis.

As used herein, the term "lectin pathway" refers to complement activation that occurs via the specific binding of serum and non-serum carbohydrate-binding proteins
10 including mannan-binding lectin (MBL), CL-11 and the ficolins (H-ficolin, M-ficolin, or L-ficolin). As described herein, the lectin pathway is MASP-2-dependent. As used herein, activation of the lectin pathways are assessed using Ca^{++} containing buffers.

As used herein, the term "classical pathway" refers to complement activation that is triggered by antibody bound to a foreign particle and requires binding of the
15 recognition molecule C1q.

As used herein, the term "MASP-2 inhibitory antibody" refers to any antibody that binds to or directly interacts with MASP-2 and inhibits MASP-2-dependent complement activation including MASP-2 antibodies and MASP-2 antigen-binding fragments thereof. MASP-2 inhibitory antibodies useful in the method of the invention
20 may reduce MASP-2-dependent complement activation by greater than 10%, such as greater than 20%, greater than 50%, or greater than 90%. In one embodiment, the MASP-2 inhibitory antibodies reduce MASP-2-dependent complement activation by greater than 90% (i.e., resulting in MASP-2 complement activation of only 10% or less). An example of a direct MASP-2 inhibitory antibody is a MASP-2-specific inhibitory
25 antibody, such as a MASP-2 inhibitory antibody that specifically binds to a portion of MASP-2 (SEQ ID NO:2) with a binding affinity of at least 10 times greater than to other components in the complement system.

As used herein, the term "antibody" encompasses antibodies and antibody fragments thereof, derived from any antibody-producing mammal (e.g., mouse, rat,
30 rabbit, and primate including human), or from a hybridoma, phage selection, recombinant expression or transgenic animals (or other methods of producing antibodies or antibody

fragments”), that specifically bind to a target polypeptide, such as, MASP-polypeptides or portions thereof. It is not intended that the term “antibody” limited as regards to the source of the antibody or the manner in which it is made (e.g., by hybridoma, phage selection, recombinant expression, transgenic animal, peptide synthesis, etc). Exemplary antibodies include polyclonal, monoclonal and recombinant antibodies; pan-specific, multispecific antibodies (e.g., bispecific antibodies, trispecific antibodies); humanized antibodies; murine antibodies; chimeric, mouse-human, mouse-primate, primate-human monoclonal antibodies; and anti-idiotypic antibodies, and may be any intact antibody or fragment thereof. As used herein, the term “antibody” encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as dAb, Fab, Fab', F(ab')₂, Fv), single chain (ScFv), synthetic variants thereof, naturally occurring variants, fusion proteins comprising an antibody portion with an antigen-binding fragment of the required specificity, humanized antibodies, chimeric antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding site or fragment (epitope recognition site) of the required specificity.

A “monoclonal antibody” refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring and non-naturally occurring) that are involved in the selective binding of an epitope. Monoclonal antibodies are highly specific for the target antigen. The term “monoclonal antibody” encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂, Fv), single chain (ScFv), variants thereof, fusion proteins comprising an antigen-binding portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding fragment (epitope recognition site) of the required specificity and the ability to bind to an epitope. It is not intended to be limited as regards the source of the antibody or the manner in which it is made (e.g., by hybridoma, phage selection, recombinant expression, transgenic animals, etc.). The term includes whole immunoglobulins as well as the fragments etc. described above under the definition of “antibody”.

As used herein, the term “antibody fragment” refers to a portion derived from or related to a full-length antibody, such as, for example, a MASP-2 antibody, generally

including the antigen binding or variable region thereof. Illustrative examples of antibody fragments include Fab, Fab', F(ab)₂, F(ab')₂ and Fv fragments, scFv fragments, diabodies, linear antibodies, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

5 As used herein, a "single-chain Fv" or "scFv" antibody fragment comprises the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains, which enables the scFv to form the desired structure for antigen binding.

10 As used herein, a "chimeric antibody" is a recombinant protein that contains the variable domains and complementarity-determining regions derived from a non-human species (e.g., rodent) antibody, while the remainder of the antibody molecule is derived from a human antibody.

 As used herein, a "humanized antibody" is a chimeric antibody that comprises a
15 minimal sequence that conforms to specific complementarity-determining regions derived from non-human immunoglobulin that is transplanted into a human antibody framework. Humanized antibodies are typically recombinant proteins in which only the antibody complementarity-determining regions are of non-human origin (including antibodies generated from phage display or yeast).

20 As used herein, the term "mannan-binding lectin" ("MBL") is equivalent to mannan-binding protein ("MBP").

 As used herein, the "membrane attack complex" ("MAC") refers to a complex of the terminal five complement components (C5b combined with C6, C7, C8 and C9) that inserts into and disrupts membranes (also referred to as C5b-9).

25 As used herein, "a subject" includes all mammals, including without limitation humans, non-human primates, dogs, cats, horses, sheep, goats, cows, rabbits, pigs and rodents.

 As used herein, the amino acid residues are abbreviated as follows: alanine
(Ala;A), asparagine (Asn;N), aspartic acid (Asp;D), arginine (Arg;R), cysteine (Cys;C),
30 glutamic acid (Glu;E), glutamine (Gln;Q), glycine (Gly;G), histidine (His;H), isoleucine (Ile;I), leucine (Leu;L), lysine (Lys;K), methionine (Met;M), phenylalanine (Phe;F),

proline (Pro;P), serine (Ser;S), threonine (Thr;T), tryptophan (Trp;W), tyrosine (Tyr;Y), and valine (Val;V).

In the broadest sense, the naturally occurring amino acids can be divided into groups based upon the chemical characteristic of the side chain of the respective amino acids. By "hydrophobic" amino acid is meant either Ile, Leu, Met, Phe, Trp, Tyr, Val, Ala, Cys or Pro. By "hydrophilic" amino acid is meant either Gly, Asn, Gln, Ser, Thr, Asp, Glu, Lys, Arg or His. This grouping of amino acids can be further subclassed as follows. By "uncharged hydrophilic" amino acid is meant either Ser, Thr, Asn or Gln. By "acidic" amino acid is meant either Glu or Asp. By "basic" amino acid is meant either Lys, Arg or His.

As used herein the term "conservative amino acid substitution" is illustrated by a substitution among amino acids within each of the following groups: (1) glycine, alanine, valine, leucine, and isoleucine, (2) phenylalanine, tyrosine, and tryptophan, (3) serine and threonine, (4) aspartate and glutamate, (5) glutamine and asparagine, and (6) lysine, arginine and histidine.

The term "oligonucleotide" as used herein refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term also covers those oligonucleobases composed of naturally-occurring nucleotides, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring modifications.

As used herein, an "epitope" refers to the site on a protein (e.g., a human MASP-2 protein) that is bound by an antibody. "Overlapping epitopes" include at least one (e.g., two, three, four, five, or six) common amino acid residue(s), including linear and non-linear epitopes.

As used herein, the terms "polypeptide," "peptide," and "protein" are used interchangeably and mean any peptide-linked chain of amino acids, regardless of length or post-translational modification. The MASP-2 protein described herein can contain or be wild-type proteins or can be variants that have not more than 50 (e.g., not more than one, two, three, four, five, six, seven, eight, nine, ten, 12, 15, 20, 25, 30, 35, 40, or 50) conservative amino acid substitutions. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and

leucine; aspartic acid and glutamic acid; asparagine, glutamine, serine and threonine; lysine, histidine and arginine; and phenylalanine and tyrosine.

The human MASP-2 protein (set forth as SEQ ID NO:2) described herein also include "peptide fragments" of the proteins, which are shorter than full-length and/or immature (pre-pro) MASP-2 proteins, including peptide fragments of a MASP-2 protein include terminal as well internal deletion variants of the protein. Deletion variants can lack one, two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid segments (of two or more amino acids) or noncontiguous single amino acids.

10 In some embodiments, the human MASP-2 protein can have an amino acid sequence that is, or is greater than, 70 (e.g., 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100) % identical to the human MASP-2 protein having the amino acid sequence set forth in SEQ ID NO:2.

15 In some embodiments, peptide fragments can be at least 6 (e.g., at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, or 600 or more) amino acid residues in length (e.g., at least 6 contiguous amino acid residues in SEQ ID NO: 2 or 3). In some embodiments, an antigenic peptide fragment of
20 a human MASP-2 protein is fewer than 500 (e.g., fewer than 450, 400, 350, 325, 300, 275, 250, 225, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, or 6) amino acid residues in length (e.g., fewer than 500 contiguous amino acid residues in
25 any one of SEQ ID NOS:2 or 3).

In some embodiments, in the context of generating an antibody that binds MASP-2, the peptide fragments are antigenic and retain at least 10% (e.g., at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least
30 98%, at least 99%, at least 99.5%, or 100% or more) of the ability of the full-length

protein to induce an antigenic response in a mammal (see below under "Methods for Producing an Antibody").

Percent (%) amino acid sequence identity is defined as the percentage of amino acids in a candidate sequence that are identical to the amino acids in a reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared can be determined by known methods.

In representative embodiments, the human MASP-2 protein (SEQ ID NO:2) is encoded by the cDNA sequence set forth as SEQ ID NO:1; Those skilled in the art will recognize that the cDNA sequences disclosed in SEQ ID NO:1 represent a single allele of human MASP-2, and that allelic variation and alternative splicing are expected to occur. Allelic variants of the nucleotide sequences shown in SEQ ID NO:1, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention. Allelic variants of the MASP-2 sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures, or may be identified by homology comparison search (e.g., BLAST searching) of databases containing such information.

Lectin Pathway

MASP-2 is activated upon binding of the recognition components to their respective pattern, and may also be activated by MASP-1, and subsequently cleaves the complement component C4 into C4a and C4b. After the binding of the cleavage product C4b to plasma C2, C4b-bound C2 becomes substrate of a second MASP-2-mediated cleavage step which converts C4b-bound C2 into the enzymatically active complex C4bC2a and a small C2b cleavage fragment. C4b2a is the C3-converting C3 convertase of the lectin pathway, converting the abundant plasma component C3 into C3a and C3b. C3b binds to any surface in close proximity via a thioester bond. If several C3b fragments bind in

close proximity to the C3 convertase complex C4b2a, this convertase alters its specificity to convert C5 into C5b and C5a, forming the C5 convertase complex C4b2a(C3b)n. While this C5 convertase can initiate formation of MAC, this process is thought to be insufficiently effective to promote lysis on its own. Rather, the initial C3b opsonins produced by the lectin pathway form the nucleus for the formation of new alternative pathway C3 convertase and C5 convertase sites, which ultimately lead to abundant MAC formation and lysis. There is also a MASP-2-dependent C4-bypass activation route to activate C3 in the absence of C4, which plays an important role in the pathophysiology of ischemia-reperfusion injury, since C4-deficient mice are not protected from ischemia-reperfusion injury while MASP-2-deficient mice are (Schwaeble et al., *PNAS*, 2011 *supra*). As described herein, MASP-2-dependent complement activation is also tied to the coagulation pathway, involving the cleavage of prothrombin to thrombin (common pathway) and also the cleavage of factor XII (Hageman factor) to convert into its enzymatically active form XIIa. Factor XIIa in turn cleaves factor XI to XIa (intrinsic pathway). The intrinsic pathway activation of the clotting cascade leads to fibrin formation, which is of critical importance for thrombus formation.

A MASP-2-dependent activation route leading to the formation of the lectin pathway C3 convertase C4b2a and, upon accumulation of the C3 cleavage product C3b, subsequently to the C5 convertase C4b2a(C3b)n. In the absence of complement C4, MASP-2 can form an alternative C3 convertase complex which involves C2 and clotting factor XI. As demonstrated in Example 14 herein, lectin pathway activation, via MASP-2, triggers activation of the coagulation and contact systems. This is expected to result in additional coagulation, thereby driving a feedback cycle that results in excessive and unchecked clot formation. This feedback cycle is expected to promote thrombotic disorders. As further demonstrated herein, MASP-2 forms the nexus of this crosstalk, and the inhibition of MASP-2 by MASP-2 inhibitory antibodies blocked Factor XII activation and Kallikrein activation, thereby indicating that the therapeutic use of MASP-2 inhibitory antibodies is expected to reduce excessive activation of the coagulation and contact cascades.

In addition to its role in lysis, the MASP-2-driven activation route plays an important role in bacterial opsonization leading to microbes being coated with covalently

bound C3b and cleavage products thereof (i.e., iC3b and C3dg), which will be targeted for the uptake and killing by C3 receptor-bearing phagocytes, such as granulocytes, macrophages, monocytes, microglia cells and the reticuloendothelial system. This is the most effective route of clearance of bacteria and microorganisms that are resistant to complement lysis. These include most of the gram-positive bacteria.

Background of MASP-2

Three mannan-binding lectin-associated serine proteases (MASP-1, MASP-2 and MASP-3) are presently known to be associated in human serum with the mannan-binding lectin (MBL). Mannan-binding lectin is also called 'mannose-binding protein' or 'mannose-binding lectin' in the recent literature. The MBL-MASP-2 complex plays an important role in innate immunity by virtue of the binding of MBL to carbohydrate structures present on a wide variety of microorganisms. The interaction of MBL with specific arrays of carbohydrate structures brings about the activation of the MASP proenzymes which, in turn, activate complement by cleaving the complement components C4 and C2 to form the C3 convertase C4b2b (Kawasaki *et al.*, *J. Biochem* 106:483-489 (1989); Matsushita & Fujita, *J. Exp Med.* 176:1497-1502 (1992); Ji *et al.*, *J. Immunol* 150:571-578 (1993)).

The MBL-MASP proenzyme complex was, until recently, considered to contain only one type of protease (MASP-1), but it is now clear that there are two other distinct proteases (i.e., MASP-2 and MASP-3) associated with MBL (Thiel *et al.*, *Nature* 386:506-510 (1997); Dahl *et al.*, *Immunity* 15:127-135 (2001)), as well as an additional serum protein of 19 kDa, referred to as "MAp19" or "sMAP" (Stover *et al.*, *J. Immunol* 162:3481-3490 (1999); Stover *et al.*, *J. Immunol* 163:6848-6859 (1999); Takahashi *et al.*, *Int. Immunol* 11:859-63 (1999)).

MAp19 is an alternatively spliced gene product of the structural gene for MASP-2 and lacks the four C-terminal domains of MASP-2, including the serine endopeptidase domain. The abundantly expressed truncated mRNA transcript encoding MAp19 is generated by an alternative splicing/polyadenylation event of the MASP-2 gene. By a similar mechanism, the MASP-1/3 gene gives rise to three major gene products, the two serine proteases MASP-1 and MASP-3 and a truncated gene product of 44 kDa referred

to as "MAp44" (Degn *et al.*, *J. Immunol* 183(11):7371-8 (2009); Skjoedt *et al.*, *J Biol Chem* 285:8234-43 (2010)).

MASP-1 was first described as the P-100 protease component of the serum Ra-reactive factor, which is now recognized as being a complex composed of MBL plus
5 MASP (Matsushita *et al.*, *Collectins and Innate Immunity*, (1996); Ji *et al.*, *J Immunol* 150:571-578 (1993). The ability of an MBL-associated endopeptidase within the MBL-MASPs complex to act on the complement components C4 and C2 in a manner apparently identical to that of the C1s enzyme within the C1q-(C1r)₂-(C1s)₂ complex of the classical pathway of complement suggests that there is a MBL-MASPs complex
10 which is functionally analogous to the C1q-(C1r)₂-(C1s)₂ complex. The C1q-(C1r)₂-(C1s)₂ complex is activated by the interaction of C1q with the Fc regions of antibody IgG or IgM present in immune complexes. This brings about the autoactivation of the C1r proenzyme which, in turn, activates the C1s proenzyme which then acts on complement components C4 and C2.

15 The stoichiometry of the MBL-MASPs complex differs from the one found for the C1q-(C1r)₂-(C1s)₂ complex in that different MBL oligomers appear to associate with different proportions of MASP-1/MAp19 or MASP-2/MASP-3 (Dahl *et al.*, *Immunity* 15:127-135 (2001). The majority of MASPs and MAp19 found in serum are not complexed with MBL (Thiel *et al.*, *J Immunol* 165:878-887 (2000)) and may associate in
20 part with ficolins, a recently described group of lectins having a fibrinogen-like domain able to bind to N-acetylglucosamine residues on microbial surfaces (Le *et al.*, *FEBS Lett* 425:367 (1998); Sugimoto *et al.*, *J. Biol Chem* 273:20721 (1998)). Among these, human L-ficolin, H-ficolin and M-ficolin associate with MASPs as well as with MAp19 and may activate the lectin pathway upon binding to the specific carbohydrate structures
25 recognized by ficolins (Matsushita *et al.*, *J Immunol* 164:2281-2284 (2000); Matsushita *et al.*, *J Immunol* 168:3502-3506 (2002)). In addition to the ficolins and MBL, an MBL-like lectin collectin, called CL-11, has been identified as a lectin pathway recognition molecule (Hansen *et al.* *J Immunol* 185:6096-6104 (2010); Schwaeble *et al.* *PNAS* 108:7523-7528 (2011)). There is overwhelming evidence underlining the physiological
30 importance of these alternative carbohydrate recognition molecules and it is therefore important to understand that MBL is not the only recognition component of the lectin

activation pathway and that MBL deficiency is not to be mistaken for lectin-pathway deficiency. The existence of possibly an array of alternative carbohydrate-recognition complexes structurally related to MBL may broaden the spectrum of microbial structures that initiate a direct response of the innate immune system via activation of complement.

5 All lectin pathway recognition molecules are characterized by a specific MASPs-binding motif within their collagen-homologous stalk region (Wallis et al. *J. Biol Chem* 279:14065-14073 (2004)). The MASP-binding site in MBLs, CL-11 and ficolins is characterized by a distinct motif within this domain: Hyp-Gly-Lys-Xaa-Gly-Pro, where Hyp is hydroxyproline and Xaa is generally an aliphatic residue. Point mutations in this
10 sequence disrupt MASP binding.

FIGURE 1 is a schematic diagram illustrating the domain structure of the MASP-2 polypeptide (SEQ ID NO:2) and MAP19 polypeptide and the exons encoding the same. As shown in **FIGURE 1** the serine proteases MASP-2 consists of six distinct domains arranged as found in C1r and C1s; *i.e.*, (I) an N-terminal C1r/C1s/sea urchin VEGF/bone
15 morphogenic protein (or CUBI) domain; (II) an epidermal growth factor (EGF)-like domain; (III) a second CUB domain (CUBII); (IV and V) two complement control protein (CCP1 and CCP2) domains; and (VI) a serine protease (SP) domain.

The cDNA-derived amino acid sequences of human and mouse MASP-1 (Sato *et al.*, *Int Immunol* 6:665-669 (1994); Takada *et al.*, *Biochem Biophys Res Commun*
20 196:1003-1009 (1993); Takayama *et al.*, *J. Immunol* 152:2308-2316 (1994)), human, mouse, and rat MASP-2 (Thiel *et al.*, *Nature* 386:506-510 (1997); Endo *et al.*, *J Immunol* 161:4924-30 (1998); Stover *et al.*, *J. Immunol* 162:3481-3490 (1999); Stover *et al.*, *J. Immunol* 163:6848-6859 (1999)), as well as human MASP-3 (Dahl *et al.*, *Immunity* 15:127-135 (2001)) indicate that these proteases are serine peptidases having the
25 characteristic triad of His, Asp and Ser residues within their putative catalytic domains (Genbank Accession numbers: human MASP-1: BAA04477.1; mouse MASP-1: BAA03944; rat MASP-1: AJ457084; Human MASP-3: AAK84071; mouse MASP-3: AB049755, as accessed on Genbank on 2/15/2012, each of which is hereby incorporated herein by reference).

30 As further shown in **FIGURE 1**, upon conversion of the zymogen to the active form, the heavy chain (alpha, or A chain) and light chain (beta, or B chain) are split to

yield a disulphide-linked A-chain and a smaller B-chain representing the serine protease domain.

The human MASP-2 gene is located on chromosome 1p36.3-2 (Stover *et al.*, *Cytogenet and Cell Genet* 84:148-149 (1999) and encompasses twelve exons, as shown in

5 **FIGURE 1.** MASP-2 (SEQ ID NO:2) and MAP19 are encoded by transcripts of a single structural gene generated by alternative splicing/polyadenylation (Stover *et al.*, *Genes and Immunity* 2:119-127 (2001)). The human MASP-2 cDNA (SEQ ID NO:1) is encoded by exons 2, 3, 4, 6, 7, 8, 9, 10, 11 and 12. The 20 kDa protein termed MBL-associated protein 19 ("MAP19", also referred to as "sMAP") arises from exons 2, 3, 4 and 5.
10 MAP19 is a nonenzymatic protein containing the N-terminal CUB1-EGF region of MASP-2 with four additional residues (EQSL) derived from exon 5 as shown in **FIGURE 1.**

The MASP-2 polypeptide (SEQ ID NO:2) has 686 amino acid residues, which includes a leader peptide of 15 residues that is cleaved off after secretion, resulting in the
15 mature form of human MASP-2 (SEQ ID NO:3). As shown in **FIGURE 1**, the MASP-2 amino acid sequence does not contain any N-linked glycosylation sites. The MASP-2 polypeptide exhibits a molecular structure similar to MASP-1, MASP-3, and C1r and C1s, the proteases of the C1 complement system. The domains of the human MASP-2 protein (numbered with reference to SEQ ID NO:2) are shown in **FIGURE 1** and include
20 an N-terminal C1r/C1s/sea urchin VEGF/bone morphogenic protein (CUBI) domain (aa 24-136 of SEQ ID NO:2), an epidermal growth factor-like domain (aa 138-180 of SEQ ID NO:2), a second CUB domain (CUBII) (aa 184-295 of SEQ ID NO:2), as well as a tandem of complement control protein (CCP1 aa 300-359 and CCP2 aa 364-431 of SEQ ID NO:2) domains and a serine protease domain (aa 445-682 of SEQ ID NO:2).

25 As shown in **FIGURE 1**, the MASP-2 polypeptide has an alpha chain (heavy chain) containing the CUB-1-EGF-CUB-2-CCP-1-CCP-2 domains (alpha chain: aa 1-443 of SEQ ID NO:2) and a beta chain (light chain) containing the serine protease domain (beta chain: aa 444-686). The CUB-1, EGF and CUB-2 domains are required for dimerization and the CUB-1, EGF, CUB-2 and CCP-1 domains contain the binding site
30 for MBP. As described in Wallis *et al.*, *J. Biol Chem* 279:14065-14073 (2004), each MASP-2 dimer binds to two MBL subunits.

The role of the MBL/Ficolin-MASPs complexes in innate immunity is mediated via the calcium-dependent binding of the C-type lectin domains (present in the MBL molecule) or via the binding of the fibrinogen-like domains (present in the ficolin molecule) to carbohydrate structures found on yeast, bacteria, viruses, and fungi. This recognition phase brings about the activation of the proenzyme MASP-2, which then mimics the action of the activated C1s within the C1q-(C1r)₂-(C1s)₂ complex by cleaving C4 and C2 to form the C3 convertase C4b2b. This allows deposition of C4b and C3b on target pathogens and thus promotes killing and clearance through phagocytosis.

Evidence in the recent literature suggests that the lectin pathway activation complex only requires the activity of MASP-2 to cleave C4 and C2: i) the reconstitution of a minimal lectin-pathway activation complex using recombinant MBL and recombinantly expressed MASP-2 appears to be sufficient to effectively cleave both C4 and C2 *in vitro* (Vorup-Jensen *et al.*, *J. Immunol* 165:2093-2100 (2000); Rossi *et al.*, *J Biol Chem* 276:40880-40887 (2001); Ambrus *et al.*, *J Immunol* 170:1374-1382 (2003); Gál *et al.*, *J Biol Chem* 280:33435-33444 (2005)); while ii) the serum of mice with a gene-targeted deficiency of MASP-2 is devoid of any lectin pathway functional activity (Schwaeble *et al.*, *PNAS* 108:7523-7528 (2011)). Recently, a genetically determined deficiency of MASP-2 was described (Stengaard-Pedersen *et al.*, *New Eng. J. Med.* 349:554-560, (2003)). The mutation of a single nucleotide leads to an Asp-Gly exchange in the CUB1 domain and renders MASP-2 incapable of binding to MBL.

The data provided herein demonstrate that MASP-2-dependent lectin pathway complement activation provides a link between lectin-dependent complement activation and coagulation. Thus, in view of the above, MASP-2 inhibitors are expected to have therapeutic benefits in treating a subject suffering from coagulation and thrombotic disorders as described herein.

In accordance with the foregoing, an aspect of the invention thus provides a method for inhibiting MASP-2-dependent complement activation for treating, preventing, or reducing the severity of coagulation and thrombotic disorders as disclosed herein in a subject in need thereof comprising administering a composition comprising a therapeutically effective amount of a MASP-2 inhibitory antibody in a pharmaceutical carrier to a subject in need thereof. The MASP-2 inhibitory composition may be

administered to the subject systemically, such as by intra-arterial, intravenous, intramuscular, inhalational, nasal, subcutaneous or other parenteral administration, or potentially by oral administration for non-peptidergic agents. Administration may be repeated as determined by a physician until the condition has been resolved or is controlled. For treatment or prevention of a thrombotic or coagulation disease or disorder secondary to trauma or other acute event, the MASP-2 inhibitory composition may be administered immediately following the traumatic injury or prophylactically prior to, during, immediately following, or within one to seven days or longer, such as within 24 hours to 72 hours, after trauma-inducing injury or situations such as surgery in patients deemed at risk. In some embodiments, the MASP-2 inhibitory composition may suitably be administered in a fast-acting dosage form, such as by intravenous or intra-arterial delivery of a bolus of a solution containing the MASP-2 inhibitory agent composition.

Application of the MASP-2 inhibitory compositions of the present invention may be carried out by a single administration of the composition), or a limited sequence of administrations, for treating, preventing, or reducing the severity of coagulation or thrombosis in subject in need thereof. Alternatively, the composition may be administered at periodic intervals such as daily, biweekly, weekly, every other week, monthly or bimonthly over an extended period of time for treatment of a subject experiencing, or at risk for developing a thrombotic or coagulation disorder, disease or condition.

XVII. MASP-2 INHIBITORY AGENTS

A MASP-2 inhibitory antibody may effectively block MASP-2 protein-to-protein interactions, interfere with MASP-2 dimerization or assembly, block Ca^{++} binding, or interfere with the MASP-2 serine protease active site, preventing MASP-2 from activating the lectin pathway. The MASP-2 inhibitory antibodies can be used alone as a primary therapy or in combination with other therapeutics as an adjuvant therapy to enhance the therapeutic benefits of other medical treatments, as further described herein.

In one embodiment, the MASP-2 inhibitory antibody specifically binds to a portion of MASP-2 (SEQ ID NO:2) with a binding affinity of at least 10 times greater than to other antigens in the complement system. In another embodiment, the MASP-2

inhibitory antibody specifically binds to a portion of MASP-2 (SEQ ID NO:2) with a binding affinity of at least 100 times greater than to other antigens in the complement system. In one embodiment, the MASP-2 inhibitory antibody specifically binds to at least one of (i) the CCP1-CCP2 domain (aa 300-431 of SEQ ID NO:2) or the serine protease domain of MASP-2 (aa 445-682 of SEQ ID NO:2) and inhibits MASP-2-dependent complement activation, with the proviso that the inhibitory antibody does not bind to the serine protease domain of MASP-1 and it does not bind to the serine protease domain of MASP-3. In one embodiment, the MASP-2 inhibitory antibody is a MASP-2 monoclonal antibody, or antigen-binding fragment thereof that specifically binds to MASP-2.

The binding affinity of the MASP-2 inhibitory antibody can be determined using a suitable binding assay.

The inhibition of MASP-2-dependent complement activation is characterized by at least one of the following changes in a component of the complement system that occurs as a result of administration of a MASP-2 inhibitory antibody in accordance with the methods of the invention: the inhibition of the generation or production of MASP-2-dependent complement-activation-system products C4b, C3a, C5a and/or C5b-9 (MAC) (measured, for example, as described in Example 2 of US Patent No. 7,919,094), the reduction of C4 cleavage and C4b deposition (measured, for example as described in Example 1 or Example 2), or the reduction of C3 cleavage and C3b deposition (measured, for example, as described in Example 4).

In some embodiments, the MASP-2 inhibitory antibodies selectively inhibit MASP-2 complement activation leaving the C1q-dependent complement activation system functionally intact.

MASP-2 ANTIBODIES

The MASP-2 antibodies useful in this aspect of the invention include polyclonal, monoclonal or recombinant antibodies derived from any antibody producing mammal and may be multispecific (i.e., bispecific or trispecific), chimeric, humanized, fully human, anti-idiotypic, and antibody fragments. Antibody fragments include Fab, Fab', F(ab)₂, F(ab')₂, Fv fragments, scFv fragments and single-chain antibodies as further described herein.

For example, as described in Examples 4-6 herein, anti-rat MASP-2 Fab2 antibodies have been identified that block MASP-2-dependent complement activation. As further described in Example 7, fully human MASP-2 scFv antibodies have been identified that block MASP-2-dependent complement activation.

5

TABLE 1: MASP-2 SPECIFIC ANTIBODIES

TARGET	ANTIGEN	ANTIBODY TYPE	REFERENCE
MASP-2	Recombinant MASP-2	Rat Polyclonal	Peterson, S.V., et al., <i>Mol. Immunol.</i> 37:803-811, 2000
MASP-2	Recombinant human CCP1/2-SP fragment (MoAb 8B5)	Rat MoAb (subclass IgG1)	Moller-Kristensen, M., et al., <i>J. of Immunol. Methods</i> 282:159-167, 2003
MASP-2	Recombinant human MASP19 (MoAb 6G12) (cross-reacts with MASP-2)	Rat MoAb (subclass IgG1)	Moller-Kristensen, M., et al., <i>J. of Immunol. Methods</i> 282:159-167, 2003
MASP-2	hMASP-2	Mouse MoAb (S/P) Mouse MoAb (N-term)	Peterson, S.V., et al., <i>Mol. Immunol.</i> 35:409, April 1998
MASP-2	hMASP-2 (CCP1-CCP2-SP domain)	rat MoAb: Nimoab101, produced by hybridoma cell line 03050904 (ECACC)	WO 2004/106384

TARGET	ANTIGEN	ANTIBODY TYPE	REFERENCE
MASP-2	hMASP-2 (full-length his-tagged)	murine MoAbs: NimoAb104, produced by hybridoma cell line M0545YM035 (DSMZ) NimoAb108, produced by hybridoma cell line M0545YM029 (DSMZ) NimoAb109 produced by hybridoma cell line M0545YM046 (DSMZ) NimoAb110 produced by hybridoma cell line M0545YM048 (DSMZ)	WO 2004/106384
MASP-2	Rat MASP-2 (full-length)	MASP-2 Fab2 antibody fragments	Examples 4-5
MASP-2	hMASP-2 (full-length)	Fully human scFv clones	Example 7

MASP-2 antibodies with reduced effector function

In some embodiments of this aspect of the invention, the MASP-2 antibodies described herein have reduced effector function in order to reduce inflammation that may arise from the activation of the classical complement pathway. The ability of IgG molecules to trigger the classical complement pathway has been shown to reside within the Fc portion of the molecule (Duncan, A.R., et al., *Nature* 332:738-740 (1988)). IgG molecules in which the Fc portion of the molecule has been removed by enzymatic cleavage are devoid of this effector function (see Harlow, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, 1988). Accordingly, antibodies

with reduced effector function can be generated as the result of lacking the Fc portion of the molecule by having a genetically engineered Fc sequence that minimizes effector function, or being of either the human IgG₂ or IgG₄ isotype.

Antibodies with reduced effector function can be produced by standard molecular biological manipulation of the Fc portion of the IgG heavy chains as described in Jolliffe et al., *Int'l Rev. Immunol.* 10:241-250, (1993), and Rodrigues et al., *J. Immunol.* 151:6954-6961, (1998). Antibodies with reduced effector function also include human IgG₂ and IgG₄ isotypes that have a reduced ability to activate complement and/or interact with Fc receptors (Ravetch, J.V., et al., *Annu. Rev. Immunol.* 9:457-492, (1991); Isaacs, J.D., et al., *J. Immunol.* 148:3062-3071, 1992; van de Winkel, J.G., et al., *Immunol. Today* 14:215-221, (1993)). Humanized or fully human antibodies specific to human MASP-MASP-2 comprised of IgG₂ or IgG₄ isotypes can be produced by one of several methods known to one of ordinary skilled in the art, as described in Vaughan, T.J., et al., *Nature Biotechnical* 16:535-539, (1998).

15 Production of MASP-2 antibodies

 MASP-2 antibodies can be produced using MASP-2 polypeptides (e.g., full-length MASP-2) or using antigenic MASP-2 epitope-bearing peptides (e.g., a portion of the MASP-2 polypeptide). Immunogenic peptides may be as small as five amino acid residues. For example, the MASP-2 polypeptide including the entire amino acid sequence of SEQ ID NO:2 may be used to induce MASP-2 antibodies useful in the method of the invention. Particular MASP-2 domains known to be involved in protein-protein interactions, such as the CUBI, and CUBI-EGF domains, as well as the region encompassing the serine-protease active site, may be expressed as recombinant polypeptides using methods well known in the art and used as antigens. In addition, peptides comprising a portion of at least 6 amino acids of the MASP-2 polypeptide (SEQ ID NO:2) are also useful to induce MASP-2 antibodies. The MASP-2 peptides and polypeptides used to raise antibodies may be isolated as natural polypeptides, or recombinant or synthetic peptides and catalytically inactive recombinant polypeptides. Antigens useful for producing MASP-2 antibodies also include fusion polypeptides, such as fusions of a MASP-2 polypeptide or a portion thereof with an immunoglobulin polypeptide or with maltose-binding protein. The polypeptide immunogen may be a

full-length molecule or a portion thereof. If the polypeptide portion is hapten-like, such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

5 Polyclonal antibodies

Polyclonal antibodies against MASP-2 can be prepared by immunizing an animal with MASP-2 polypeptide or an immunogenic portion thereof using methods well known to those of ordinary skill in the art. See, for example, Green et al., "Production of Polyclonal Antisera," in *Immunochemical Protocols* (Manson, ed.). The immunogenicity
10 of a MASP-2 polypeptide can be increased through the use of an adjuvant, including mineral gels, such as aluminum hydroxide or Freund's adjuvant (complete or incomplete), surface active substances such as lysolecithin, pluronic polyols, polyanions, oil emulsions, KLH and dinitrophenol. Polyclonal antibodies are typically raised in animals such as horses, cows, dogs, chicken, rats, mice, rabbits, guinea pigs, goats, or sheep.
15 Alternatively, a MASP-2 antibody useful in the present invention may also be derived from a subhuman primate. General techniques for raising diagnostically and therapeutically useful antibodies in baboons may be found, for example, in Goldenberg et al., International Patent Publication No. WO 91/11465, and in Losman, M.J., et al., *Int. J. Cancer* 46:310, (1990). Sera containing immunologically active antibodies are then
20 produced from the blood of such immunized animals using standard procedures well known in the art.

Monoclonal antibodies

In some embodiments, the MASP-2 inhibitory antibody is a MASP-2 monoclonal
25 antibody. As described above, in some embodiments, MASP-2 monoclonal antibodies are highly specific, being directed against a single MASP-2 epitope. As used herein, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogenous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. Monoclonal antibodies
30 can be obtained using any technique that provides for the production of antibody molecules by continuous cell lines in culture, such as the hybridoma method described by

Kohler, G., et al., *Nature* 256:495, (1975), or they may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567 to Cabilly). Monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson, T., et al., *Nature* 352:624-628, (1991), and Marks, J.D., et al., *J. Mol. Biol.* 222:581-597, 5 (1991). Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof.

For example, monoclonal antibodies can be obtained by injecting a suitable mammal (e.g., a BALB/c mouse) with a composition comprising a MASP-2 polypeptide, or portion thereof. After a predetermined period of time, splenocytes are removed from 10 the mouse and suspended in a cell culture medium. The splenocytes are then fused with an immortal cell line to form a hybridoma. The formed hybridomas are grown in cell culture and screened for their ability to produce a monoclonal antibody against MASP-2. (See also *Current Protocols in Immunology*, Vol. 1., John Wiley & Sons, pages 2.5.1-2.6.7, 1991.)

15 Human monoclonal antibodies may be obtained through the use of transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human immunoglobulin heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous immunoglobulin heavy chain 20 and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, such as the MASP-2 antigens described herein, and the mice can be used to produce human MASP-2 antibody-secreting hybridomas by fusing B-cells from such animals to suitable myeloma cell lines using conventional Kohler-Milstein technology. Methods for obtaining human antibodies from transgenic mice are described, for 25 example, by Green, L.L., et al., *Nature Genet.* 7:13, 1994; Lonberg, N., et al., *Nature* 368:856, 1994; and Taylor, L.D., et al., *Int. Immun.* 6:579, 1994.

Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and 30 ion-exchange chromatography (see, for example, Coligan at pages 2.7.1-2.7.12 and

pages 2.9.1-2.9.3; Baines et al., "Purification of Immunoglobulin G (IgG)," in *Methods in Molecular Biology*, The Humana Press, Inc., Vol. 10, pages 79-104, 1992).

Once produced, polyclonal, monoclonal or phage-derived antibodies are first tested for specific MASP-2 binding. Methods for determining whether an antibody binds to a protein antigen and/or the affinity for an antibody to a protein antigen are known in the art. For example, the binding of an antibody to a protein antigen can be detected and/or quantified using a variety of techniques such as, but not limited to, Western blot, dot blot, plasmon surface resonance method (e.g., BIAcore system; Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ), or enzyme-linked immunosorbent assays (ELISA). See, e.g., Harlow and Lane (1988) "Antibodies: A Laboratory Manual" Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y.; Benny K. C. Lo (2004) "Antibody Engineering: Methods and Protocols," Humana Press (ISBN: 1588290921); Borrebaek (1992) "Antibody Engineering, A Practical Guide," W.H. Freeman and Co., NY; Borrebaek (1995) "Antibody Engineering," 2nd Edition, Oxford University Press, NY, Oxford; Johne et al. (1993), *Immunol. Meth.* 160:191-198; Jonsson et al. (1993) *Ann. Biol. Clin.* 51: 19-26; and Jonsson et al. (1991) *Biotechniques* 11:620-627. See also, U.S. Patent No. 6,355,245.

The affinity of MASP-2 monoclonal antibodies can be readily determined by one of ordinary skill in the art (see, e.g., Scatchard, A., *NY Acad. Sci.* 51:660-672, 1949). In one embodiment, the MASP-2 monoclonal antibodies useful for the methods of the invention bind to MASP-2 with a binding affinity of <100 nM, preferably <10 nM and most preferably <2 nM.

Once antibodies are identified that specifically bind to MASP-2, the MASP-2 antibodies are tested for the ability to function as a MASP-2 inhibitory antibody in one of several functional assays. For example, antibodies identified that specifically bind to MASP-2 are tested for the ability to function as a MASP-2 inhibitory antibody in one of several assays, such as, for example, (e.g., a lectin-specific C4 cleavage assay (such as the assay described in Example 1 or Example 2), or a C3b deposition assay (such as the assay described in Example 4)).

30

Chimeric/humanized antibodies

Monoclonal antibodies useful in the method of the invention include chimeric antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies (U.S. Patent No. 4,816,567, to Cabilly; and Morrison, S.L., et al., *Proc. Nat'l Acad. Sci. USA* 81:6851-6855, (1984)).

One form of a chimeric antibody useful in the invention is a humanized monoclonal MASP-2 antibody. Humanized forms of non-human (e.g., murine) antibodies are chimeric antibodies, which contain minimal sequence derived from non-human immunoglobulin. Humanized monoclonal antibodies are produced by transferring the non-human (e.g., mouse) complementarity determining regions (CDR), from the heavy and light variable chains of the mouse immunoglobulin into a human variable domain. Typically, residues of human antibodies are then substituted in the framework regions of the non-human counterparts. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the Fv framework regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones, P.T., et al., *Nature* 321:522-525, (1986); Reichmann, L., et al., *Nature* 332:323-329, (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596, (1992).

The humanized antibodies useful in the invention include human monoclonal antibodies including at least a MASP-2 binding CDR3 region. In addition, the Fc portions may be replaced so as to produce IgA or IgM as well as human IgG antibodies. Such humanized antibodies will have particular clinical utility because they will specifically recognize human MASP-2 but will not evoke an immune response in humans

against the antibody itself. Consequently, they are better suited for *in vivo* administration in humans, especially when repeated or long-term administration is necessary.

Techniques for producing humanized monoclonal antibodies are also described, for example, by Jones, P.T., et al., *Nature* 321:522, (1986); Carter, P., et al., *Proc. Nat'l. Acad. Sci. USA* 89:4285, (1992); Sandhu, J.S., *Crit. Rev. Biotech.* 12:437, (1992); Singer, I.I., et al., *J. Immun.* 150:2844, (1993); Sudhir (ed.), *Antibody Engineering Protocols*, Humana Press, Inc., (1995); Kelley, "Engineering Therapeutic Antibodies," in *Protein Engineering: Principles and Practice*, Cleland et al. (eds.), John Wiley & Sons, Inc., pages 399-434, (1996); and by U.S. Patent No. 5,693,762, to Queen, 1997. In addition, there are commercial entities that will synthesize humanized antibodies from specific murine antibody regions, such as Protein Design Labs (Mountain View, CA).

Recombinant antibodies

MASP-2 antibodies can also be made using recombinant methods. For example, human antibodies can be made using human immunoglobulin expression libraries (available for example, from Stratagene, Corp., La Jolla, CA) to produce fragments of human antibodies (V_H , V_L , Fv, Factor D, Fab or $F(ab')_2$). These fragments are then used to construct whole human antibodies using techniques similar to those for producing chimeric antibodies.

Anti-idiotypic antibodies

Once MASP-2 antibodies are identified with the desired inhibitory activity, these antibodies can be used to generate anti-idiotypic antibodies that resemble a portion of MASP-2 using techniques that are well known in the art. See, e.g., Greenspan, N.S., et al., *FASEB J.* 7:437, (1993). For example, antibodies that bind to MASP-2 and competitively inhibit a MASP-2 protein interaction required for complement activation can be used to generate anti-idiotypes that resemble the MBL binding site on MASP-2 protein and therefore bind and neutralize a binding ligand of MASP-2 such as, for example, MBL.

Immunoglobulin fragments

The MASP-2 inhibitory antibodies or antigen-binding fragments thereof useful in the method of the invention encompass not only intact immunoglobulin molecules but

also the well known fragments including Fab, Fab', F(ab)₂, F(ab')₂ and Fv fragments, scFv fragments, diabodies, linear antibodies, single-chain antibody molecules and multispecific (e.g., bispecific and trispecific) antibodies formed from antibody fragments.

It is well known in the art that only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, e.g., Clark, W.R., *The Experimental Foundations of Modern Immunology*, Wiley & Sons, Inc., NY, 1986). The pFc' and Fc regions of the antibody are effectors of the classical complement pathway but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, is designated an F(ab')₂ fragment and retains both of the antigen binding sites of an intact antibody. An isolated F(ab')₂ fragment is referred to as a bivalent monoclonal fragment because of its two antigen binding sites. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, is designated a Fab fragment, and retains one of the antigen binding sites of an intact antibody molecule.

Antibody fragments can be obtained by proteolytic hydrolysis, such as by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulphydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using pepsin produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, U.S. Patent No. 4,331,647 to Goldenberg; Nisonoff, A., et al., *Arch. Biochem. Biophys.* 89:230, (1960); Porter, R.R., *Biochem. J.* 73:119, (1959); Edelman, et al., in *Methods in Enzymology* 1:422, Academic Press, (1967); and by Coligan at pages 2.8.1-2.8.10 and 2.10.-2.10.4.

In some embodiments, the use of antibody fragments lacking the Fc region are preferred to avoid activation of the classical complement pathway which is initiated upon binding Fc to the Fcγ receptor. There are several methods by which one can produce a monoclonal antibody that avoids Fcγ receptor interactions. For example, the Fc region of

a monoclonal antibody can be removed chemically using partial digestion by proteolytic enzymes (such as ficin digestion), thereby generating, for example, antigen-binding antibody fragments such as Fab or F(ab)₂ fragments (Mariani, M., et al., *Mol. Immunol.* 28:69-71, (1991)). Alternatively, the human γ 4 IgG isotype, which does not bind Fc γ receptors, can be used during construction of a humanized antibody as described herein. Antibodies, single chain antibodies and antigen-binding domains that lack the Fc domain can also be engineered using recombinant techniques described herein.

Single-chain antibody fragments

Alternatively, one can create single peptide chain binding molecules specific for MASP-2 in which the heavy and light chain Fv regions are connected. The Fv fragments may be connected by a peptide linker to form a single-chain antigen binding protein (scFv). These single-chain antigen binding proteins are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains which are connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell, such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing scFvs are described for example, by Whitlow, et al., "Methods: A Companion to Methods in Enzymology" 2:97, (1991); Bird, et al., *Science* 242:423, (1988); U.S. Patent No. 4,946,778, to Ladner; Pack, P., et al., *Bio/Technology* 11:1271, (1993).

As an illustrative example, a MASP-2-specific scFv can be obtained by exposing lymphocytes to MASP-2 polypeptide *in vitro* and selecting antibody display libraries in phage or similar vectors (for example, through the use of immobilized or labeled MASP-2 protein or peptide). Genes encoding polypeptides having potential MASP-2 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage or on bacteria such as *E. coli*. These random peptide display libraries can be used to screen for peptides which interact with MASP-2. Techniques for creating and screening such random peptide display libraries are well known in the art (U.S. Patent No. 5,223,409, to Lardner; U.S. Patent No. 4,946,778, to Ladner; U.S. Patent No. 5,403,484, to Lardner; U.S. Patent No. 5,571,698, to Lardner; and Kay et al., *Phage Display of Peptides and Proteins* Academic Press, Inc., 1996) and random peptide

display libraries and kits for screening such libraries are available commercially, for instance from CLONTECH Laboratories, Inc. (Palo Alto, Calif.), Invitrogen Inc. (San Diego, Calif.), New England Biolabs, Inc. (Beverly, Mass.), and Pharmacia LKB Biotechnology Inc. (Piscataway, N.J.).

5 Another form of a MASP-2 antibody fragment useful in this aspect of the invention is a peptide coding for a single complementarity-determining region (CDR) that binds to an epitope on a MASP-2 antigen and inhibits MASP-2-dependent complement activation (i.e., the lectin pathway). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes
10 are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (see, for example, Larrick et al., *Methods: A Companion to Methods in Enzymology* 2:106, (1991); Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Ritter et al. (eds.), page 166,
15 Cambridge University Press, (1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, Birch et al. (eds.), page 137, Wiley-Liss, Inc., 1995).

The MASP-2 antibodies described herein are administered to a subject in need thereof to inhibit MASP-2-dependent complement activation. In some embodiments, the
20 MASP-2 inhibitory antibody is a high-affinity human or humanized monoclonal MASP-2 antibody with reduced effector function.

PHARMACEUTICAL COMPOSITIONS AND DELIVERY METHODS

DOSING

25 In another aspect, the invention provides compositions for inhibiting the adverse effects of MASP-2-dependent complement activation in a subject suffering from, or at risk of developing, a disease or disorder associated with fibrin-induced activation of the complement system and the associated activation of the coagulation and/or contact systems, wherein the compositions comprise a therapeutic amount of a MASP-2
30 inhibitory antibody sufficient to inhibit MASP-2-dependent complement activation in a subject in need thereof and a pharmaceutically acceptable carrier. In some embodiments,

the methods of the invention provide anticoagulation and/or antithrombosis and/or antithrombogenesis without affecting hemostasis.

Toxicity and therapeutic efficacy of MASP-2 inhibitory antibodies can be determined by standard pharmaceutical procedures employing experimental animal models. Using such animal models, the NOAEL (no observed adverse effect level) and the MED (the minimally effective dose) can be determined using standard methods. The dose ratio between NOAEL and MED effects is the therapeutic ratio, which is expressed as the ratio NOAEL/MED. MASP-2 inhibitory antibodies that exhibit large therapeutic ratios or indices are most preferred. The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. The dosage of the MASP-2 inhibitory antibody preferably lies within a range of circulating concentrations that include the MED with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

For any compound formulation, the therapeutically effective dose can be estimated using animal models. For example, a dose may be formulated in an animal model to achieve a circulating plasma concentration range that includes the MED. Quantitative levels of the MASP-2 inhibitory antibody in plasma may also be measured, for example, by high performance liquid chromatography.

In addition to toxicity studies, effective dosage may also be estimated based on the amount of target MASP-2 protein present in a living subject and the binding affinity of the MASP-2 inhibitory antibody.

It has been shown that MASP-2 levels in normal human subjects is present in serum in low levels in the range of 500 ng/mL, and MASP-2 levels in a particular subject can be determined using a quantitative assay for MASP-2 described in Moller-Kristensen M., et al., *J. Immunol. Methods* 282:159-167 (2003) and Csuka et al., *Mol. Immunol.* 54:271 (2013).

Generally, the dosage of administered compositions comprising MASP-2 inhibitory antibodies varies depending on such factors as the subject's age, weight, height, sex, general medical condition, and previous medical history. As an illustration, MASP-2 inhibitory antibodies can be administered in dosage ranges from about 0.010 to

100.0 mg/kg, preferably 0.010 to 10 mg/kg, preferably 0.010 to 1.0 mg/kg, more preferably 0.010 to 0.1 mg/kg of the subject body weight. In some embodiments, MASP-2 inhibitory antibodies are administered in dosage ranges from about preferably 0.010 to 10 mg/kg, preferably 0.010 to 1.0 mg/kg, more preferably 0.010 to 0.1 mg/kg of the subject body weight. Therapeutic efficacy of MASP-2 inhibitory compositions, and methods of the present invention in a given subject, and appropriate dosages, can be determined in accordance with complement assays well known to those of skill in the art. Complement generates numerous specific products. During the last decade, sensitive and specific assays have been developed and are available commercially for most of these activation products, including the small activation fragments C3a, C4a, and C5a and the large activation fragments iC3b, C4d, Bb, and sC5b-9. Most of these assays utilize monoclonal antibodies that react with new antigens (neoantigens) exposed on the fragment, but not on the native proteins from which they are formed, making these assays very simple and specific. Most rely on ELISA technology, although radioimmunoassay is still sometimes used for C3a and C5a. These latter assays measure both the unprocessed fragments and their 'desArg' fragments, which are the major forms found in the circulation. Unprocessed fragments and C5a_{desArg} are rapidly cleared by binding to cell surface receptors and are hence present in very low concentrations, whereas C3a_{desArg} does not bind to cells and accumulates in plasma. Measurement of C3a provides a sensitive, pathway-independent indicator of complement activation. Alternative pathway activation can be assessed by measuring the Bb fragment and/or measurement of factor D activation. Detection of the fluid-phase product of membrane attack pathway activation, sC5b-9, provides evidence that complement is being activated to completion. Because both the lectin and classical pathways generate the same activation products, C4a and C4d, measurement of these two fragments does not provide any information about which of these two pathways has generated the activation products.

The inhibition of MASP-2-dependent complement activation is characterized by at least one of the following changes in a component of the complement system that occurs as a result of administration of a MASP-2 inhibitory antibody in accordance with the methods of the invention: the inhibition of the generation or production of MASP-2-dependent complement activation system products C4b, C3a, C5a and/or C5b-9 (MAC)

(measured, for example, as described in measured, for example, as described in Example 2 of US Patent No. 7,919,094), the reduction of C4 cleavage and C4b deposition (measured, for example as described in Example 1 or Example 2), or the reduction of C3 cleavage and C3b deposition (measured, for example, as described in Example 4).

5

Pharmaceutical carriers and delivery vehicles

In general, the MASP-2 inhibitory agent compositions of the present invention may be combined with any other selected therapeutic agents, are suitably contained in a pharmaceutically acceptable carrier. The carrier is non-toxic, biocompatible and is selected so as not to detrimentally affect the biological activity of the MASP-2 inhibitory agent (and any other therapeutic agents combined therewith). Exemplary pharmaceutically acceptable carriers for peptides are described in U.S. Patent No. 5,211,657 to Yamada. The MASP-2 antibodies useful in the invention, as described herein, may be formulated into preparations in solid, semi-solid, gel, liquid or gaseous forms such as tablets, capsules, powders, granules, ointments, solutions, depositories, inhalants and injections allowing for oral, parenteral or surgical administration. The invention also contemplates local administration of the compositions by coating medical devices and the like.

Suitable carriers for parenteral delivery via injectable, infusion or irrigation and topical delivery include distilled water, physiological phosphate-buffered saline, normal or lactated Ringer's solutions, dextrose solution, Hank's solution, or propanediol. In addition, sterile, fixed oils may be employed as a solvent or suspending medium. For this purpose any biocompatible oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. The carrier and agent may be compounded as a liquid, suspension, polymerizable or non-polymerizable gel, paste or salve.

The carrier may also comprise a delivery vehicle to sustain (i.e., extend, delay or regulate) the delivery of the agent(s) or to enhance the delivery, uptake, stability or pharmacokinetics of the therapeutic agent(s). Such a delivery vehicle may include, by way of non-limiting example, microparticles, microspheres, nanospheres or nanoparticles

composed of proteins, liposomes, carbohydrates, synthetic organic compounds, inorganic compounds, polymeric or copolymeric hydrogels and polymeric micelles. Suitable hydrogel and micelle delivery systems include the PEO:PHB:PEO copolymers and copolymer/cyclodextrin complexes disclosed in WO 2004/009664 A2 and the PEO and
5 PEO/cyclodextrin complexes disclosed in U.S. Patent Application Publication No. 2002/0019369 A1. Such hydrogels may be injected locally at the site of intended action, or subcutaneously or intramuscularly to form a sustained release depot.

Compositions of the present invention may be formulated for delivery subcutaneously, intra-muscularly, intravenously, intra-arterially or as an inhalant.

10 For intra-articular delivery, the MASP-2 inhibitory antibody may be carried in above-described liquid or gel carriers that are injectable, above-described sustained-release delivery vehicles that are injectable, or a hyaluronic acid or hyaluronic acid derivative.

For topical administration, the MASP-2 inhibitory antibody may be carried in
15 ointment, lotion, cream, gel, drop, suppository, spray, liquid or powder, or in gel or microcapsular delivery systems via a transdermal patch.

Various nasal and pulmonary delivery systems, including aerosols, metered-dose inhalers, dry powder inhalers, and nebulizers, are being developed and may suitably be adapted for delivery of the present invention in an aerosol, inhalant, or nebulized delivery
20 vehicle, respectively.

For intrathecal (IT) or intracerebroventricular (ICV) delivery, appropriately sterile delivery systems (e.g., liquids; gels, suspensions, etc.) can be used to administer the present invention.

The compositions of the present invention may also include biocompatible
25 excipients, such as dispersing or wetting agents, suspending agents, diluents, buffers, penetration enhancers, emulsifiers, binders, thickeners, flavoring agents (for oral administration).

Pharmaceutical carriers for antibodies and peptides

30 More specifically with respect to MASP-2 antibodies, as described herein, exemplary formulations can be parenterally administered as injectable dosages of a

solution or suspension of the compound in a physiologically acceptable diluent with a pharmaceutical carrier that can be a sterile liquid such as water, oils, saline, glycerol or ethanol. Additionally, auxiliary substances such as wetting or emulsifying agents, surfactants, pH buffering substances and the like can be present in compositions comprising MASP-2 antibodies. Additional components of pharmaceutical compositions include petroleum (such as of animal, vegetable or synthetic origin), for example, soybean oil and mineral oil. In general, glycols such as propylene glycol or polyethylene glycol are preferred liquid carriers for injectable solutions.

The MASP-2 antibodies can also be administered in the form of a depot injection or implant preparation that can be formulated in such a manner as to permit a sustained or pulsatile release of the active agents.

MODES OF ADMINISTRATION

The pharmaceutical compositions comprising the MASP-2 inhibitory antibodies may be administered in a number of ways depending on whether a local or systemic mode of administration is most appropriate for the condition being treated. Further, the compositions of the present invention can be delivered by coating or incorporating the compositions on or into an implantable medical device.

Systemic delivery

As used herein, the terms "systemic delivery" and "systemic administration" are intended to include but are not limited to oral and parenteral routes including intramuscular (IM), subcutaneous, intravenous (IV), intraarterial, inhalational, sublingual, buccal, topical, transdermal, nasal, rectal, vaginal and other routes of administration that effectively result in dispersement of the delivered agent to a single or multiple sites of intended therapeutic action. Preferred routes of systemic delivery for the present compositions include intravenous, intramuscular, subcutaneous, intraarterial and inhalational. It will be appreciated that the exact systemic administration route for selected agents utilized in particular compositions of the present invention will be determined in part to account for the agent's susceptibility to metabolic transformation pathways associated with a given route of administration. For example, peptidergic agents may be most suitably administered by routes other than oral.

The MASP-2 inhibitory antibodies, as described herein, can be delivered into a subject in need thereof by any suitable means. Methods of delivery of MASP-2 antibodies and polypeptides include administration by oral, pulmonary, parenteral (e.g., intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), inhalation (such as via a fine powder formulation), transdermal, nasal, vaginal, rectal, or sublingual routes of administration, and can be formulated in dosage forms appropriate for each route of administration.

By way of representative example, MASP-2 inhibitory antibodies can be introduced into a living body by application to a bodily membrane capable of absorbing the polypeptides, for example the nasal, gastrointestinal and rectal membranes. The polypeptides are typically applied to the absorptive membrane in conjunction with a permeation enhancer. (See, e.g., Lee, V.H.L., *Crit. Rev. Ther. Drug Carrier Sys.* 5:69, (1988); Lee, V.H.L., *J. Controlled Release* 13:213, (1990); Lee, V.H.L., Ed., *Peptide and Protein Drug Delivery*, Marcel Dekker, New York (1991); DeBoer, A.G., et al., *J. Controlled Release* 13:241, (1990). For example, STDHF is a synthetic derivative of fusidic acid, a steroidal surfactant that is similar in structure to the bile salts, and has been used as a permeation enhancer for nasal delivery. (Lee, W.A., *Biopharm.* 22, Nov./Dec. 1990.)

The MASP-2 inhibitory antibodies as described herein may be introduced in association with another molecule, such as a lipid, to protect the polypeptides from enzymatic degradation. For example, the covalent attachment of polymers, especially polyethylene glycol (PEG), has been used to protect certain proteins from enzymatic hydrolysis in the body and thus prolong half-life (Fuertges, F., et al., *J. Controlled Release* 11:139, (1990)). Many polymer systems have been reported for protein delivery (Bae, Y.H., et al., *J. Controlled Release* 9:271, (1989); Hori, R., et al., *Pharm. Res.* 6:813, (1989); Yamakawa, I., et al., *J. Pharm. Sci.* 79:505, (1990); Yoshihiro, I., et al., *J. Controlled Release* 10:195, (1989); Asano, M., et al., *J. Controlled Release* 9:111, (1989); Rosenblatt, J., et al., *J. Controlled Release* 9:195, (1989); Makino, K., *J. Controlled Release* 12:235, (1990); Takakura, Y., et al., *J. Pharm. Sci.* 78:117, (1989); Takakura, Y., et al., *J. Pharm. Sci.* 78:219, (1989)).

Recently, liposomes have been developed with improved serum stability and circulation half-times (see, e.g., U.S. Patent No. 5,741,516, to Webb). Furthermore, various methods of liposome and liposome-like preparations as potential drug carriers have been reviewed (see, e.g., U.S. Patent No. 5,567,434, to Szoka; U.S. Patent No. 5,552,157, to Yagi; U.S. Patent No. 5,565,213, to Nakamori; U.S. Patent No. 5,738,868, to Shinkarenko; and U.S. Patent No. 5,795,587, to Gao).

For transdermal applications, the MASP-2 inhibitory antibodies, as described herein, may be combined with other suitable ingredients, such as carriers and/or adjuvants. There are no limitations on the nature of such other ingredients, except that they must be pharmaceutically acceptable for their intended administration, and cannot degrade the activity of the active ingredients of the composition. Examples of suitable vehicles include ointments, creams, gels, or suspensions, with or without purified collagen. The MASP-2 inhibitory antibodies may also be impregnated into transdermal patches, plasters, and bandages, preferably in liquid or semi-liquid form.

The compositions of the present invention may be systemically administered on a periodic basis at intervals determined to maintain a desired level of therapeutic effect. For example, compositions may be administered, such as by subcutaneous injection, every two to four weeks or at less frequent intervals. The dosage regimen will be determined by the physician considering various factors that may influence the action of the combination of agents. These factors will include the extent of progress of the condition being treated, the patient's age, sex and weight, and other clinical factors. The dosage for each individual agent will vary as a function of the MASP-2 inhibitory antibody that is included in the composition, as well as the presence and nature of any drug delivery vehicle (e.g., a sustained release delivery vehicle). In addition, the dosage quantity may be adjusted to account for variation in the frequency of administration and the pharmacokinetic behavior of the delivered agent(s).

Local delivery

As used herein, the term "local" encompasses application of a drug in or around a site of intended localized action, and may include for example topical delivery to the skin or other affected tissues, ophthalmic delivery, intrathecal (IT), intracerebroventricular (ICV), intra-articular, intracavity, intracranial or intravesicular administration, placement

or irrigation. Local administration may be preferred to enable administration of a lower dose, to avoid systemic side effects, and for more accurate control of the timing of delivery and concentration of the active agents at the site of local delivery. Local administration provides a known concentration at the target site, regardless of interpatient
5 variability in metabolism, blood flow, etc. Improved dosage control is also provided by the direct mode of delivery.

Local delivery of a MASP-2 inhibitory antibody may be achieved in the context of surgical methods for treating a disease or condition, such as for example during procedures such as arterial bypass surgery, atherectomy, laser procedures, ultrasonic
10 procedures, balloon angioplasty and stent placement. For example, a MASP-2 inhibitory antibody can be administered to a subject in conjunction with a balloon angioplasty procedure. A balloon angioplasty procedure involves inserting a catheter having a deflated balloon into an artery. The deflated balloon is positioned in proximity to the atherosclerotic plaque and is inflated such that the plaque is compressed against the
15 vascular wall. As a result, the balloon surface is in contact with the layer of vascular endothelial cells on the surface of the blood vessel. The MASP-2 inhibitory antibody may be attached to the balloon angioplasty catheter in a manner that permits release of the agent at the site of the atherosclerotic plaque. The agent may be attached to the balloon catheter in accordance with standard procedures known in the art. For example,
20 the agent may be stored in a compartment of the balloon catheter until the balloon is inflated, at which point it is released into the local environment. Alternatively, the agent may be impregnated on the balloon surface, such that it contacts the cells of the arterial wall as the balloon is inflated. The agent may also be delivered in a perforated balloon catheter such as those disclosed in Flugelman, M.Y., et al., *Circulation* 85:1110-1117,
25 (1992). See also published PCT Application WO 95/23161 for an exemplary procedure for attaching a therapeutic protein to a balloon angioplasty catheter. Likewise, the MASP-2 inhibitory antibody may be included in a gel or polymeric coating applied to a stent, or may be incorporated into the material of the stent, such that the stent elutes the MASP-2 inhibitory antibody after vascular placement.

TREATMENT REGIMENS

In prophylactic applications, the pharmaceutical compositions are administered to a subject susceptible to, or otherwise at risk of, a disease or disorder associated with fibrin-induced activation of the complement system and the associated activation of the coagulation and/or contact systems as described herein in an amount sufficient to eliminate or reduce the risk of developing symptoms of the condition. In therapeutic applications, the pharmaceutical compositions are administered to a subject suspected of, or already suffering from, a disease or disorder associated with fibrin-induced activation of the complement system and the associated activation of the coagulation and/or contact systems as described herein in a therapeutically effective amount sufficient to relieve, or at least partially reduce, the symptoms of the condition.

In both prophylactic and therapeutic regimens for the treatment, prevention or reduction in severity of a disease or disorder associated with fibrin-induced activation of the complement system and the associated activation of the coagulation and/or contact systems as described herein the pharmaceutical compositions may be administered in several dosages until a sufficient therapeutic outcome has been achieved in the subject. In one embodiment of the invention, the MASP-2 inhibitory antibody may be administered to an adult patient (e.g., an average adult weight of 70 kg) in a dosage of from 0.1 mg to 10,000 mg, more suitably from 1.0 mg to 5,000 mg, more suitably 10.0 mg to 2,000 mg, more suitably 10.0 mg to 1,000 mg and still more suitably from 50.0 mg to 500 mg, or 10 to 200 mg. For pediatric patients, dosage can be adjusted in proportion to the patient's weight.

Application of the therapeutic compositions of the present invention may be carried out by a single administration of the composition (e.g., a single composition comprising MASP-2 inhibitory antibodies) or a limited sequence of administrations. Alternatively, the composition may be administered at periodic intervals such as daily, biweekly, weekly, every other week, monthly or bimonthly over an extended period of time for as determined by a physician for optimal therapeutic effect.

In accordance with the foregoing, the invention features the following embodiments:

1. A method of preventing, reducing and/or treating a disease, disorder or condition associated with fibrin-induced activation of the complement system and the associated activation of the coagulation and/or contact systems comprising administering a therapeutic amount of a MASP-2 inhibitory antibody to a subject in need thereof.

5 2. The method of paragraph 1, wherein the subject in need thereof is suffering from, or at risk of developing, a disease, disorder or condition associated with complement-related inflammation, excessive coagulation or contact system activation initiated by fibrin or activated platelets.

 3. The method of paragraph 2, wherein the subject is suffering from a disease or
10 disorder selected from the group consisting of arterial thrombosis, venous thrombosis, deep vein thrombosis, post-surgical thrombosis, restenosis following coronary artery bypass graft and/or an interventional cardiovascular procedure (e.g., angioplasty or stent placement), atherosclerosis, plaque rupture, plaque instability, restenosis, hypotension, acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome
15 (SIRS), disseminated intravascular coagulation (DIC), veno-occlusive disease (VOD), sickle cell disease, thrombotic microangiopathy, lupus nephritis, superficial thrombophlebitis, Factor V Leiden mutation, ischemic/reperfusion injury, human immunodeficiency virus (HIV) infection, undergoing hormone-replacement therapy (HRT), Alzheimer's disease and/or suffering from a hypercoagulable state.

20 4. The method of paragraph 3, wherein the subject is suffering from, or at risk for developing an acquired hypercoagulable state due to at least one or more of the following: undergoing therapy with a drug selected from the group consisting of 5-FU, GM-CSF, cisplatin, heparin, COX-2 inhibitor, contrast media, corticosteroids and antipsychotics; venous stasis (immobilization, surgery, etc), antiphospholipid syndrome,
25 cancer (promyelocytic leukemia, lung, breast, prostate, pancreas, stomach and colon tumors), tissue injury due to trauma or surgery, presence of a catheter in a central vein, acquired deficiency of a protein involved in clot formation (e.g., protein C), paroxysmal nocturnal hemoglobinuria (PNH), elevated levels of homocysteine, heart failure, presence of a mechanical valve, pulmonary hypertension with in-situ thrombosis, atrial fibrillation,
30 heparin-induced thrombocytopenia (HIT), heparin-induced thrombocytopenia and thrombosis (HITT), Kawasaki disease with in-situ thrombus, Takayasu arteritis with in-

situ thrombus, thrombophilia of metastatic cancer, elevated Factor VIII levels, pregnancy, and inflammatory bowel disease (IBD).

5. The method of paragraph 1, wherein the subject is suffering from, or at risk for developing, a disease or disorder that is amenable to treatment with a kallikrein inhibitor.

5 6. The method of paragraph 5, wherein the disease or disorder amenable to treatment with a kallikrein inhibitor is selected from the group consisting of hereditary angioedema, diabetic macular edema and bleeding during cardiopulmonary bypass.

7. The method of paragraph 1, wherein the subject is suffering from, or at risk for developing, a disease or disorder that is amenable to treatment with a thrombin inhibitor.

8. The method of paragraph 7, wherein the disease or disorder amenable to treatment with a thrombin inhibitor is selected from the group consisting of arterial thrombosis, venous thrombosis, pulmonary embolism, atrial fibrillation, heparin-induced thrombocytopenia, conversion from one anticoagulant to another, and off-label use for extracorporeal circuit patency of continuous renal replacement therapy (CRRT) in critically ill patients with HIT (maintenance).

9. The method of paragraph 1, wherein the subject has previously experienced, is currently suffering from, or is at risk for developing atrial fibrillation and the MASP-2 inhibitory antibody is administered in an amount sufficient to reduce the risk of stroke in said subject.

10. The method of paragraph 1, wherein the subject is suffering from, or at risk for developing, a disease or disorder that is amenable to treatment with a factor XII inhibitor.

11. The method of paragraph 10, wherein the disease or disorder amenable to treatment with a factor XII inhibitor is selected from the group consisting of deep vein thrombosis (both primary prophylaxis and extended therapy), pulmonary embolism, nonvalvular atrial fibrillation, prevention of recurrent ischemia after acute coronary syndrome in subjects with or without atrial fibrillation, end-stage renal disease, cerebral ischemia, angina, reduce or prevent clotting associated with medical devices (e.g., valves, small caliber grafts, etc) and/or extracorporeal circuits.

12. The method of paragraph 1, wherein the subject has previously experienced, is currently suffering from, or is at risk for developing nonvalvular atrial fibrillation and the MASP-2 inhibitory antibody is administered in an amount sufficient to reduce the risk of stroke and/or embolism in said subject.

5 13. The method of paragraph 3, wherein the subject has a genetic defect that causes or increases the risk of developing, a hypercoagulable state.

 14. The method of paragraph 13, wherein the genetic defect is selected from the group consisting of a Prothrombin 20210 gene mutation, an MTHFR mutation, a deficiency of protein C, a deficiency of protein S, a deficiency of protein A, a deficiency
10 of protein Z, an antithrombin deficiency and a genetic disorder producing thrombophilia.

 15. The method of paragraph 1, wherein the subject has an acquired disease, disorder or condition that increases the propensity for thromboembolism.

 16. The method of paragraph 15, wherein the acquired disease or disorder that increases the propensity for thromboembolism is selected from the group consisting of
15 atherosclerosis, antiphospholipid antibodies, cancer, hyperhomocysteinemia, infection, tissue injury, venous stasis (such as due to surgery, orthopedic or paralytic immobilization, heart failure, pregnancy, or obesity) and a subject taking oral contraceptives that contain estrogen.

 17. The method of paragraph 16, wherein the cancer is selected from the
20 group consisting of promyelocytic leukemia, lung, breast, prostate, pancreatic, stomach and colon.

 18. The method of paragraph 1, wherein the subject is in need of anticoagulant therapy and the MASP-2 inhibitory antibody is used as a replacement for standard anticoagulant therapy (e.g., Warfarin).

25 19. The method of paragraph 18, wherein the subject has a condition that normally prohibits standard anticoagulant therapy, such as CNS amyloid angiopathy.

 20. The method of paragraph 18, wherein the MASP-2 inhibitory antibody is administered as a bridging agent perioperatively in a subject otherwise on standard anticoagulation therapy.

21. The method of any of paragraphs 1-20, wherein the MASP-2 inhibitory agent is a MASP-2 monoclonal antibody, or fragment thereof that specifically binds to a portion of SEQ ID NO:5.

22. The method of paragraph 21, wherein the MASP-2 antibody is a chimeric,
5 humanized or human antibody.

23. The method of paragraph 21, wherein said MASP-2 inhibitory antibody is an antibody fragment selected from the group consisting of Fv, Fab, Fab', F(ab)₂ and F(ab')₂.

24. The method of paragraph 21, wherein said MASP-2 inhibitory antibody is
10 a single-chain molecule.

25. The method of paragraph 21, wherein said MASP-2 inhibitory antibody is selected from the group consisting of an IgG1 molecule, an IgG2 and an IgG4 molecule.

26. The method of paragraph 25, wherein the IgG4 molecule comprises a S228P mutation.

15 27. The method of any of paragraphs 1-26, wherein said MASP-2 inhibitory antibody does not substantially inhibit the classical pathway.

28. The method of any of paragraphs 1-27, wherein the MASP-2 inhibitory monoclonal antibody, or antigen-binding fragment thereof, comprises:

(a) a heavy-chain variable region comprising: i) a heavy chain CDR-H1
20 comprising the amino acid sequence from 31-35 of SEQ ID NO:6; and ii) a heavy-chain CDR-H2 comprising the amino acid sequence from 50-65 of SEQ ID NO:6; and iii) a heavy-chain CDR-H3 comprising the amino acid sequence from 95-107 of SEQ ID NO:6 and

(b) a light-chain variable region comprising: i) a light-chain CDR-L1 comprising
25 the amino acid sequence from 24-34 of SEQ ID NO:7; and ii) a light-chain CDR-L2 comprising the amino acid sequence from 50-56 of SEQ ID NO:7; and iii) a light-chain CDR-L3 comprising the amino acid sequence from 89-97 of SEQ ID NO:7.

29. The method of any of paragraphs 1-28, wherein the MASP-2 inhibitory monoclonal antibody comprises a heavy-chain variable region set forth as SEQ ID NO:6
30 and a light-chain variable region set forth as SEQ ID NO:7.

30. The method of any of paragraphs 1-27, wherein the MASP-2 inhibitory antibody or antigen binding-fragment thereof specifically recognizes at least part of an epitope recognized by a reference antibody comprising a heavy chain variable region as set forth in SEQ ID NO:6 and a light-chain variable region as set forth in SEQ ID NO:7.

5

XXI. EXAMPLES

The following examples merely illustrate the best mode now contemplated for practicing the invention, but should not be construed to limit the invention. All literature citations herein are expressly incorporated by reference.

10

EXAMPLE 1

This example describes exemplary methods for producing murine monoclonal antibodies against human MASP-2 polypeptides.

1. Methods for generating MASP-2 antibodies

15 Male A/J mice (Harlan, Houston, Tex.), 8 to 12 weeks of age, are injected subcutaneously with 100 μ g human full-length polypeptides: rMASP-2 (SEQ ID NO:2), or antigen fragments thereof, in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) in 200 μ l of phosphate buffered saline (PBS) pH 7.4. Two weeks later, the mice are injected subcutaneously with 50 μ g of the same human polypeptide in incomplete
20 Freund's adjuvant. At the sixth week, the mice are injected with 50 μ g of the same human polypeptide in PBS and are fused 4 days later.

For each fusion, single-cell suspensions are prepared from the spleen of an immunized mouse and used for fusion with Sp2/0 myeloma cells. 5×10^8 of the Sp2/0 and 5×10^8 spleen cells are fused in a medium containing 50% polyethylene glycol
25 (M.W. 1450) (Kodak, Rochester, N.Y.) and 5% dimethylsulfoxide (Sigma Chemical Co., St. Louis, Mo.). The cells are then adjusted to a concentration of 1.5×10^5 spleen cells per 200 μ l of the suspension in Iscove medium (Gibco, Grand Island, N.Y.), supplemented with 10% fetal bovine serum, 100 units/mL of penicillin, 100 μ g/mL of streptomycin, 0.1 mM hypoxanthine, 0.4 μ M aminopterin and 16 μ M thymidine. Two hundred
30 microliters of the cell suspension are added to each well contained in roughly twenty 96-well microculture plates. After about ten days, culture supernatants are withdrawn for

screening for reactivity with the target purified MASP-2 or the antigen fragment in an ELISA assay.

ELISA Assay: Wells of Immulon 2 (Dynatech Laboratories, Chantilly, Va.) microtest plates are coated by adding 50 μ l of purified hMASP-2 at 50 ng/mL overnight at room temperature. The low concentration of MASP-2 used for coating enables the selection of high-affinity antibodies. After the coating solution is removed by flicking the plate, 200 μ l of BLOTTO (non-fat dry milk) in PBS is added to each well for one hour to block the non-specific sites. An hour later, the wells are then washed with a buffer PBST (PBS containing 0.05% Tween 20). The culture supernatants from each fusion well (50 μ L) are mixed with 50 μ l of BLOTTO and then added to individual MASP-2-coated wells of the microtest plates. After one hour of incubation, the wells are washed with PBST and antibody binding to MASP-2 is detected by adding horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Fc specific) (Jackson ImmunoResearch Laboratories, West Grove, Pa.). The HRP-conjugated anti-mouse IgG is diluted appropriately in BLOTTO to provide an appropriate signal to noise ratio, and added to each sample-containing well. After washing, the bound HRP-conjugated antibody is detected with the peroxidase substrate solution. Peroxidase substrate solution containing 0.1% 3,3,5,5-tetramethyl benzidine (Sigma, St. Louis, Mo.) and 0.0003% hydrogen peroxide (Sigma) is added to the wells for color development for 30 minutes. The reaction is terminated by addition of 50 μ l of 2M H_2SO_4 per well and the optical density at 450 nm of the reaction mixture is measured with a BioTek ELISA Reader (BioTek Instruments, Winooski, Vt.).

Binding Assay:

Culture supernatants that test positive in the MASP-2 ELISA assay described above can be tested in a binding assay to determine the binding affinity that the MASP-2 inhibitory antibodies have for MASP-2. A similar assay can also be used to determine if the inhibitory agents bind to other antigens in the complement system.

Polystyrene microtiter plate wells (96-well medium binding plates, Corning Costar, Cambridge, MA) are coated with MASP-2 (20 ng/100 μ l/well, Advanced Research Technology, San Diego, CA) in phosphate-buffered saline (PBS) pH 7.4 overnight at 4°C. After aspirating the MASP-2 solution, wells are blocked with PBS containing 1% bovine serum albumin (BSA; Sigma Chemical) for 2 hours at room

temperature. Wells without MASP-2 coating serve as the background controls. Aliquots of hybridoma supernatants or purified MASP-2 MoAbs, at varying concentrations in BSA PBS blocking solution, are added to the wells. Following a two-hour incubation at room temperature, the wells are extensively rinsed with PBS. MASP-2-bound MASP-2
5 MoAb is detected by the addition of peroxidase-conjugated goat anti-mouse IgG (Sigma Chemical) in blocking solution, which is allowed to incubate for 1 hour at room temperature. The plate is rinsed again thoroughly with PBS, and 100 μ l of 3,3',5,5'-tetramethyl benzidine (TMB) substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) is added. The reaction of TMB is quenched by the addition of 100 μ l
10 of 1M phosphoric acid, and the plate is read at 450 nm in a microplate reader (SPECTRA MAX 250, Molecular Devices, Sunnyvale, CA).

The culture supernatants from the positive wells are then tested for the ability to inhibit complement activation in a functional assay such as the C4 cleavage assay as described herein (Example 2). The cells in positive wells are then cloned by limiting
15 dilution. The MoAbs are tested again for reactivity with hMASP-2 in an ELISA assay as described above. The selected hybridomas are grown in spinner flasks and the spent culture supernatant collected for antibody purification by protein A affinity chromatography.

MASP-2 antibodies may be assayed for lectin pathway inhibitory activity in a C4
20 cleavage assay, for example as described in Example 2.

EXAMPLE 2

This example describes an *in vitro* C4 cleavage assay used as a functional screen to identify MASP-2 inhibitory agents capable of blocking MASP-2-dependent
25 complement activation via L-ficolin/P35, H-ficolin, M-ficolin or mannan.

C4 Cleavage Assay: A C4 cleavage assay has been described by Petersen, S.V., et al., *J. Immunol. Methods* 257:107, 2001, which measures lectin pathway activation resulting from lipoteichoic acid (LTA) on *S. aureus*, which binds to L-ficolin.

Reagents: Formalin-fixed *S. aureus* (DSM20233) is prepared as follows:
30 bacteria is grown overnight at 37°C in tryptic soy blood medium, washed three times with PBS, then fixed for 1 hour at room temperature in PBS/0.5% formalin, and washed a

further three times with PBS, before being resuspended in coating buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, pH 9.6).

Assay: The wells of a Nunc MaxiSorb microtiter plate (Nalgene Nunc International, Rochester, NY) are coated with: 100 µl of formalin-fixed *S. aureus* DSM20233 (OD₅₅₀ = 0.5) in coating buffer with 1 µg of L-ficolin in coating buffer. After overnight incubation, wells are blocked with 0.1% human serum albumin (HSA) in TBS (10 mM Tris-HCl, 140 mM NaCl, pH 7.4), then are washed with TBS containing 0.05% Tween 20 and 5 mM CaCl₂ (wash buffer). Human serum samples are diluted in 20 mM Tris-HCl, 1 M NaCl, 10 mM CaCl₂, 0.05% Triton X-100, 0.1% HSA, pH 7.4, which prevents activation of endogenous C4 and dissociates the C1 complex (composed of C1q, C1r and C1s). MASP-2 inhibitory agents, including MASP-2 MoAbs, are added to the serum samples in varying concentrations. The diluted samples are added to the plate and incubated overnight at 4°C. After 24 hours, the plates are washed thoroughly with wash buffer, then 0.1 µg of purified human C4 (obtained as described in Dodds, A.W., *Methods Enzymol.* 223:46, 1993) in 100 µl of 4 mM barbital, 145 mM NaCl, 2 mM CaCl₂, 1 mM MgCl₂, pH 7.4 is added to each well. After 1.5 hours at 37°C, the plates are washed again and C4b deposition is detected using alkaline phosphatase-conjugated chicken anti-human C4c (obtained from Immunsystem, Uppsala, Sweden) and measured using the colorimetric substrate p-nitrophenyl phosphate.

C4 Assay on mannan: The assay described above is adapted to measure lectin pathway activation via MBL by coating the plate with LSP and mannan prior to adding serum mixed with various MASP-2 inhibitory agents.

C4 assay on H-ficolin (Hakata Ag): The assay described above is adapted to measure lectin pathway activation via H-ficolin by coating the plate with LPS and H-ficolin prior to adding serum mixed with various MASP-2 inhibitory agents.

EXAMPLE 3

The following assay is used to test whether a MASP-2 inhibitory antibody blocks the classical pathway by analyzing the effect of a MASP-2 inhibitory antibody under conditions in which the classical pathway is initiated by immune complexes.

Methods: To test the effect of a MASP-2 inhibitory antibody on conditions of complement activation where the classical pathway is initiated by immune complexes, triplicate 50 μ L samples containing 90% NHS are incubated at 37°C in the presence of 10 μ g/mL immune complex or PBS, and parallel triplicate samples (+/- immune complexes) are also included containing 200 nM anti-properdin monoclonal antibody during the 37°C incubation. After a two-hour incubation at 37°C, 13 mM EDTA is added to all samples to stop further complement activation and the samples are immediately cooled to 5°C. The samples are then stored at -70°C prior to being assayed for complement activation products (C3a and sC5b-9) using ELISA kits (Quidel, Catalog Nos. A015 and A009) following the manufacturer's instructions.

EXAMPLE 4

This example describes the identification of high-affinity MASP-2 Fab2 antibody fragments that block MASP-2 activity.

Background and rationale: MASP-2 is a complex protein with many separate functional domains, including: binding site(s) for MBL and ficolins, a serine protease catalytic site, a binding site for proteolytic substrate C2, a binding site for proteolytic substrate C4, a MASP-2 cleavage site for autoactivation of MASP-2 zymogen, and two Ca^{++} binding sites. Fab2 antibody fragments were identified that bind with high affinity to MASP-2, and the identified Fab2 fragments were tested in a functional assay to determine if they were able to block MASP-2 functional activity.

To block MASP-2 functional activity, an antibody or Fab2 antibody fragment must bind and interfere with a structural epitope on MASP-2 that is required for MASP-2 functional activity. Therefore, many or all of the high-affinity binding MASP-2 Fab2s may not inhibit MASP-2 functional activity unless they bind to structural epitopes on MASP-2 that are directly involved in MASP-2 functional activity.

A functional assay that measures inhibition of lectin pathway C3 convertase formation was used to evaluate the "blocking activity" of MASP-2 Fab2s. It is known that the primary physiological role of MASP-2 in the lectin pathway is to generate the next functional component of the lectin-mediated complement pathway, namely the lectin pathway C3 convertase. The lectin pathway C3 convertase is a critical enzymatic

complex (C4bC2a) that proteolytically cleaves C3 into C3a and C3b. MASP-2 is not a structural component of the lectin pathway C3 convertase (C4bC2a); however, MASP-2 functional activity is required in order to generate the two protein components (C4b, C2a) that comprise the lectin pathway C3 convertase. Furthermore, all of the separate functional activities of MASP-2 listed above appear to be required in order for MASP-2 to generate the lectin pathway C3 convertase. For these reasons, a preferred assay to use in evaluating the "blocking activity" of MASP-2 Fab2s is believed to be a functional assay that measures inhibition of lectin pathway C3 convertase formation.

Generation of High Affinity Fab2s: A phage display library of human variable light and heavy chain antibody sequences and automated antibody selection technology for identifying Fab2s that react with selected ligands of interest was used to create high-affinity Fab2s to rat MASP-2 protein (SEQ ID NO:5). A known amount of rat MASP-2 (~1 mg, >85% pure) protein was utilized for antibody screening. Three rounds of amplification were utilized for selection of the antibodies with the best affinity. Approximately 250 different hits expressing antibody fragments were picked for ELISA screening. High affinity hits were subsequently sequenced to determine uniqueness of the different antibodies.

Fifty unique MASP-2 antibodies were purified and 250 µg of each purified Fab2 antibody was used for characterization of MASP-2 binding affinity and complement pathway functional testing, as described in more detail below.

Assays used to Evaluate the Inhibitory (blocking) Activity of MASP-2 Fab2s

1. Assay to Measure Inhibition of Formation of Lectin Pathway C3 Convertase:

Background: The lectin pathway C3 convertase is the enzymatic complex (C4bC2a) that proteolytically cleaves C3 into the two potent proinflammatory fragments, anaphylatoxin C3a and opsonic C3b. Formation of C3 convertase appears to be a key step in the lectin pathway in terms of mediating inflammation. MASP-2 is not a structural component of the lectin pathway C3 convertase (C4bC2a); therefore MASP-2 antibodies (or Fab2) will not directly inhibit activity of preexisting C3 convertase. However, MASP-2 serine protease activity is required in order to generate the two protein components (C4b, C2a) that comprise the lectin pathway C3 convertase. Therefore,

MASP-2 Fab2, which inhibits MASP-2 functional activity (i.e., blocking MASP-2 Fab2) will inhibit *de novo* formation of lectin pathway C3 convertase. C3 contains an unusual and highly reactive thioester group as part of its structure. Upon cleavage of C3 by C3 convertase in this assay, the thioester group on C3b can form a covalent bond with
5 hydroxyl or amino groups on macromolecules immobilized on the bottom of the plastic wells via ester or amide linkages, thus facilitating detection of C3b in the ELISA assay.

Yeast mannan is a known activator of the lectin pathway. In the following method to measure formation of C3 convertase, plastic wells coated with mannan were incubated for 30 minutes at 37°C with diluted rat serum to activate the lectin pathway.
10 The wells were then washed and assayed for C3b immobilized onto the wells using standard ELISA methods. The amount of C3b generated in this assay is a direct reflection of the *de novo* formation of lectin pathway C3 convertase. MASP-2 Fab2s at selected concentrations were tested in this assay for their ability to inhibit C3 convertase formation and consequent C3b generation.

15 **Methods:**

96-well Costar Medium Binding plates were incubated overnight at 5°C with mannan diluted in 50 mM carbonate buffer, pH 9.5 at 1 µg/50 µl/well. After overnight incubation, each well was washed three times with 200 µl PBS. The wells were then blocked with 100 µl/well of 1% bovine serum albumin in PBS and incubated for one hour
20 at room temperature with gentle mixing. Each well was then washed three times with 200 µl of PBS. The MASP-2 Fab2 samples were diluted to selected concentrations in Ca⁺⁺ and Mg⁺⁺ containing GVB buffer (4.0 mM barbital, 141 mM NaCl, 1.0 mM MgCl₂, 2.0 mM CaCl₂, 0.1% gelatin, pH 7.4) at 5°C. A 0.5% rat serum was added to the above samples at 5°C and 100 µl was transferred to each well. Plates were covered and
25 incubated for 30 minutes in a 37°C waterbath to allow complement activation. The reaction was stopped by transferring the plates from the 37°C waterbath to a container containing an ice-water mix. Each well was washed five times with 200 µl with PBS-Tween 20 (0.05% Tween 20 in PBS), then washed two times with 200 µl PBS. A
30 100 µl/well of 1:10,000 dilution of the primary antibody (rabbit anti-human C3c, DAKO A0062) was added in PBS containing 2.0 mg/mL bovine serum albumin and incubated 1 hour at room temperature with gentle mixing. Each well was washed 5 times with 200 µl

PBS. 100 μ l/well of 1:10,000 dilution of the secondary antibody (peroxidase-conjugated goat anti-rabbit IgG, American Qualex A102PU) was added in PBS containing 2.0 mg/mL bovine serum albumin and incubated for one hour at room temperature on a shaker with gentle mixing. Each well was washed five times with 200 μ l with PBS. 100 μ l/well of the peroxidase substrate TMB (Kirkegaard & Perry Laboratories) was added and incubated at room temperature for 10 minutes. The peroxidase reaction was stopped by adding 100 μ l/well of 1.0 M H_3PO_4 and the OD_{450} was measured.

2. *Assay to Measure Inhibition of MASP-2-dependent C4 Cleavage*

Background: The serine protease activity of MASP-2 is highly specific and only two protein substrates for MASP-2 have been identified; C2 and C4. Cleavage of C4 generates C4a and C4b. MASP-2 Fab2 may bind to structural epitopes on MASP-2 that are directly involved in C4 cleavage (e.g., MASP-2 binding site for C4; MASP-2 serine protease catalytic site) and thereby inhibit the C4 cleavage functional activity of MASP-2.

Yeast mannan is a known activator of the lectin pathway. In the following method to measure the C4 cleavage activity of MASP-2, plastic wells coated with mannan were incubated for 30 minutes at 37°C with diluted rat serum to activate the lectin pathway. Since the primary antibody used in this ELISA assay only recognizes human C4, the diluted rat serum was also supplemented with human C4 (1.0 μ g/mL). The wells were then washed and assayed for human C4b immobilized onto the wells using standard ELISA methods. The amount of C4b generated in this assay is a measure of MASP-2-dependent C4 cleavage activity. MASP-2 Fab2 at selected concentrations was tested in this assay for ability to inhibit C4 cleavage.

Methods: 96-well Costar Medium Binding plates were incubated overnight at 5°C with mannan diluted in 50 mM carbonate buffer, pH 9.5 at 1.0 Tg/50 μ l/well. Each well was washed 3 times with 200 μ l PBS. The wells were then blocked with 100 μ l/well of 1% bovine serum albumin in PBS and incubated for one hour at room temperature with gentle mixing. Each well was washed 3 times with 200 μ l of PBS. MASP-2 Fab2 samples were diluted to selected concentrations in Ca^{++} and Mg^{++} containing GVB buffer (4.0 mM barbital, 141 mM NaCl, 1.0 mM MgCl_2 , 2.0 mM CaCl_2 , 0.1% gelatin, pH 7.4) at 5°C. 1.0 μ g/mL human C4 (Quidel) was also included in these samples. 0.5% rat

serum was added to the above samples at 5°C and 100 µl was transferred to each well. The plates were covered and incubated for 30 minutes in a 37°C waterbath to allow complement activation. The reaction was stopped by transferring the plates from the 37°C waterbath to a container containing an ice-water mix. Each well was washed
 5 5 times with 200 µl with PBS-Tween 20 (0.05% Tween 20 in PBS), then each well was washed with 2 times with 200 µl PBS. 100 µl/well of 1:700 dilution of biotin-conjugated chicken anti-human C4c (Immunsystem AB, Uppsala, Sweden) was added in PBS containing 2.0 mg/mL bovine serum albumin (BSA) and incubated one hour at room temperature with gentle mixing. Each well was washed 5 times with 200 µl PBS. 100
 10 µl/well of 0.1 µg/mL of peroxidase-conjugated streptavidin (Pierce Chemical #21126) was added in PBS containing 2.0 mg/mL BSA and incubated for one hour at room temperature on a shaker with gentle mixing. Each well was washed 5 x 200 µl with PBS. 100 µl/well of the peroxidase substrate TMB (Kirkegaard & Perry Laboratories) was added and incubated at room temperature for 16 min. The peroxidase reaction was
 15 stopped by adding 100 µl/well of 1.0 M H₃PO₄ and the OD₄₅₀ was measured.

3. *Binding Assay of anti-rat MASP-2 Fab2 to 'Native' rat MASP-2*

Background: MASP-2 is usually present in plasma as a MASP-2 dimer complex that also includes specific lectin molecules (mannose-binding protein (MBL) and ficolins). Therefore, if one is interested in studying the binding of MASP-2 Fab2 to the
 20 physiologically relevant form of MASP-2, it is important to develop a binding assay in which the interaction between the Fab2 and 'native' MASP-2 in plasma, rather than purified recombinant MASP-2, is used. In this binding assay, the 'native' MASP-2-MBL complex from 10% rat serum was first immobilized onto mannan-coated wells. The binding affinity of various MASP-2 Fab2s to the immobilized 'native' MASP-2 was then
 25 studied using a standard ELISA methodology.

Methods: 96-well Costar High Binding plates were incubated overnight at 5°C with mannan diluted in 50 mM carbonate buffer, pH 9.5 at 1 µg/50 µl/well. Each well was washed 3 times with 200 µl PBS. The wells were blocked with 100 µl/well of 0.5% nonfat dry milk in PBST (PBS with 0.05% Tween 20) and incubated for one hour at room
 30 temperature with gentle mixing. Each well was washed 3 times with 200 µl of TBS/Tween/Ca⁺⁺ Wash Buffer (Tris-buffered saline, 0.05% Tween 20, containing

5.0 mM CaCl₂, pH 7.4. 10% rat serum in High Salt Binding Buffer (20 mM Tris, 1.0 M NaCl, 10 mM CaCl₂, 0.05% Triton-X100, 0.1% (w/v) bovine serum albumin, pH 7.4) was prepared on ice. 100 µl/well was added and incubated overnight at 5°C. Wells were washed 3 times with 200 µl of TBS/Tween/Ca⁺⁺ Wash Buffer. Wells were then washed 2 times with 200 µl PBS. 100 µl/well of selected concentration of MASP-2 Fab2 diluted in Ca⁺⁺ and Mg⁺⁺ containing GVB Buffer (4.0 mM barbital, 141 mM NaCl, 1.0 mM MgCl₂, 2.0 mM CaCl₂, 0.1% gelatin, pH 7.4) was added and incubated for one hour at room temperature with gentle mixing. Each well was washed 5 times with 200 µl PBS. 100 µl/well of HRP-conjugated goat anti-Fab2 (Biogenesis Cat No 0500-0099) diluted 1:5000 in 2.0 mg/mL bovine serum albumin in PBS was added and incubated for one hour at room temperature with gentle mixing. Each well was washed 5 times with 200 µl PBS. 100 µl/well of the peroxidase substrate TMB (Kirkegaard & Perry Laboratories) was added and incubated at room temperature for 70 minutes. The peroxidase reaction was stopped by adding 100 µl/well of 1.0 M H₃PO₄ and OD₄₅₀ was measured.

15 **RESULTS:**

Approximately 250 different Fab2s that reacted with high affinity to the rat MASP-2 protein were picked for ELISA screening. These high-affinity Fab2s were sequenced to determine the uniqueness of the different antibodies, and 50 unique MASP-2 antibodies were purified for further analysis. 250 µg of each purified Fab2 antibody was used for characterization of MASP-2 binding affinity and complement pathway functional testing. The results of this analysis are shown below in **TABLE 2**.

TABLE 2: MASP-2 FAB2 THAT BLOCK LECTIN PATHWAY COMPLEMENT ACTIVATION

Fab2 antibody #	C3 Convertase (IC ₅₀ (nM))	K _d (nM)	C4 Cleavage IC ₅₀ (nM)
88	0.32	4.1	ND
41	0.35	0.30	0.81
11	0.46	0.86	<2 nM
86	0.53	1.4	ND
81	0.54	2.0	ND
66	0.92	4.5	ND

Fab2 antibody #	C3 Convertase (IC ₅₀ (nM))	K _d (nM)	C4 Cleavage IC ₅₀ (nM)
57	0.95	3.6	<2 nM
40	1.1	7.2	0.68
58	1.3	2.6	ND
60	1.6	3.1	ND
52	1.6	5.8	<2 nM
63	2.0	6.6	ND
49	2.8	8.5	<2 nM
89	3.0	2.5	ND
71	3.0	10.5	ND
87	6.0	2.5	ND
67	10.0	7.7	ND

As shown above in **TABLE 2**, of the 50 MASP-2 Fab2s tested, 17 were identified as MASP-2-blocking Fab2s that potentially inhibit C3 convertase formation with IC₅₀ equal to or less than 10 nM Fab2s (a 34% positive hit rate). Eight of the 17 Fab2s identified have IC₅₀s in the subnanomolar range. Furthermore, all seventeen of the

5 MASP-2 blocking Fab2s shown in **TABLE 2** gave essentially complete inhibition of C3 convertase formation in the lectin pathway C3 convertase assay. This is an important consideration, since it is theoretically possible that a "blocking" Fab2 may only fractionally inhibit MASP-2 function even when each MASP-2 molecule is bound by the Fab2.

10 Although mannan is a known activator of the lectin pathway, it is theoretically possible that the presence of anti-mannan antibodies in the rat serum might also activate the classical pathway and generate C3b via the classical pathway C3 convertase. However, each of the seventeen blocking MASP-2 Fab2s listed in this example potentially inhibits C3b generation (>95 %), thus demonstrating the specificity of this assay for

15 lectin pathway C3 convertase.

Binding assays were also performed with all seventeen of the blocking Fab2s in order to calculate an apparent K_d for each. The results of the binding assays of anti-rat MASP-2 Fab2s to native rat MASP-2 for six of the blocking Fab2s are also shown in **TABLE 2**. Similar binding assays were also carried out for the other Fab2s, the results of which are shown in **TABLE 2**. In general, the apparent K_ds obtained for binding of each

20

of the six Fab2s to 'native' MASP-2 corresponds reasonably well with the IC_{50} for the Fab2 in the C3 convertase functional assay. There is evidence that MASP-2 undergoes a conformational change from an 'inactive' to an 'active' form upon activation of its protease activity (Feinberg et al., *EMBO J* 22:2348-59 (2003); Gal et al., *J. Biol. Chem.* 280:33435-44 (2005)). In the normal rat plasma used in the C3 convertase formation assay, MASP-2 is present primarily in the 'inactive' zymogen conformation. In contrast, in the binding assay, MASP-2 is present as part of a complex with MBL bound to immobilized mannan; therefore, the MASP-2 would be in the 'active' conformation (Petersen et al., *J. Immunol Methods* 257:107-16, 2001). Consequently, one would not necessarily expect an exact correspondence between the IC_{50} and K_d for each of the seventeen blocking Fab2 tested in these two functional assays because, in each assay, the Fab2 would be binding a different conformational form of MASP-2. Nevertheless, with the exception of Fab2 #88, there appears to be a reasonably close correspondence between the IC_{50} and apparent K_d for each of the other sixteen Fab2 tested in the two assays (see **TABLE 2**).

Several of the blocking Fab2s were evaluated for inhibition of MASP-2-mediated cleavage of C4. As shown in **TABLE 2**, all of the Fab2s tested were found to inhibit C4 cleavage with IC_{50} s similar to those obtained in the C3 convertase assay.

Although mannan is a known activator of the lectin pathway, it is theoretically possible that the presence of anti-mannan antibodies in the rat serum might also activate the classical pathway and thereby generate C4b by C1s-mediated cleavage of C4. However, several MASP-2 Fab2s have been identified that potently inhibit C4b generation (>95 %), thus demonstrating the specificity of this assay for MASP-2-mediated C4 cleavage. C4, like C3, contains an unusual and highly reactive thioester group as part of its structure. Upon cleavage of C4 by MASP-2 in this assay, the thioester group on C4b can form a covalent bond with hydroxyl or amino groups on macromolecules immobilized on the bottom of the plastic wells *via* ester or amide linkages, thus facilitating detection of C4b in the ELISA assay.

These studies clearly demonstrate the creation of high-affinity FAB2s to rat MASP-2 protein that functionally block both C4 and C3 convertase activity, thereby preventing lectin pathway activation.

EXAMPLE 5

This Example describes the epitope mapping for several of the blocking anti-rat MASP-2 Fab2 antibodies that were generated as described in Example 4.

5

Methods:

The following proteins, all with N-terminal 6X His tags were expressed in CHO cells using the pED4 vector:

rat MASP-2A, a full-length MASP-2 protein, inactivated by altering the serine at
10 the active center to alanine (S613A);

rat MASP-2K, a full-length MASP-2 protein altered to reduce autoactivation (R424K);

CUBI-II, an N-terminal fragment of rat MASP-2 that contains the CUBI, EGF-like and CUBII domains only; and

15 CUBI/EGF-like, an N-terminal fragment of rat MASP-2 that contains the CUBI and EGF-like domains only.

These proteins were purified from culture supernatants by nickel-affinity chromatography, as previously described (Chen et al., *J. Biol. Chem.* 276:25894-02 (2001)).

20 A C-terminal polypeptide (CCPII-SP), containing CCPII and the serine protease domain of rat MASP-2, was expressed in *E. coli* as a thioredoxin fusion protein using pTrxFus (Invitrogen). Protein was purified from cell lysates using Thiobond affinity resin. The thioredoxin fusion partner was expressed from empty pTrxFus as a negative control.

25 All recombinant proteins were dialyzed into TBS buffer and their concentrations determined by measuring the OD at 280 nm.

Dot Blot Analysis:

30 Serial dilutions of the five recombinant MASP-2 polypeptides described above (and the thioredoxin polypeptide as a negative control for CCPII-serine protease polypeptide) were spotted onto a nitrocellulose membrane. The amount of protein spotted ranged from 100 ng to 6.4 pg, in five-fold steps. In later experiments, the amount

of protein spotted ranged from 50 ng down to 16 pg, again in five-fold steps. Membranes were blocked with 5% skimmed milk powder in TBS (blocking buffer) then incubated with 1.0 $\mu\text{g/mL}$ MASP-2 Fab2s in blocking buffer (containing 5.0 mM Ca^{++}). Bound Fab2s were detected using HRP-conjugated anti-human Fab (AbD/Serotec; diluted 1/10,000) and an ECL detection kit (Amersham). One membrane was incubated with polyclonal rabbit-anti human MASP-2 Ab (described in Stover et al., *J Immunol* 163:6848-59 (1999)) as a positive control. In this case, bound Ab was detected using HRP-conjugated goat anti-rabbit IgG (Dako; diluted 1/2,000).

MASP-2 Binding Assay:

ELISA plates were coated with 1.0 $\mu\text{g/well}$ of recombinant MASP-2A or CUBI-II polypeptide in carbonate buffer (pH 9.0) overnight at 4°C. Wells were blocked with 1% BSA in TBS, then serial dilutions of the MASP-2 Fab2s were added in TBS containing 5.0 mM Ca^{++} . The plates were incubated for one hour at RT. After washing three times with TBS/tween/ Ca^{++} , HRP-conjugated anti-human Fab (AbD/Serotec) diluted 1/10,000 in TBS/ Ca^{++} was added and the plates incubated for a further one hour at room temperature. Bound antibody was detected using a TMB peroxidase substrate kit (Biorad).

Results:

Results of the dot blot analysis demonstrating the reactivity of the Fab2s with various MASP-2 polypeptides are provided below in **TABLE 3**. The numerical values provided in **TABLE 3** indicate the amount of spotted protein required to give approximately half-maximal signal strength. As shown, all of the polypeptides (with the exception of the thioredoxin fusion partner alone) were recognized by the positive control Ab (polyclonal anti-human MASP-2 sera, raised in rabbits).

TABLE 3: REACTIVITY WITH VARIOUS RECOMBINANT RAT MASP-2 POLYPEPTIDES ON DOT BLOTS

Fab2 Antibody #	MASP-2A	CUBI-II	CUBI/EGF-like	CCPII-SP	Thioredoxin
40	0.16 ng	NR	NR	0.8 ng	NR
41	0.16 ng	NR	NR	0.8 ng	NR
11	0.16 ng	NR	NR	0.8 ng	NR

Fab2 Antibody #	MASP-2A	CUBI-II	CUBI/EGF-like	CCPII-SP	Thioredoxin
49	0.16 ng	NR	NR	>20 ng	NR
52	0.16 ng	NR	NR	0.8 ng	NR
57	0.032 ng	NR	NR	NR	NR
58	0.4 ng	NR	NR	2.0 ng	NR
60	0.4 ng	0.4 ng	NR	NR	NR
63	0.4 ng	NR	NR	2.0 ng	NR
66	0.4 ng	NR	NR	2.0 ng	NR
67	0.4 ng	NR	NR	2.0 ng	NR
71	0.4 ng	NR	NR	2.0 ng	NR
81	0.4 ng	NR	NR	2.0 ng	NR
86	0.4 ng	NR	NR	10 ng	NR
87	0.4 ng	NR	NR	2.0 ng	NR
Positive Control	<0.032 ng	0.16 ng	0.16 ng	<0.032 ng	NR

NR = No reaction. The positive control antibody is polyclonal anti-human MASP-2 sera, raised in rabbits.

All of the Fab2s reacted with MASP-2A as well as MASP-2K (data not shown). The majority of the Fab2s recognized the CCPII-SP polypeptide but not the N-terminal fragments. The two exceptions are Fab2 #60 and Fab2 #57. Fab2 #60 recognizes MASP-2A and the CUBI-II fragment, but not the CUBI/EGF-like polypeptide or the CCPII-SP polypeptide, suggesting it binds to an epitope in CUBII, or spanning the CUBII and the EGF-like domain. Fab2 # 57 recognizes MASP-2A but not any of the MASP-2 fragments tested, perhaps indicating that this Fab2 recognizes an epitope in CCP1. Fab2 #40 and #49 bound only to complete MASP-2A. In the ELISA binding assay, Fab2 #60 also bound to the CUBI-II polypeptide, albeit with a slightly lower apparent affinity (data not shown).

These finding demonstrate the identification of unique blocking Fab2s to multiple regions of the MASP-2 protein.

EXAMPLE 6

This Example describes the pharmacodynamic analysis of representative high-affinity MASP-2 Fab2 antibodies that were identified as described in Example 4.

Background/Rationale:

As described in **Example 4**, in order to identify high-affinity antibodies that block
5 the rat lectin pathway, rat MASP-2 protein was utilized to pan a phage display library. This library was designed to provide for high immunological diversity and was constructed using entirely human immunoglobulin gene sequences. As shown in **Example 4**, approximately 250 individual phage clones were identified that bound with high affinity to the rat MASP-2 protein by ELISA screening. Sequencing of these clones
10 identified 50 unique MASP-2 antibody-encoding phage. Fab2 protein was expressed from these clones, purified and analyzed for MASP-2 binding affinity and lectin complement pathway functional inhibition.

As shown in **TABLE 2** of **Example 4**, 17 MASP-2 Fab2s with functional blocking activity were identified as a result of this analysis (a 34% hit rate for blocking
15 antibodies). Functional inhibition of the lectin complement pathway by Fab2s was apparent at the level of C4 deposition, which is a direct measure of C4 cleavage by MASP-2. Importantly, inhibition was equally evident when C3 convertase activity was assessed, demonstrating functional blockade of the lectin complement pathway. The 17 MASP-2 blocking Fab2s identified as described in **Example 4** potently inhibit C3
20 convertase formation with IC_{50} values equal to or less than 10 nM. Eight of the 17 Fab2s identified have IC_{50} values in the sub-nanomolar range. Furthermore, all 17 of the MASP-2 blocking Fab2s gave essentially complete inhibition of the C3 convertase formation in the lectin pathway C3 convertase assay, as summarized in **TABLE 2** of Example 4. Moreover, each of the 17 blocking MASP-2 Fab2s shown in **TABLE 2**
25 potently inhibit C3b generation (>95%), thus demonstrating the specificity of this assay for lectin pathway C3 convertase.

Rat IgG2c and mouse IgG2a full-length antibody isotype variants were derived from Fab2 #11. This Example describes the *in vivo* characterization of these isotypes for pharmacodynamic parameters.

Methods:

As described in **Example 4**, rat MASP-2 protein was utilized to pan a Fab phage display library, from which Fab2 #11 was identified. Rat IgG2c and mouse IgG2a full-length antibody isotype variants were derived from Fab2 #11. Both rat IgG2c and mouse
5 IgG2a full-length antibody isotypes were characterized *in vivo* for pharmacodynamic parameters as follows.

In vivo study in mice:

A pharmacodynamic study was carried out in mice to investigate the effect of MASP-2 antibody dosing on the plasma lectin pathway activity *in vivo*. In this study, C4
10 deposition was measured *ex vivo* in a lectin pathway assay at various time points following subcutaneous (sc) and intraperitoneal (ip) administration of 0.3 mg/kg or 1.0 mg/kg of the mouse MASP-2 MoAb (mouse IgG2a full-length antibody isotype derived from Fab2#11).

FIGURE 2A graphically illustrates lectin pathway specific C4b deposition on a
15 zymosan-coated microtiter plate, measured *ex vivo* in undiluted serum samples taken from mice (n=3 mice/group) at various time points after subcutaneous dosing of either 0.3 mg/kg or 1.0 mg/kg of the mouse MASP-2 MoAb. Serum samples from mice collected prior to antibody dosing served as negative controls (100% activity), while serum supplemented *in vitro* with 100 nM of the same blocking MASP-2 antibody was
20 used as a positive control (0% activity).

The results shown in **FIGURE 2A** demonstrate a rapid and complete inhibition of C4b deposition following subcutaneous administration of 1.0 mg/kg dose of mouse MASP-2 MoAb. A partial inhibition of C4b deposition was seen following subcutaneous administration of a dose of 0.3 mg/kg of mouse MASP-2 MoAb.

25 The time course of lectin pathway recovery was followed for three weeks following a single ip administration of mouse MASP-2 MoAb at 0.6 mg/kg in mice. As shown in **FIGURE 2B**, a precipitous drop in lectin pathway activity occurred after antibody dosing followed by complete lectin pathway inhibition that lasted for about 7 days after i.p. administration. Slow restoration of lectin pathway activity was observed
30 over the second and third weeks, with complete lectin pathway restoration in the mice by 17 days following MASP-2 MoAb administration.

These results demonstrate that the mouse MASP-2 Moab derived from Fab2 #11 inhibits the lectin pathway of mice in a dose-responsive manner when delivered systemically.

5

EXAMPLE 7

This example describes the identification, using phage display, of fully human scFv antibodies that bind to MASP-2 and inhibit lectin-mediated complement activation while leaving the classical (C1q-dependent) pathway component of the immune system intact.

10

Overview:

Fully human, high-affinity MASP-2 antibodies were identified by screening a phage display library. The variable light and heavy chain fragments of the antibodies were isolated in both a scFv format and in a full-length IgG format. The human MASP-2 antibodies are useful for inhibiting cellular injury associated with lectin pathway-mediated alternative complement pathway activation while leaving the classical (C1q-dependent) pathway component of the immune system intact. In some embodiments, the subject MASP-2 inhibitory antibodies have the following characteristics: (a) high affinity for human MASP-2 (e.g., a K_D of 10 nM or less), and (b) inhibit MASP-2-dependent complement activity in 90% human serum with an IC_{50} of 30 nM or less.

20

Methods:

Expression of full-length catalytically inactive MASP-2:

The full-length cDNA sequence of human MASP-2 (SEQ ID NO: 1), encoding the human MASP-2 polypeptide with leader sequence (SEQ ID NO:2) was subcloned into the mammalian expression vector pCI-Neo (Promega), which drives eukaryotic expression under the control of the CMV enhancer/promoter region (described in Kaufman R.J. et al., *Nucleic Acids Research* 19:4485-90, 1991; Kaufman, *Methods in Enzymology*, 185:537-66 (1991)).

25

In order to generate catalytically inactive human MASP-2A protein, site-directed mutagenesis was carried out as described in US2007/0172483, hereby incorporated herein by reference. The PCR products were purified after agarose gel electrophoresis

30

and band preparation and single adenosine overlaps were generated using a standard tailing procedure. The adenosine-tailed MASP-2A was then cloned into the pGEM-T easy vector and transformed into *E. coli*. The human MASP-2A was further subcloned into either of the mammalian expression vectors pED or pCI-Neo.

5 The MASP-2A expression construct described above was transfected into DXB1 cells using the standard calcium phosphate transfection procedure (Maniatis et al., 1989). MASP-2A was produced in serum-free medium to ensure that preparations were not contaminated with other serum proteins. Media was harvested from confluent cells every second day (four times in total). The level of recombinant MASP-2A averaged
10 approximately 1.5 mg/liter of culture medium. The MASP-2A (Ser-Ala mutant described above) was purified by affinity chromatography on MBP-A-agarose columns

MASP-2A ELISA on ScFv Candidate Clones identified by panning/scFv conversion and filter screening

15 A phage display library of human immunoglobulin light- and heavy-chain variable region sequences was subjected to antigen panning followed by automated antibody screening and selection to identify high-affinity scFv antibodies to human MASP-2 protein. Three rounds of panning the scFv phage library against HIS-tagged or biotin-tagged MASP-2A were carried out. The third round of panning was eluted first with MBL and then with TEA (alkaline). To monitor the specific enrichment of phages
20 displaying scFv fragments against the target MASP-2A, a polyclonal phage ELISA against immobilized MASP-2A was carried out. The scFv genes from panning round 3 were cloned into a pHOG expression vector and run in a small-scale filter screening to look for specific clones against MASP-2A.

25 Bacterial colonies containing plasmids encoding scFv fragments from the third round of panning were picked, gridded onto nitrocellulose membranes and grown overnight on non-inducing medium to produce master plates. A total of 18,000 colonies were picked and analyzed from the third panning round, half from the competitive elution and half from the subsequent TEA elution. Panning of the scFv phagemid library against MASP-2A followed by scFv conversion and a filter screen yielded 137 positive clones.
30 108/137 clones were positive in an ELISA assay for MASP-2 binding (data not shown),

of which 45 clones were further analyzed for the ability to block MASP-2 activity in normal human serum.

Assay to Measure Inhibition of Formation of Lectin Pathway C3 Convertase

A functional assay that measures inhibition of lectin pathway C3 convertase formation was used to evaluate the "blocking activity" of the MASP-2 scFv candidate clones. MASP-2 serine protease activity is required in order to generate the two protein components (C4b, C2a) that comprise the lectin pathway C3 convertase. Therefore, a MASP-2 scFv that inhibits MASP-2 functional activity (i.e., a blocking MASP-2 scFv), will inhibit *de novo* formation of lectin pathway C3 convertase. C3 contains an unusual and highly reactive thioester group as part of its structure. Upon cleavage of C3 by C3 convertase in this assay, the thioester group on C3b can form a covalent bond with hydroxyl or amino groups on macromolecules immobilized on the bottom of the plastic wells via ester or amide linkages, thus facilitating detection of C3b in the ELISA assay.

Yeast mannan is a known activator of the lectin pathway. In the following method to measure formation of C3 convertase, plastic wells coated with mannan were incubated with diluted human serum to activate the lectin pathway. The wells were then washed and assayed for C3b immobilized onto the wells using standard ELISA methods. The amount of C3b generated in this assay is a direct reflection of the *de novo* formation of lectin pathway C3 convertase. MASP-2 scFv clones at selected concentrations were tested in this assay for their ability to inhibit C3 convertase formation and consequent C3b generation.

Methods:

The 45 candidate clones identified as described above were expressed, purified and diluted to the same stock concentration, which was again diluted in Ca^{++} and Mg^{++} containing GVB buffer (4.0 mM barbital, 141 mM NaCl, 1.0 mM MgCl_2 , 2.0 mM CaCl_2 , 0.1% gelatin, pH 7.4) to assure that all clones had the same amount of buffer. The scFv clones were each tested in triplicate at the concentration of 2 $\mu\text{g/mL}$. The positive control was OMS100 Fab2 and was tested at 0.4 $\mu\text{g/mL}$. C3c formation was monitored in the presence and absence of the scFv/IgG clones.

Mannan was diluted to a concentration of 20 $\mu\text{g/mL}$ (1 $\mu\text{g/well}$) in 50mM carbonate buffer (15mM Na_2CO_3 + 35mM NaHCO_3 + 1.5 mM NaN_3), pH 9.5 and coated

on an ELISA plate overnight at 4°C. The next day, the mannan-coated plates were washed 3 times with 200 µl PBS. 100 µl of 1% HSA blocking solution was then added to the wells and incubated for 1 hour at room temperature. The plates were washed 3 times with 200 µl PBS, and stored on ice with 200 µl PBS until addition of the samples.

5 Normal human serum was diluted to 0.5% in CaMgGVB buffer, and scFv clones or the OMS100 Fab2 positive control were added in triplicates at 0.01 µg/mL; 1 µg/mL (only OMS100 control) and 10 µg/mL to this buffer and preincubated 45 minutes on ice before addition to the blocked ELISA plate. The reaction was initiated by incubation for one hour at 37°C and was stopped by transferring the plates to an ice bath. C3b
10 deposition was detected with a Rabbit α-Mouse C3c antibody followed by Goat α-Rabbit HRP. The negative control was buffer without antibody (no antibody = maximum C3b deposition), and the positive control was buffer with EDTA (no C3b deposition). The background was determined by carrying out the same assay except that the wells were mannan-free. The background signal against plates without mannan was subtracted from
15 the signals in the mannan-containing wells. A cut-off criterion was set at half of the activity of an irrelevant scFv clone (VZV) and buffer alone.

Results: Based on the cut-off criterion, a total of 13 clones were found to block the activity of MASP-2. All 13 clones producing > 50% pathway suppression were selected and sequenced, yielding 10 unique clones. All ten clones were found to have the
20 same light chain subclass, λ3, but three different heavy chain subclasses: VH2, VH3 and VH6. In the functional assay, five out of the ten candidate scFv clones gave IC₅₀ nM values less than the 25 nM target criteria using 0.5% human serum.

To identify antibodies with improved potency, the three mother scFv clones, identified as described above, were subjected to light-chain shuffling. This process
25 involved the generation of a combinatorial library consisting of the VH of each of the mother clones paired up with a library of naïve, human lambda light chains (VL) derived from six healthy donors. This library was then screened for scFv clones with improved binding affinity and/or functionality.

30 **TABLE 4:** Comparison of functional potency in IC₅₀ (nM) of the lead daughter clones and their respective mother clones (all in scFv format)

scFv clone	1% human serum C3 assay (IC ₅₀ nM)	90% human serum C3 assay (IC ₅₀ nM)	90% human serum C4 assay (IC ₅₀ nM)
17D20mc	38	nd	nd
17D20m_d3521N11 (OMS646)	26	>1000	140

Presented below are the heavy-chain variable region (VH) sequences for the mother clones and daughter clones shown above in **TABLE 4**,

The Kabat CDRs (31-35 (H1), 50-65 (H2) and 95-107 (H3)) are bolded; and the
5 Chothia CDRs (26-32 (H1), 52-56 (H2) and 95-101 (H3)) are underlined.

17D20_35VH-21N11VL (OMS646) heavy chain variable region (VH) (SEQ ID NO:6

QVTLKESGPVLVKPTETLTCTVSGFSL**SRGKMGV**SWIRQPPGKALEW
10 **LAHIFSSDEKSYRTSLKSRLTISKDTSKNQVVL**TMTNMDPVD**TATYYCARIRRG**
GIDYWGQGTLVTVSS

Presented below are the light-chain variable region (VL) sequences for the mother clones and daughter clones.

15 The Kabat CDRs (24-34 (L1); 50-56 (L2); and 89-97 (L3) are bolded; and the Chothia CDRs (24-34 (L1); 50-56 (L2) and 89-97 (L3) are underlined. These regions are the same whether numbered by the Kabat or Chothia system.

17D20m_d3521N11 (OMS646) light chain variable region (VL) (SEQ ID NO:7)
20 QPVL**TQPPSLSVSPGQTASITCSGEKLGDKYAYWYQQKPGQSPVLV**MYQ
DKQRPSGIPERFSGSNSGNTATLTISGTQAMDEADYYCQ**AWDSSTAVF**GGG**TKL**
TVL

25 The MASP-2 antibodies OMS100 and MoAb_d3521N11VL (OMS646), which have both been demonstrated to bind to human MASP-2 with high affinity and have the

ability to block functional complement activity, were analyzed with regard to epitope binding by dot blot analysis. The results show that d3521N11 and OMS100 antibodies are highly specific for MASP-2 and do not bind to MASP-1/3. Neither antibody bound to MASP-19 nor to MASP-2 fragments that did not contain the CCP1 domain of MASP-2, leading to the conclusion that the binding sites encompass CCP1.

Accordingly, in one embodiment, a MASP-2 inhibitory agent for use in the compositions and methods of the claimed invention comprises a human antibody that binds a polypeptide consisting of human MASP-2 (SEQ ID NO:2), wherein the antibody comprises:

I) a) a heavy chain variable region comprising: i) a heavy chain CDR1 comprising the amino acid sequence from 31-35 of SEQ ID NO:6; and ii) a heavy chain CDR2 comprising the amino acid sequence from 50-65 of SEQ ID NO:6; and iii) a heavy chain CDR3 comprising the amino acid sequence from 95-107 of SEQ ID NO:6; and

b) a light chain variable region comprising: i) a light chain CDR1 comprising the amino acid sequence from 24-34 of SEQ ID NO:7; and ii) a light chain CDR2 comprising the amino acid sequence from 50-56 of SEQ ID NO:7; and iii) a light chain CDR3 comprising the amino acid sequence from 89-97 of either SEQ ID NO:7, wherein the antibody inhibits MASP-2-dependent complement activation.

EXAMPLE 8

This Example demonstrates that thrombin activation can occur following lectin pathway activation under physiological conditions, and demonstrates the extent of MASP-2 involvement. In normal rat serum, activation of the lectin pathway leads to thrombin activation (assessed as thrombin deposition) concurrent with complement activation (assessed as C4 deposition). As can be seen in **FIGURES 3A** and **3B**, thrombin activation in this system is inhibited by the MASP-2 blocking antibody, H1 (Fab2 format), exhibiting an inhibition concentration-response curve (**FIGURE 3B**) that parallels that for complement activation (**FIGURE 3A**). These data suggest that activation of the lectin pathway as it occurs in trauma will lead to activation of both complement and coagulation systems in a process that is entirely dependent on MASP-2. Therefore, it is expected that MASP-2 blocking antibodies will prove efficacious in

mitigating cases of excessive systemic coagulation, e.g., disseminated intravascular coagulation, which is one of the hallmarks leading to mortality in major trauma cases.

EXAMPLE 9

5 This Example provides results generated using a localized Schwartzman reaction model of disseminated intravascular coagulation ("DIC") in MASP-2 (-/-) and WT (+/+) mice to evaluate the role of lectin pathway in DIC.

Background/Rationale:

10 As described supra, blockade of MASP-2 inhibits lectin pathway activation and reduces the generation of both anaphylatoxins C3a and C5a. C3a anaphylatoxins can be shown to be potent platelet aggregators *in vitro*, but their involvement *in vivo* is less well defined and the release of platelet substances and plasmin in wound repair may only secondarily involve complement C3. In this Example, the role of the lectin pathway was
15 analyzed in MASP-2 (-/-) and WT (+/+) mice in order to address whether prolonged elevation of C3 activation is necessary to generate disseminated intravascular coagulation.

Methods:

20 The MASP-2 knockout mice (MASP-2 -/- mice) used in this study were generated as described in Example 1 of US 7,919,094, hereby incorporated herein by reference.

25 The localized Schwartzman reaction (LSR) model was used in this experiment. The LSR is a lipopolysaccharide (LPS) -induced response with well-characterized contributions from cellular and humoral elements of the innate immune system. Dependence of the LSR on complement is well established (Polak, L., et al., *Nature* 223:738-739 (1969); Fong J.S. et al., *J Exp Med* 134:642-655 (1971)). In the LSR model,
30 the mice were primed for 4 hours with TNF alpha (500 ng, intrascrotal), then the mice were anaesthetized and prepared for intravital microscopy of the cremaster muscle. Networks of post-capillary venules (15-60 μ m diameter) with good blood flow (1-4 mm/s) were selected for observation. Animals were treated with fluorescent antibodies to selectively label neutrophils, or platelets. The network of vessels was sequentially scanned and images of all vessels were digitally recorded for later analysis. After

recording the basal state of the microcirculation, mice received a single intravenous injection of LPS (100 μ g), either alone or with the agents listed below. The same network of vessels was then scanned every 10 minutes for 1 hour. Specific accumulation of fluorophores was identified by subtraction of background fluorescence and enhanced by thresholding the image. The magnitude of reactions was measured from recorded images. The primary measure of LSR was aggregate data.

The studies compared the WT (+/+) mice exposed to either a known complement pathway depletory agent, cobra venom factor (CVF), or a terminal pathway inhibitor (C5aR antagonist). The results (**FIGURE 4A**) demonstrate that CVF as well as a C5aR antagonist both prevented the appearance of aggregates in the vasculature. In addition, the MASP-2 (-/-) mice (**FIGURE 4B**) also demonstrated complete inhibition of the localized Schwartzman reaction, supporting lectin pathway involvement. These results clearly demonstrate the role of MASP-2 in DIC generation and support the use of MASP-2 inhibitors for the treatment and prevention of DIC.

EXAMPLE 10

This Example describes activation of C3 by thrombin substrates and C3 deposition on mannan in WT (+/+), MASP-2 (-/-), F11 (-/-), F11/C4 (-/-) and C4 (-/-) mice.

Rationale:

As described in **Example 8**, it was determined that thrombin activation can occur following lectin pathway activation under physiological conditions, and demonstrates the extent of MASP-2 involvement. C3 plays a central role in the activation of the complement system. C3 activation is required for both classical and alternative complement activation pathways. An experiment was carried out to determine whether C3 is activated by thrombin substrates.

Methods:

C3 Activation by thrombin substrates

Activation of C3 was measured in the presence of the following activated forms of thrombin substrates; human FXIa, human FVIIa, bovine FXa, human FXa, human activated protein C, and human thrombin. C3 was incubated with the various thrombin

substrates, then separated under reducing conditions on 10% SDS-polyacrylamide gels. After electrophoretic transfer using cellulose membrane, the membrane was incubated with monoclonal biotin-coupled rat anti-mouse C3, detected with a streptavidin-HRP kit and developed using ECL reagent.

5 **Results:**

Activation of C3 involves cleavage of the intact α -chain into the truncated α' chain and soluble C3a. **FIGURE 5** shows the results of a Western blot analysis on the activation of human C3 by thrombin substrates, wherein the uncleaved C3 α chain, and the activation product α' chain are shown by arrows. As shown in **FIGURE 5**,
10 incubation of C3 with the activated forms of human clotting factor XI and factor X, as well as activated bovine clotting factor X, can cleave C3 in vitro in the absence of any complement proteases.

Discussion:

The data described in this Example show that in the physiological context of
15 whole serum the lectin pathway can activate components of the coagulation cascade. Thus, it is demonstrated that there is cross-talk between complement and coagulation involving MASP-2.

EXAMPLE 11

This study investigates the effect of MASP-2-deficiency in a mouse model of LPS
20 (lipopolysaccharide)-induced thrombosis.

Rationale:

Hemolytic uremic syndrome (HUS), which is caused by Shiga toxin-producing *E. coli* infection, is the leading cause of acute renal failure in children. In this Example, a Schwartzman model of LPS-induced thrombosis (microvascular coagulation) was carried
25 out in MASP-2 (-/-) mice to determine whether MASP-2 inhibition is effective to inhibit or prevent the formation of intravascular thrombi.

Methods:

MASP-2 (-/-) (n=9) and WT (n=10) mice were analyzed in a Schwartzman model of LPS-induced thrombosis (microvascular coagulation). Mice were administered

Serratia LPS and thrombus formation was monitored over time. A comparison of the incidence of microthrombi and LPS-induced microvascular coagulation was carried out.

Results:

Notably, all MASP-2 $-/-$ mice tested (9/9) did not form intravascular thrombi after Serratia LPS administration. In contrast, microthrombi were detected in 7 of 10 of the WT mice tested in parallel ($p=0.0031$, Fischer's exact). As shown in **FIGURE 6**, the time to onset of microvascular occlusion following LPS infection was measured in MASP-2 ($-/-$) and WT mice, showing the percentage of WT mice with thrombus formation measured over 60 minutes, with thrombus formation detected as early as about 15 minutes. Up to 80% of the WT mice demonstrated thrombus formation at 60 minutes. In contrast, as shown in **FIGURE 6**, none of the MASP-2 ($-/-$) had thrombus formation at 60 minutes (log rank: $p=0.0005$).

These results demonstrate that MASP-2 inhibition is protective against the development of intravascular thrombi in an HUS model.

EXAMPLE 12

This Example describes the effect of MASP-2 deficiency and MASP-2 inhibition in a murine FITC-dextran/light induced endothelial cell injury model of thrombosis.

Background/Rationale:

The following experiments were carried out to analyze the effect of MASP-2 deficiency and MASP-2 inhibition in a fluorescein isothiocyanate (FITC)-dextran-induced endothelial cell injury model of thrombotic microangiopathy (TMA) in order to demonstrate further the benefit of MASP-2 inhibitors for the treatment of HUS, aHUS, TTP, and TMA's with other etiologies.

Methods:

Intravital microscopy

Mice were prepared for intravital microscopy as described by Frommhold et al., *BMC Immunology* 12:56-68, 2011. Briefly, mice were anesthetized with intraperitoneal (i.p.) injection of ketamine (125 mg/kg bodyweight, Ketanest, Pfitzer GmbH, Karlsruhe, Germany) and xylazine (12.5 mg/kg body weight; Rompun, Bayer, Leverkusen, Germany) and placed on a heating pad to maintain body temperature at 37°C. Intravital microscopy was conducted on an upright microscope (Leica, Wetzlar, Germany) with a saline immersion objective (SW 40/0.75 numerical aperture, Zeiss, Jena, Germany). To ease breathing, mice were intubated using PE 90 tubing (Becton Dickson and Company, Sparks, MD, USA). The left carotid artery was cannuled with PE10 tubing (Becton Dickson and Company, Sparks, MD, USA) for blood sampling and systemic monoclonal antibody (mAb) administration.

Cremaster muscle preparation

The surgical preparation of the cremaster muscle for intravital microscopy was performed as described by Sperandio et al., *Blood*, 97:3812-3819, 2001. Briefly, the scrotum was opened and the cremaster muscle mobilized. After longitudinal incision and spreading of the muscle over a cover glass, the epididymis and testis were moved and pinned to the side, giving full microscopic access to the cremaster muscle microcirculation. Cremaster muscle venules were recorded via a CCD camera (CF8/1; Kappa, Gleichen, Germany) on a Panasonic S-VHS recorder. The cremaster muscle was superfused with thermo-controlled (35°C bicarbonate-buffered saline) as previously described by Frommhold et al., *BMC Immunology* 12:56-68, 2011.

Light excitation FITC dextran injury model

A controlled, light-dose-dependent vascular injury of the endothelium of cremaster muscle venules and arterioles was induced by light excitation of phototoxic (FITC)-dextran (Cat. No. FD150S, Sigma Aldrich, Poole, U.K.). This procedure initiates

localized thrombosis. As a phototoxic reagent, 60 μ L of a 10% w/v solution of FITC-dextran was injected through the left carotid artery access and allowed to spread homogenously throughout the circulating blood for 10 minutes. After selecting a well-perfused venule, halogen light of low to midrange intensity (800-1500) was focused on the vessel of interest to induce FITC-dextran fluorescence and mild to moderate phototoxicity to the endothelial surface in order to stimulate thrombosis in a reproducible, controlled manner. The necessary phototoxic light intensity for the excitation of FITC-dextran was generated using a halogen lamp (12V, 100W, Zeiss, Oberkochen, Germany). The phototoxicity resulting from light-induced excitation of the fluorochrome requires a threshold of light intensity and/or duration of illumination and is caused by either direct heating of the endothelial surface or by generation of reactive oxygen radicals as described by Steinbauer et al., *Langenbecks Arch Surg* 385:290-298, 2000.

The intensity of the light applied to each vessel was measured for adjustment by a wavelength-correcting diode detector for low power measurements (Labmaster LM-2, Coherent, Auburn, USA). Off-line analysis of video scans was performed by means of a computer assisted microcirculation analyzing system (CAMAS, Dr. Zeintl, Heidelberg) and red blood cell velocity was measured as described by Zeintl et al., *Int J Microcirc Clin Exp*, 8(3):293-302, 2000.

Application of monoclonal anti-human MASP-2 inhibitory antibody (mAbH6) and vehicle control prior to induction of thrombosis

Using a blinded study design, 9-week-old male C57BL/6 WT littermate mice were given i.p. injections of either the recombinant monoclonal human MASP-2 antibody (mAbH6), an inhibitor of MASP-2 functional activity (given at a final concentration of 10mg/kg body weight), or the same quantity of an isotype control antibody (without MASP-2 inhibitory activity) 16 hours before the phototoxic induction of thrombosis in the cremaster model of intravital microscopy. One hour prior to thrombosis induction, a

second dose of either mAbH6 or the control antibody was given. MASP-2 knockout (KO) mice were also evaluated in this model.

mAbH6 (established against recombinant human MASP-2) is a potent inhibitor of human MASP-2 functional activity, which cross-reacts with, binds to and inhibits mouse MASP-2 but with lower affinity due to its species specificity (data not shown). In order to compensate for the lower affinity of mAbH6 to mouse MASP-2, mAbH6 was given at a high concentration (10mg/kg body weight) to overcome the variation in species specificity, and the lesser affinity for mouse MASP-2, to provide effective blockade of murine MASP-2 functional activity under *in vivo* conditions.

In this blinded study, the time required for each individual venuole tested (selection criteria were by comparable diameters and blood flow velocity) to fully occlude was recorded.

The percentage of mice with microvascular occlusion, the time of onset, and the time to occlusion were evaluated over a 60-minute observation period using intravital microscopy video recordings.

Results:

FIGURE 7 graphically illustrates, as a function of time after injury induction, the percentage of mice with microvascular occlusion in the FITC/Dextran UV model after treatment with isotype control or human MASP-2 antibody mAbH6 (10mg/kg) dosed at 16 hours and 1 hour prior to injection of FITC/Dextran. As shown in FIGURE 7, 85% of the wild-type mice receiving the isotype control antibody occluded within 30 minutes or less, whereas only 19% of the wild-type mice pre-treated with the human MASP-2 antibody (mAbH6) occluded within the same time period, and the time to occlusion was delayed in the mice that did eventually occlude in the human MASP-2 antibody-treated group. It is further noted that three of the MASP-2 mAbH6 treated mice did not occlude

at all within the 60-minute observation period (i.e., were protected from thrombotic occlusion).

FIGURE 8 graphically illustrates the occlusion time in minutes for mice treated with the human MASP-2 antibody (mAbH6) and the isotype control antibody. The data are reported as scatter-dots with mean values (horizontal bars) and standard error bars (vertical bars). This figure shows the occlusion time in the mice where occlusion was observable. Thus, the three MASP-2 antibody-treated mice that did not occlude during the 60 minute observation period were not included in this analysis (there was no control treated mouse that did not occlude). The statistical test used for analysis was the unpaired t test; wherein the symbol “*” indicates $p=0.0129$. As shown in FIGURE 8, in the four MASP-2 antibody (mAbH6)-treated mice that occluded, treatment with MASP-2 antibody significantly increased the venous occlusion time in the FITC-dextran/light-induced endothelial cell injury model of thrombosis with low light intensity (800-1500) as compared to the mice treated with the isotype control antibody. The average of the full occlusion time of the isotype control was 19.75 minutes, while the average of the full occlusion time for the MASP-2 antibody treated group was 32.5 minutes.

FIGURE 9 graphically illustrates the time until occlusion in minutes for wild-type mice, MASP-2 KO mice, and wild-type mice pre-treated with human MASP-2 antibody (mAbH6) administered i.p. at 10mg/kg 16 hours before, and then administered again i.v. 1 hour prior to the induction of thrombosis in the FITC-dextran/light-induced endothelial cell injury model of thrombosis with low light intensity (800-1500). Only the animals that occluded were included in FIGURE 9; $n=2$ for wild-type mice receiving isotype control antibody; $n=2$ for MASP-2 KO; and $n=4$ for wild-type mice receiving human MASP-2 antibody (mAbH6). The symbol “*” indicates $p<0.01$. As shown in FIGURE 9, MASP-2 deficiency and MASP-2 inhibition (mAbH6 at 10mg/kg) increased the venous occlusion time in the FITC-dextran/light-induced endothelial cell injury model of thrombosis with low light intensity (800-1500).

Conclusions:

The results in this Example further demonstrate that a MASP-2 inhibitory agent that blocks the lectin pathway (e.g., antibodies that block MASP-2 function), inhibits microvascular coagulation and thrombosis, the hallmarks of multiple microangiopathic disorders, in a mouse model of TMA. Therefore, it is expected that administration of a MASP-2 inhibitory agent, such as a MASP-2 inhibitory antibody, will be an effective therapy in patients suffering from HUS, aHUS, TTP, or other microangiopathic disorders and provide protection from microvascular coagulation and thrombosis.

10

EXAMPLE 13

This Example describes a study demonstrating that human MASP-2 inhibitory antibody (mAbH6) has no effect on platelet function in platelet-rich human plasma.

Background/Rationale: As described in Example 12, it was demonstrated that MASP-2 inhibition with human MASP-2 inhibitory antibody (mAbH6) increased the venous occlusion time in the FITC-dextran/light-induced endothelial cell injury model of thrombosis. The following experiment was carried out to determine whether the MASP-2 inhibitory antibody (mAbH6) has an effect on platelet function.

Methods: The effect of human mAbH6 MASP-2 antibody was tested on ADP-induced aggregation of platelets as follows. Human MASP-2 mAbH6 at a concentration of either 1 $\mu\text{g/ml}$ or 0.1 $\mu\text{g/ml}$ was added in a 40 μL solution to 360 μL of freshly prepared platelet-rich human plasma. An isotype control antibody was used as the negative control. After adding the antibodies to the plasma, platelet activation was induced by adding ADP at a final concentration of 2 μM . The assay was started by stirring the solutions with a small magnet in the 1 mL cuvette. Platelet aggregation was measured in a two-channel Chrono-log Platelet Aggregometer Model 700 Whole Blood/Optical Lumi-Aggregometer.

Results:

The percent aggregation in the solutions was measured over a time period of five minutes. The results are shown below in TABLE 5.

TABLE 5: Platelet Aggregation over a time period of five minutes.

Antibody	Amplitude (percent aggregation)	Slope (percent aggregation over time)
MASP-2 antibody (mAbH6) (1 µg/ml)	46%	59
Isotype control antibody (1 µg/ml)	49%	64
MASP-2 antibody (mAbH6) (0.1 µg/ml)	52%	63
Isotype control antibody (0.1 µg/ml)	46%	59

5 As shown above in TABLE 5, no significant difference was observed between the aggregation of the ADP-induced platelets treated with the control antibody or the MASP-2 mAbH6 antibody. These results demonstrate that the human MASP-2 antibody (mAbH6) has no effect on platelet function. Therefore, the results described in Example 12 demonstrating that MASP-2 inhibition with human MASP-2 inhibitory antibody
10 (mAbH6) increased the venous occlusion time in the FITC-dextran/light-induced endothelial cell injury model of thrombosis, were not due to an effect of mAbH6 on platelet function. Thus, MASP-2 inhibition prevents thrombosis without directly impacting platelet function, revealing a therapeutic mechanism that is distinct from

existing anti-thrombotic agents.

EXAMPLE 14

Background/Rationale:

This Example describes a study that was carried out to examine the cross-talk
5 between the lectin pathway and the coagulation/contact system.

Methods:

Hirudin-anticoagulated human plasma (~90%) was pre-incubated with varying concentrations of the following MASP-2 inhibitory antibodies: OMS646 (generated as described in Example 7) and NimoAb101 (described in Table 1 and generated as
10 described in WO2004/106384). Control antibody ET-904 (Eureka Therapeutics) was also used as a negative control.

The samples were stimulated by the addition of fibrin (20 µg/mL). The supernatants were evaluated for serine protease activation endpoints by sandwich ELISA assays for detection of complexes between MASP-2 and the serpins C1 inhibitor (C1-
15 INH) or antithrombin III (ATIII) to specifically detect and quantify the activated forms of MASP-2 as described in Kozarcanin H. et al., *Journal of Thrombosis and Haemostasis* 14:531-545, 2016, which is hereby incorporated herein by reference in its entirety.

Results:

FIGURE 10A graphically illustrates MASP-2 activation, as determined by
20 MASP-2-antithrombin complex formation (MASP-2-ATIII), shown in Absorbance Units (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies mAb#1 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin, demonstrating that MASP-2 inhibitory antibodies block MASP-2 activation in a dose-dependent manner, whereas the control antibody had no effect.

25 FIGURE 10B graphically illustrates MASP-2 activation, as determined by

MASP-2-serpin complex formation (MASP-2-C1-INH), shown in Absorbance Units (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies mAb#1 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin, demonstrating that MASP-2 inhibitory antibodies block MASP-2 activation in a dose-
5 dependent manner, whereas the control antibody had no effect.

FIGURE 11A graphically illustrates Kallikrein activation, as determined by Kallikrein-antithrombin complex formation (KK-ATIII), shown in Absorbance Units (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies mAb#1 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin,
10 demonstrating that MASP-2 inhibitory antibodies block Kallikrein activation in a dose-dependent manner, whereas the vehicle control had no effect.

FIGURE 11B graphically illustrates Kallikrein activation, as determined by Kallikrein-serpin complex formation (KK-C1-INH), shown in Absorbance Units (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies mAb#1
15 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin, demonstrating that MASP-2 inhibitory antibodies block Kallikrein activation in a dose-dependent manner, whereas the vehicle control had no effect.

FIGURE 12A graphically illustrates Factor XII activation, as determined by Factor XII-antithrombin complex formation (FXIIa-ATIII), shown in Absorbance Units
20 (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies mAb#1 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin, demonstrating that MASP-2 inhibitory antibodies block Factor XII activation in a dose-dependent manner, whereas the control antibody had no effect.

FIGURE 12B graphically illustrates Factor XII activation, as determined by
25 Factor XII-serpin complex formation (FXIIa-C1-INH), shown in Absorbance Units (AU), in the presence of increasing concentrations of MASP-2 inhibitory antibodies mAb#1 (NimoAb101) and mAb#2 (OMS646) in human plasma stimulated by fibrin, demonstrating that MASP-2 inhibitory antibodies block Factor XII activation in a dose-dependent manner, whereas the control antibody had no effect.

As shown in FIGs 10A, 11A and 12A, the MASP-2 inhibitory antibodies inhibited fibrin-induced activation and protease-ATIII complex formation.

As further shown in FIG. 10B, 11B and 12B, activation of protease-C1 INH complex was also inhibited by the MASP-2 antibodies, although the IC₅₀ values tended to be higher.

The negative control monoclonal antibody ET-904 showed no effect in any of the assays, as further shown in FIGs 10A, 10B, 11A, 11B, 12A and 12B.

Discussion:

The data described in this Example demonstrate the existence of an extensive bi-directional cross-talk between the complement cascade's lectin pathway, for which the effector enzyme is MASP-2, and the coagulation and contact systems. As described in Kozarcanin et al., *Journal of Thrombosis and Haemostasis* 14:531-545, 2016, it was determined that fibrin clots – the product of a coagulation event – activate the lectin pathway. As demonstrated herein, lectin pathway activation, via MASP-2, subsequently triggers activation of the coagulation and contact systems. This is expected to result in additional coagulation, thereby driving a feedback cycle that results in excessive and unchecked clot formation. This feedback cycle is expected to promote thrombotic disorders. As further demonstrated herein, MASP-2 forms the nexus of this crosstalk, and the inhibition of MASP-2 by MASP-2 inhibitory antibodies blocked Factor XII activation and Kallikrein activation, thereby indicating that the therapeutic use of MASP-2 inhibitory antibodies is expected to reduce excessive activation of the coagulation and contact cascades.

Accordingly, in one aspect, the present invention provides a method of preventing, reducing and/or treating a disease, disorder or condition associated with fibrin-induced activation of the complement system and the associated activation of the coagulation and/or contact systems comprising administering a therapeutic amount of a MASP-2 inhibitory antibody to a subject in need thereof. In some embodiments, the methods of the invention provide anticoagulation and/or antithrombosis and/or

antithrombogenesis without affecting hemostasis.

In one embodiment of this aspect of the invention, the methods are useful for treating a subject that is suffering from, or at risk of developing, a disease, disorder or condition associated with complement-related inflammation, excessive coagulation or contact system activation initiated by fibrin or activated platelets. In one embodiment, the methods of the invention are useful for treating a subject suffering from a disease, disorder or condition selected from the group consisting of arterial thrombosis, venous thrombosis, deep vein thrombosis, post-surgical thrombosis, restenosis following coronary artery bypass graft and/or an interventional cardiovascular procedure (e.g., angioplasty or stent placement), atherosclerosis, plaque rupture, plaque instability, restenosis, hypotension, acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome (SIRS), disseminated intravascular coagulation (DIC), veno-occlusive disease (VOD), sickle cell disease, thrombotic microangiopathy, lupus nephritis, superficial thrombophlebitis, Factor V Leiden mutation, ischemic/reperfusion injury, human immunodeficiency virus (HIV) infection, undergoing hormone-replacement therapy (HRT), Alzheimer's disease and/or suffering from a hypercoagulable state, such as wherein the subject is suffering from, or at risk for developing an acquired hypercoagulable state due to at least one or more of the following: undergoing drug therapy with a drug selected from the group consisting of 5-FU, GM-CSF, cisplatin, heparin, COX-2 inhibitor, contrast media, corticosteroids and antipsychotics, venous stasis (due to immobilization, surgery, etc), antiphospholipid syndrome (develops lupus anticoagulant or cardiolipin antibody), cancer (promyelocytic leukemia, lung, breast, prostate, pancreas, stomach and colon tumors: activate coagulation by (i) secreting a factor X-activating protease; (ii) by expressing/exposing tissue factor on membrane surfaces, or both; (iii) may cause DIC; and (iv) cancer treatments may increase risk of excessive clotting, tissue injury due to trauma or surgery, presence of a catheter in a central vein (disruption in the flow of blood can cause blood clots to form), acquired deficiency of a protein involved in clot formation (e.g., protein C), paroxysmal nocturnal hemoglobinuria (PNH), elevated levels of homocysteine, heart failure (causes slowing of blood flow, stasis), mechanical valves, pulmonary hypertension with in-situ thrombosis, atrial fibrillation, heparin-induced thrombocytopenia (HIT), heparin-induced

thrombocytopenia and thrombosis (HITT), Kawasaki disease with in-situ thrombus, Takayasu arteritis with in-situ thrombus, thrombophilia of metastatic cancer, elevated Factor VIII levels, pregnancy and inflammatory bowel disease (IBD). See e.g., J.L. Moake, "Overview of Thrombotic Disorders," Merck Manual Profession Version, April 5 2018, which is hereby incorporated herein by reference. See also Xu T., et al., Activated platelets contribute importantly to myocardial reperfusion injury, *Am J Physiol Heart Circ Physiol* 290:H692-H699, 2006; Banz Y. et al., Role of complement and perspectives for intervention in ischemia-reperfusion damage, *Ann Med.* 44:205-217, 2012; Cortes-Canteli M et al., Fibrinogen and altered hemostasis in Alzheimer's disease, *J Alzheimer's* 10 *Dis* 32(3):599-608, 2012. With regard to drugs that can induce a hypercoagulable state and mechanism, see Ramot Y. et al., Drug-induced thrombosis-experimental, clinical and mechanistic considerations, *Toxicol Pathol* 35(2):208-25, which is hereby incorporated by reference in its entirety.

In one embodiment, the methods of the invention are useful for treating a subject 15 suffering from, or at risk for developing one or more symptoms associated with sickle cell disease, including ameliorating at least one symptom associated with sickle cell disease, such as pain (acute or chronic) associated with veno-occlusive crisis, fatigue, thromboembolic events such as stroke associated with veno-occlusive crisis (ischemia, intense pain, necrosis), splenic sequestration crisis, acute chest syndrome, hemolytic 20 crisis, and frequent infections. Sickle cell disease (SCD) is an inherited vaso-occlusive disorder involving activation of platelets. Sickle cell disease originates from a missense mutation within the β -globin gene, leading to the substitution of valine for glutamic acid on the outer surface of the globin molecule. This amino acid substitution renders the sickle cell hemoglobin ("HbS") less soluble and prone to polymerization upon 25 deoxygenation. Erythrocytes carrying polymerized HbS are therefore less deformable and may obstruct microvessels. This vascular occlusion (vaso-occlusion) produces tissue ischemia and infarction which represents a major cause of morbidity and mortality among

SCD patients (Frenette and Atweh, *J Clin Invest.*117:850-858, 2007; Steinberg et al., *Scientific World Journal* 8:1295-1324, 2008).

In one embodiment, the methods of the invention are useful for treating a subject suffering from, or at risk for developing, a disease, disorder or condition that is amenable
5 to treatment with a kallikrein inhibitor, such as, for example, wherein the disease or disorder amenable to treatment with a kallikrein inhibitor is selected from the group consisting of hereditary angioedema (currently an FDA-approved indication), diabetic macular edema (KalVista Pharmaceutical's plasma kallikrein inhibitor known as KVD001 is currently in Phase 2 trials) and bleeding during cardiopulmonary bypass
10 (ongoing trials, see Kolte et al., *Plasma Kallikrein Inhibitors in Cardiovascular Disease*, *Cardiol Rev* 24(3):99-109, 2016). See also world-wide-web.drugs.com/condition/thrombotic-thromboembolic-disorder, accessed June 20, 2018.

In one embodiment, the methods of the invention are useful for treating a subject suffering from, or at risk for developing, a disease or disorder that is amenable to
15 treatment with a thrombin inhibitor, such as, for example, wherein the disease or disorder amenable to treatment with a thrombin inhibitor is selected from the group consisting of arterial thrombosis (especially acute coronary syndromes), venous thrombosis, pulmonary embolism, reduce risk of stroke in patients with atrial fibrillation, heparin-induced thrombocytopenia (see e.g., argatroban/Lexicomp package insert), conversion from one
20 anticoagulant to another (see, e.g., dabigatran etexilate/Pradaxa package insert, e.g., conversion from oral dabigatran to a parenteral anticoagulant) and off-label use for extracorporeal circuit patency of continuous renal replacement therapy (CRRT) in critically ill patients with HIT (maintenance). In one embodiment, the method comprises identifying a subject that has previously experienced, is currently suffering from, or is at
25 risk for developing atrial fibrillation and administering a MASP-2 inhibitory antibody in an amount sufficient to reduce the risk of stroke in said subject. See e.g., world-wide-web.drugs.com/condition/thrombotic-thromboembolic-disorder, accessed June 20, 2018.

In one embodiment, methods of the invention are useful for treating a subject suffering from, or at risk for developing, a disease or disorder that is amenable to treatment with a factor XII inhibitor, such as, for example, wherein the disease or disorder amenable to treatment with a factor XII inhibitor is selected from the group consisting of deep vein thrombosis (both primary prophylaxis and extended therapy), pulmonary embolism, nonvalvular atrial fibrillation, prevention of recurrent ischemia after acute coronary syndrome in subjects with or without atrial fibrillation, end-stage renal disease, cerebral ischemia, angina, medical devices (use for reducing clotting associated with medical devices such as valves, small caliber grafts, etc., is expected to be more effective than current therapy) and extracorporeal circuits. In one embodiment, the method comprises identifying a subject that has previously experienced, is currently suffering from, or is at risk for developing nonvalvular atrial fibrillation and administering a MASP-2 inhibitory antibody in an amount sufficient to reduce the risk of stroke and/or embolism in said subject. See e.g., world-wide-web.drugs.com/condition/thrombotic-thromboembolic-disorder, accessed June 20, 2018; world-wide-web.askhematologist.com/thrombotic-disorders, accessed June 20, 2018 and Weitz J. et al., Front Med (Lausanne) 4:19, 2017, which is hereby incorporated herein by reference in its entirety.

In one embodiment, the methods of the invention are useful for treating a subject determined to have a genetic defect that causes, or increases the risk of developing a hypercoagulable state, such as, for example, wherein the genetic defect is selected from the group consisting of a Prothrombin 20210 gene mutation, an MTHFR mutation (a mutation in this gene may predispose to high levels of homocysteine, which can increase the risk of excessive clotting), a deficiency of protein C, a deficiency of protein S, a deficiency of protein A, a deficiency of protein Z, an antithrombin deficiency, and genetic disorders producing thrombophilia.

In one embodiment, the methods of the invention are useful for providing a replacement for standard anticoagulant therapy, such as Warfarin, in a subject in need of anticoagulant therapy, such as wherein the subject has a condition that normally prohibits standard anticoagulant therapy (e.g., CNS amyloid angiopathy), or wherein the MASP-2
5 inhibitory antibody is administered as a bridging agent perioperatively in a subject otherwise on standard anticoagulation therapy.

In one embodiment, the methods of the invention are useful for treating a subject that has an acquired disease, disorder or condition that increases the propensity for thromboembolism, such as, for example, wherein the acquired disease or disorder that
10 increases the propensity for thromboembolism is selected from the group consisting of atherosclerosis, antiphospholipid antibodies, cancer (e.g., promyelocytic leukemia, lung, breast, prostate, pancreatic, stomach or colon cancer), hyperhomocysteinemia, infection, tissue injury, venous stasis (such as due to surgery, orthopedic or paralytic immobilization, heart failure, pregnancy, or obesity) and a subject taking oral
15 contraceptives that contain estrogen.

With regard to antiphospholipid antibodies, it is known that this is an autoimmune disorder with an increased risk of venous or arterial thrombi, and a higher risk of thromboembolism exists in patients with preexisting stenosis.

With regard to atherosclerosis, when atherosclerotic plaques rupture, they expose
20 or release tissue factor, activate coagulation, initiate local platelet adhesion and aggregation and thereby cause thrombosis.

With regard to a patient suffering from cancer, and in particular promyelocytic leukemia, lung, breast, prostate, pancreatic, stomach or colon cancer, these tumor types may activate coagulation by secreting a factor X-activating protease, or by
25 expressing/exposing tissue factor on membrane surfaces, or both.

With regard to infection, a severe infection (e.g., sepsis) increases expression/exposure of tissue factor by monocytes and macrophages and decreases formation of activated protein C.

With regard to the use of oral contraceptives that contain estrogen, the risk is
5 higher in patients who have a genetic abnormality that predisposes to various thromboembolism, and in women who smoke.

In accordance with any of the embodiments of the methods of the invention described herein, the MASP-2 inhibitory antibody is preferably a MASP-2 monoclonal antibody, or fragment thereof that specifically binds to a portion of SEQ ID NO:5. In
10 some embodiments, the MASP-2 antibody is a chimeric, humanized or human antibody. In some embodiments, the MASP-2 inhibitory antibody is an antibody fragment selected from the group consisting of Fv, Fab, Fab', F(ab)₂ and F(ab')₂. In some embodiments, the MASP-2 inhibitory antibody is a single-chain molecule. In some embodiments, the MASP-2 inhibitory antibody is selected from the group consisting of an IgG1 molecule,
15 an IgG2 and an IgG4 molecule. In some embodiments, the MASP-2 inhibitory antibody is an IgG4 molecule comprising a S228P mutation. In some embodiments, the MASP-2 inhibitory antibody does not substantially inhibit the classical pathway. In some embodiments, the MASP-2 inhibitory monoclonal antibody, or antigen-binding fragment thereof, comprises: (a) a heavy-chain variable region comprising: i) a heavy chain CDR-
20 H1 comprising the amino acid sequence from 31-35 of SEQ ID NO:6; and ii) a heavy-chain CDR-H2 comprising the amino acid sequence from 50-65 of SEQ ID NO:6; and iii) a heavy-chain CDR-H3 comprising the amino acid sequence from 95-107 of SEQ ID NO:6 and (b) a light-chain variable region comprising: i) a light-chain CDR-L1 comprising the amino acid sequence from 24-34 of SEQ ID NO:7; and ii) a light-chain
25 CDR-L2 comprising the amino acid sequence from 50-56 of SEQ ID NO:7; and iii) a light-chain CDR-L3 comprising the amino acid sequence from 89-97 of SEQ ID NO:7. In some embodiments, the MASP-2 inhibitory monoclonal antibody comprises a heavy-chain variable region set forth as SEQ ID NO:6 and a light-chain variable region set forth as SEQ ID NO:7. In some embodiments, the MASP-2 inhibitory antibody or antigen
30 binding-fragment thereof specifically recognizes at least part of an epitope recognized by

a reference antibody comprising a heavy chain variable region as set forth in SEQ ID NO:6 and a light-chain variable region as set forth in SEQ ID NO:7.

5 While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

Claim

5 The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A MASP-2 inhibitory antibody for use in a method of treating a subject suffering from, or at risk of developing, a disease disorder or condition associated with complement-related inflammation, excessive coagulation or contact system activation initiated by fibrin or activated platelets, wherein the MASP-2 inhibitory agent is a MASP-2 monoclonal antibody,
10 or fragment thereof that specifically binds to a portion of SEQ ID NO:5 wherein said MASP-2 inhibitory antibody does not substantially inhibit the classical pathway.

2. Use of a MASP-2 inhibitory antibody in the manufacture of a pharmaceutical composition for treating a subject suffering from, or at risk of developing, a disease disorder or condition associated with complement-related inflammation, excessive coagulation or
15 contact system activation initiated by fibrin or activated platelets, wherein the MASP-2 inhibitory agent is a MASP-2 monoclonal antibody, or fragment thereof that specifically binds to a portion of SEQ ID NO:5 wherein said MASP-2 inhibitory antibody does not substantially inhibit the classical pathway.

3. The antibody for use of Claim 1 or the use of Claim 2, wherein the subject is
20 suffering from an acquired hypercoagulable state.

4. The antibody for use or the use of Claim 2, wherein the acquired hypercoagulable state is due to a disease or condition selected from the group consisting of Kawasaki disease with in-situ thrombus, pulmonary hypertension with in-situ thrombosis, Takayasu arteritis with in-situ thrombus, atrial fibrillation, undergoing therapy with a drug selected from the
25 group consisting of 5-FU, GM-CSF, cisplatin, heparin, COX-2 inhibitor, contrast media, corticosteroids and antipsychotics; venous stasis from immobilization and/or surgery, acquired deficiency of a protein involved in clot formation (e.g., protein C), elevated levels of homocysteine, heart failure, presence of a mechanical valve, heparin-induced thrombocytopenia (HIT), heparin-induced thrombocytopenia and thrombosis (HITT), ,

thrombophilia of metastatic cancer, elevated Factor VIII levels, pregnancy, suffering from antiphospholipid syndrome, cancer (promyelocytic leukemia, lung, breast, prostate, pancreas, stomach and colon tumors); tissue injury due to trauma or surgery, presence of a catheter in a central vein, paroxysmal nocturnal hemoglobinuria (PNH), or inflammatory bowel disease (IBD).

5 5. The antibody for use of Claim 1 or the use of Claim 2, wherein the subject has an acquired disease, disorder or condition that increases the propensity for thromboembolism.

6. The antibody for use or the use of Claim 5, wherein the acquired disease, disorder or condition that increases the propensity for thromboembolism is selected from the group consisting of infection, tissue injury, atherosclerosis, antiphospholipid antibodies, cancer, hyperhomocysteinemia, venous stasis (such as due to surgery, orthopedic or paralytic immobilization, heart failure, pregnancy, or obesity) and a subject taking oral contraceptives that contain estrogen.

7. The antibody for use of Claim 1 or the use of Claim 2, wherein the subject is suffering from a disease or disorder selected from the group consisting of sickle cell disease, arterial thrombosis, venous thrombosis, deep vein thrombosis, post-surgical thrombosis, atherosclerotic, plaque rupture, and/or plaque instability, hypotension, superficial thrombophlebitis, Factor V Leiden mutation, and undergoing hormone-replacement therapy (HRT), following coronary artery bypass graft and/or an interventional cardiovascular procedure such as angioplasty or stent replacement; atherosclerosis, acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome (SIRS), disseminated intravascular coagulation (DIC), veno-occlusive disease (VOD), thrombotic microangiopathy, lupus nephritis, ischemic/reperfusion injury, human immunodeficiency virus (HIV) infection, Alzheimer's disease, hereditary angioedema, diabetic macular edema and bleeding during cardiopulmonary bypass.

8. The antibody for use of claim 1 or the use of Claim 2, wherein the subject is suffering from, or at risk for developing, a disease or disorder that is amenable to treatment with a thrombin inhibitor such as wherein the disease or disorder amenable to treatment with a thrombin inhibitor is selected from the group consisting of, pulmonary embolism,

conversion from one anticoagulant to another, and off-label use for extracorporeal circuit patency of continuous renal replacement therapy (CRRT) in critically ill patients with HIT (maintenance).

9. The antibody for use of claim 1 or the use of Claim 2, wherein the subject has
5 previously experienced, is currently suffering from, or is at risk for developing atrial fibrillation and the MASP-2 inhibitory antibody is for administration in an amount sufficient to reduce the risk of stroke in said subject.

10. The antibody for use of claim 1 or the use of Claim 2, wherein the subject is
suffering from, or at risk for developing, a disease or disorder that is amenable to treatment
10 with a factor XII inhibitor, such as wherein the disease or disorder amenable to treatment with a factor XII inhibitor is selected from the group consisting of deep vein thrombosis (both primary prophylaxis and extended therapy), nonvalvular atrial fibrillation, prevention of recurrent ischemia after acute coronary syndrome in subjects with or without atrial fibrillation, end-stage renal disease, cerebral ischemia, angina, reduce or prevent clotting
15 associated with medical devices (e.g., valves, small caliber grafts) and/or extracorporeal circuits.

11. The antibody for use of claim 1 or the use of Claim 2, wherein the subject has
previously experienced, is currently suffering from, or is at risk for developing nonvalvular atrial fibrillation and the MASP-2 inhibitory antibody is for administration in an amount
20 sufficient to reduce the risk of stroke and/or embolism in said subject.

12. The antibody for use of claim 1 or the use of Claim 2, wherein the subject has
a genetic defect that causes or increases the risk of developing, a hypercoagulable state,
wherein the genetic defect is selected from the group consisting of a Prothrombin 20210 gene
mutation, an MTHFR mutation, a deficiency of protein C, a deficiency of protein S, a
25 deficiency of protein A, a deficiency of protein Z, an antithrombin deficiency and a genetic disorder producing thrombophilia.

13. The antibody for use of Claim 1 or the use of claim 2, wherein the subject is
in need of anticoagulant therapy and the MASP-2 inhibitory antibody is used as a
replacement for standard anticoagulant therapy such as Warfarin.

14. The antibody for use or the use of Claim 13, wherein the subject has a condition that normally prohibits standard anticoagulant therapy, such as CNS amyloid angiopathy.

5 15. The antibody for use or the use of Claim 13, wherein the MASP-2 inhibitory antibody is for administration as a bridging agent perioperatively in a subject otherwise on standard anticoagulation therapy.

16. The antibody for use of Claim 1 or the use of Claim 2, wherein the MASP-2 antibody is a recombinant, chimeric, humanized or human antibody.

10 17. The antibody for use of Claim 1 or the use of Claim 2, wherein said MASP-2 inhibitory antibody is an antibody fragment selected from the group consisting of Fv, Fab, Fab', F(ab)₂ and F(ab')₂.

18. The antibody for use of Claim 1 or the use of Claim 2, wherein said MASP-2 inhibitory antibody is a single-chain molecule.

15 19. The antibody for use of Claim 1 or the use of Claim 2, wherein said MASP-2 inhibitory antibody is selected from the group consisting of an IgG1 molecule, an IgG2 and an IgG4 molecule.

20. The antibody for use of Claim 1 or the use of Claim 2, wherein the MASP-2 inhibitory monoclonal antibody, or antigen-binding fragment thereof, comprises:

20 (a) a heavy-chain variable region comprising: i) a heavy chain CDR-H1 comprising the amino acid sequence from 31-35 of SEQ ID NO:6; and ii) a heavy-chain CDR-H2 comprising the amino acid sequence from 50-65 of SEQ ID NO:6; and iii) a heavy-chain CDR-H3 comprising the amino acid sequence from 95-107 of SEQ ID NO:6 and

25 (b) a light-chain variable region comprising: i) a light-chain CDR-L1 comprising the amino acid sequence from 24-34 of SEQ ID NO:7; and ii) a light-chain CDR-L2 comprising the amino acid sequence from 50-56 of SEQ ID NO:7; and iii) a light-chain CDR-L3 comprising the amino acid sequence from 89-97 of SEQ ID NO:7.

21. The antibody for use of Claim 1 or the use of Claim 2, wherein the MASP-2 inhibitory monoclonal antibody comprises a heavy-chain variable region set forth as SEQ ID NO:6 and a light-chain variable region set forth as SEQ ID NO:7.

MA SP 2

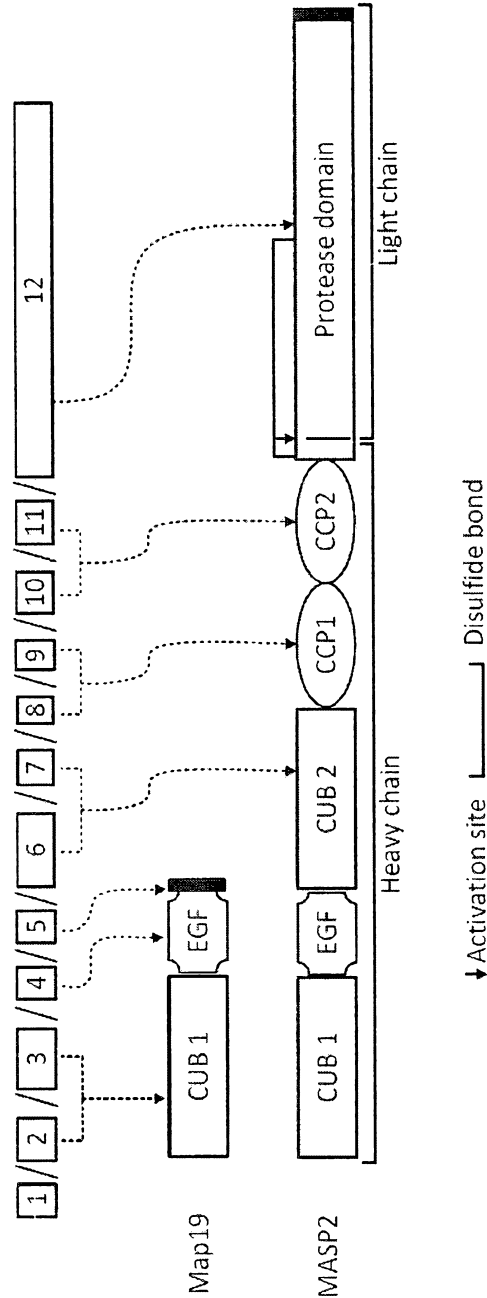


FIG. 1

2/12

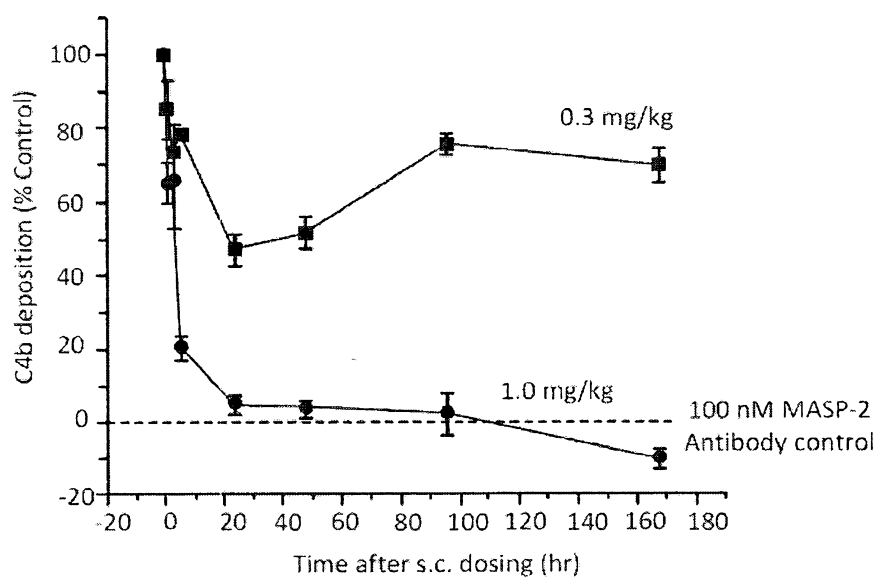


FIG. 2A

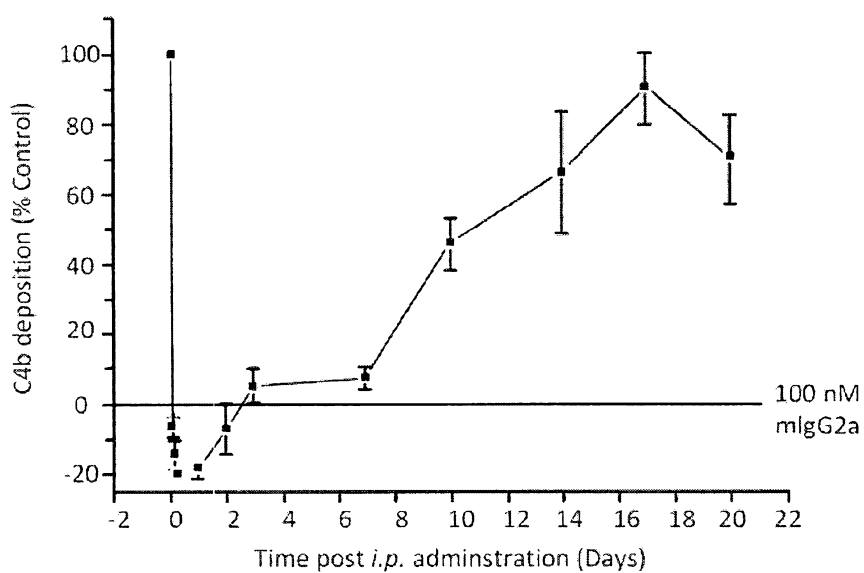


FIG. 2B

3/12

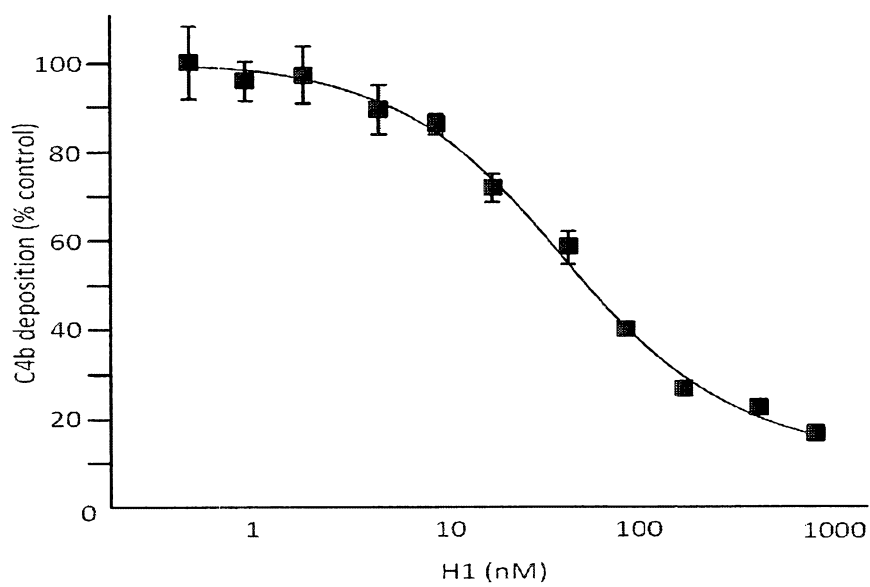


FIG. 3A

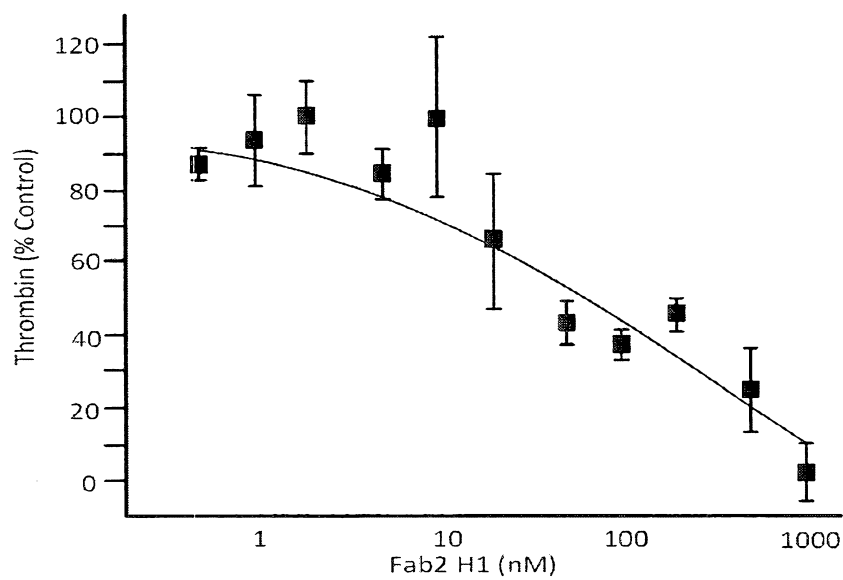


FIG. 3B

4/12

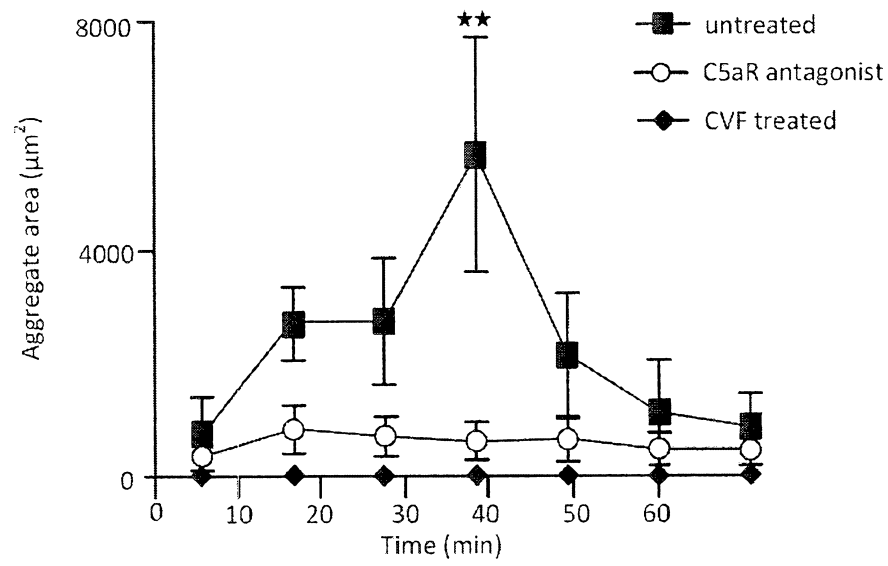


FIG. 4A

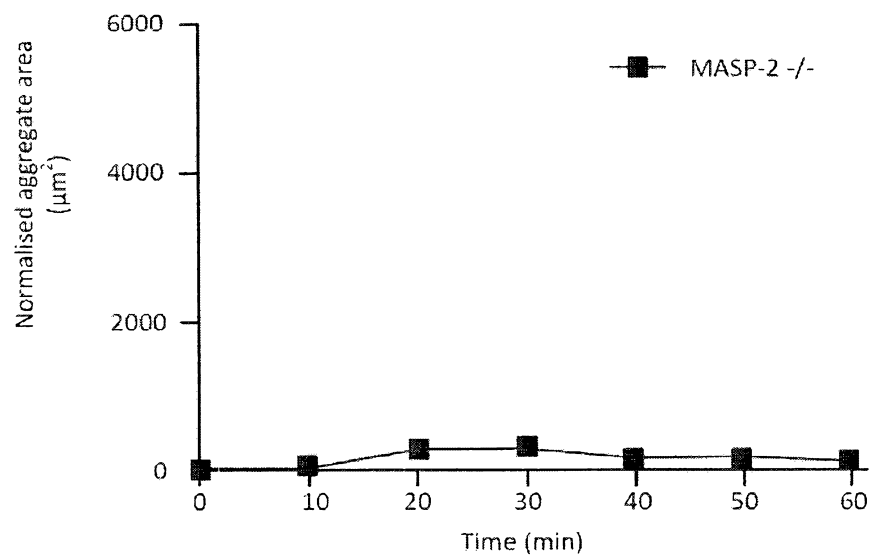


FIG. 4B

5/12

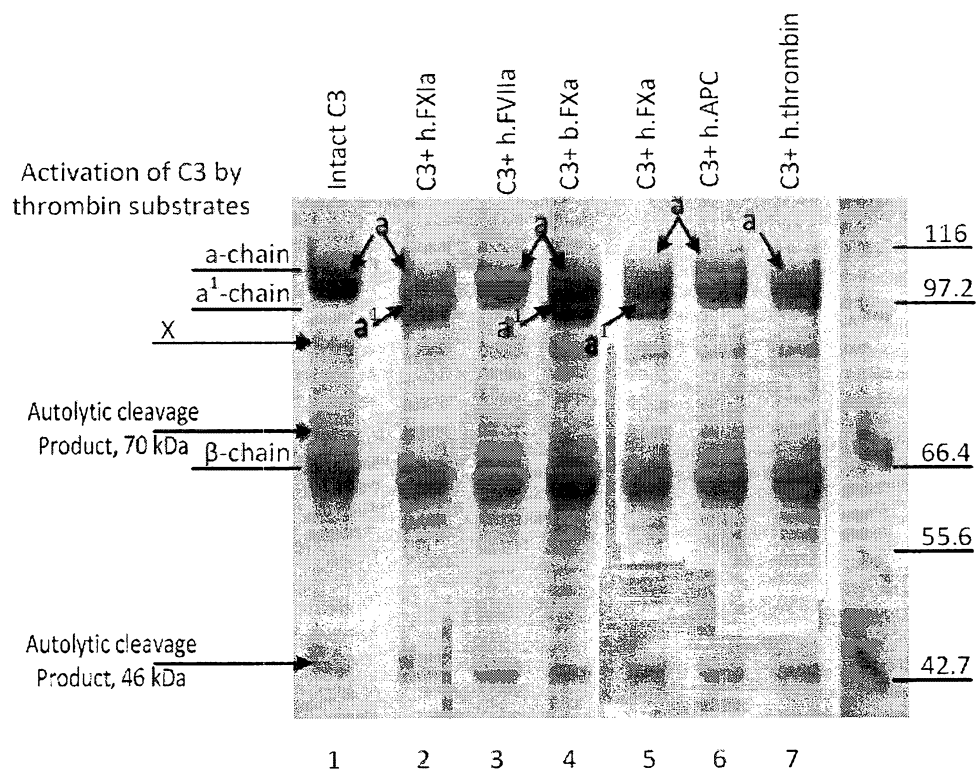


FIG. 5

6/12

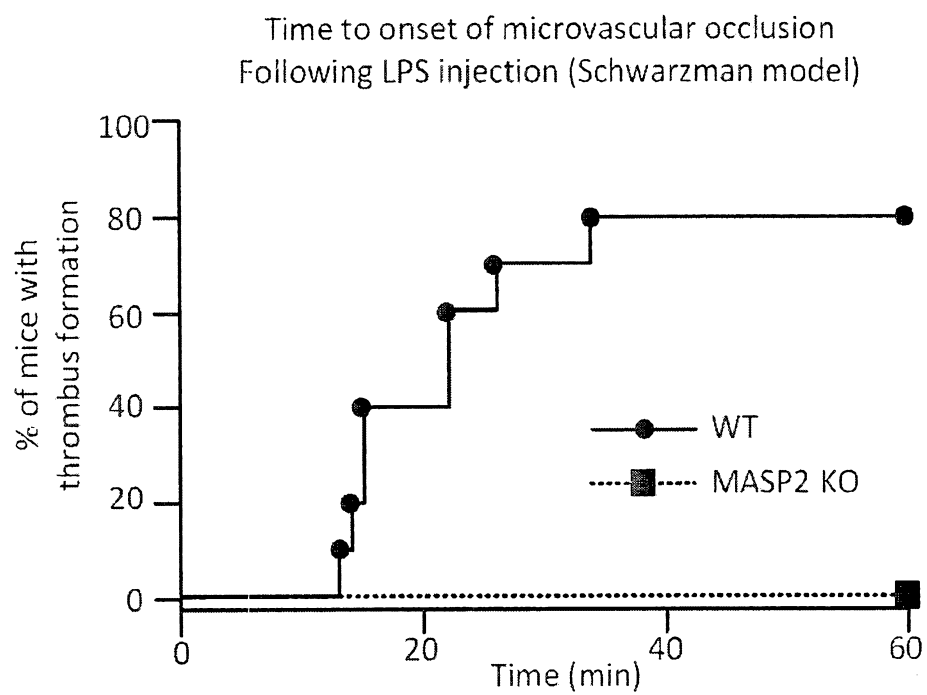


FIG. 6

7/12

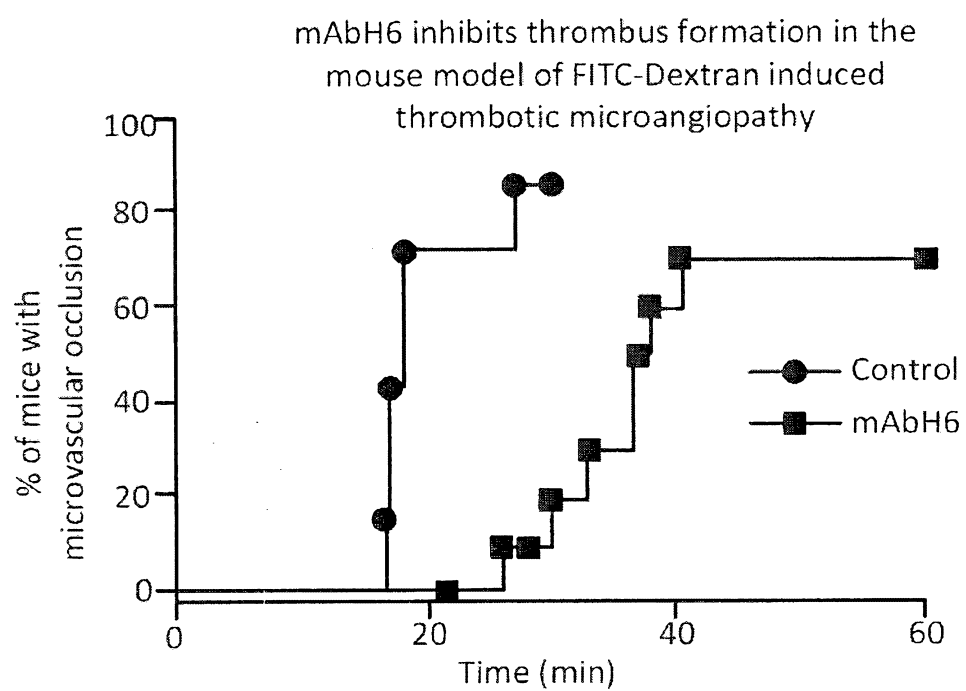


FIG. 7

8/12

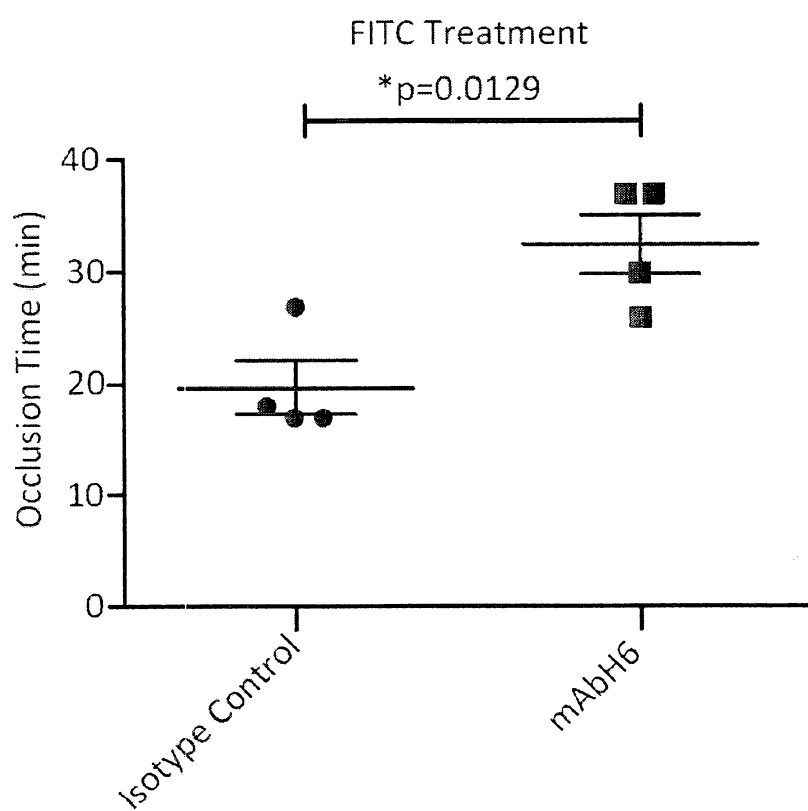


FIG. 8

9/12

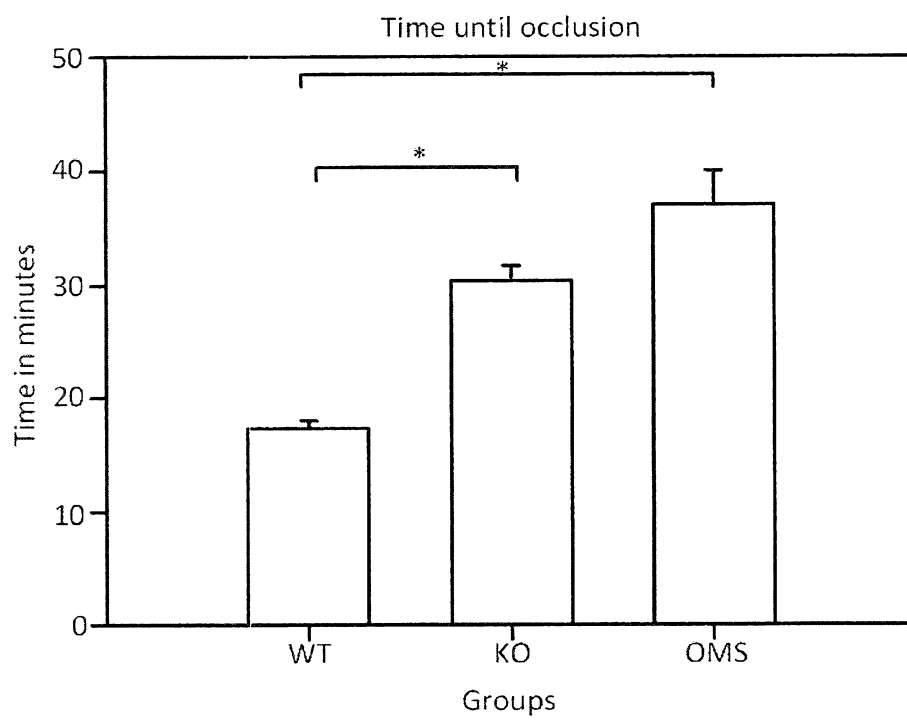


FIG. 9

10/12

**MASP-2 mAb inhibition of MASP-2-ATIII
in plasma stimulated by fibrin**

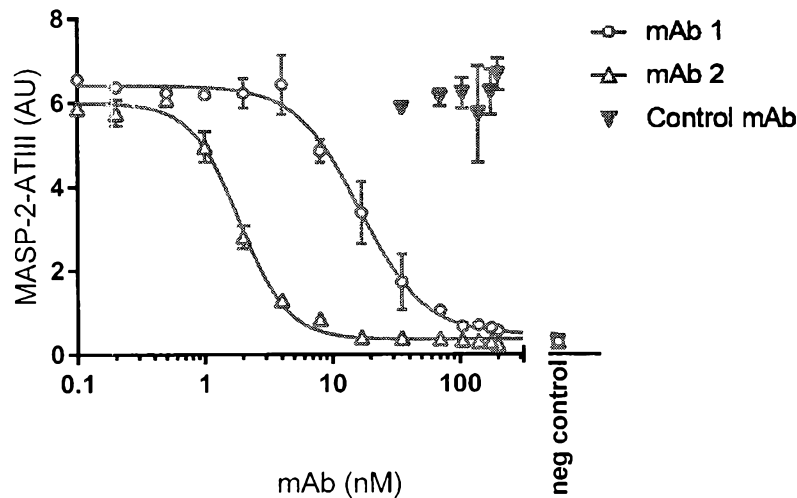


FIG. 10A

**MASP-2 mAb inhibition of MASP-2-C1-INH
in plasma stimulated by fibrin**

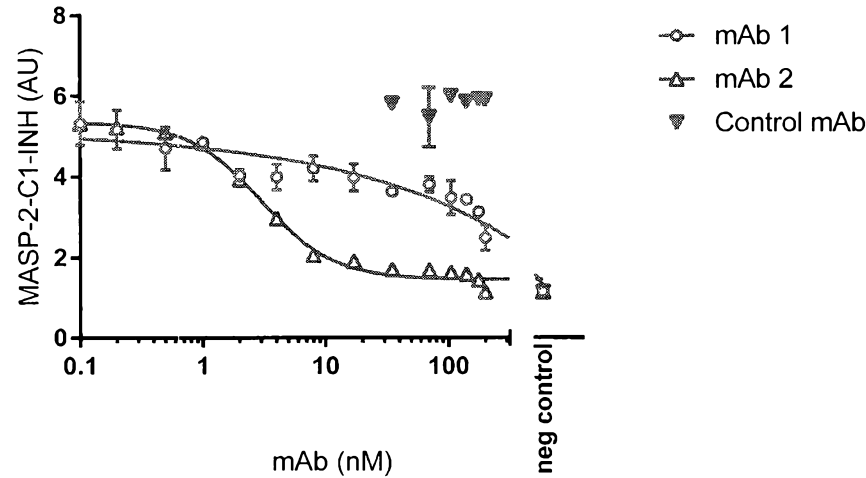


FIG. 10B

11/12

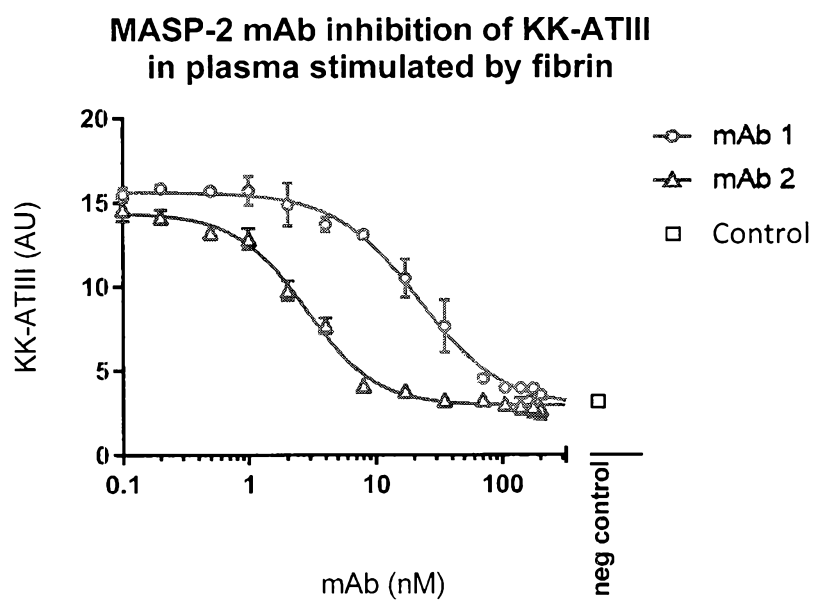


FIG. 11A

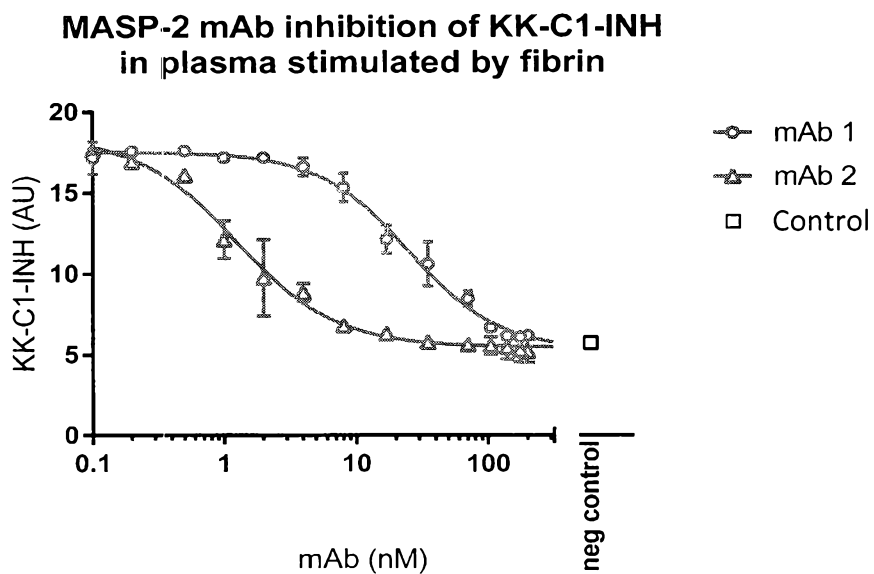


FIG. 11B

12/12

**MASP-2 mAb inhibition of FXIIa-ATIII
in plasma stimulated by fibrin**

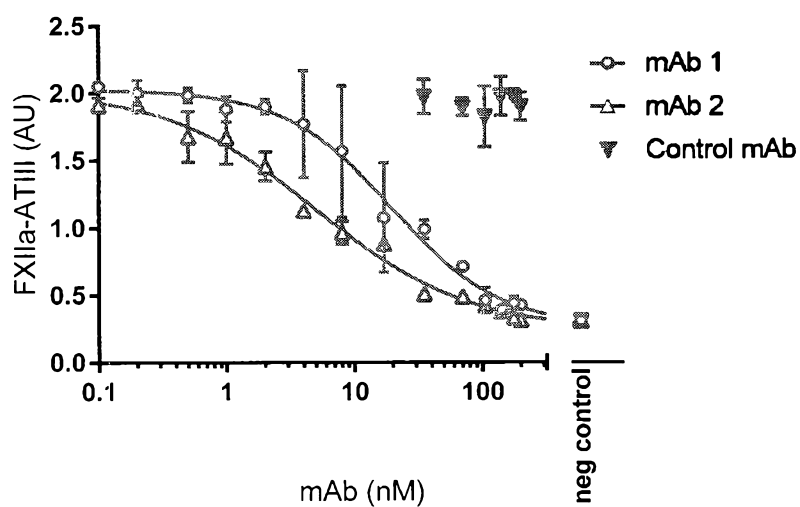


FIG. 12A

**MASP-2 mAb inhibition of FXIIa-C1-INH
in plasma stimulated by fibrin**

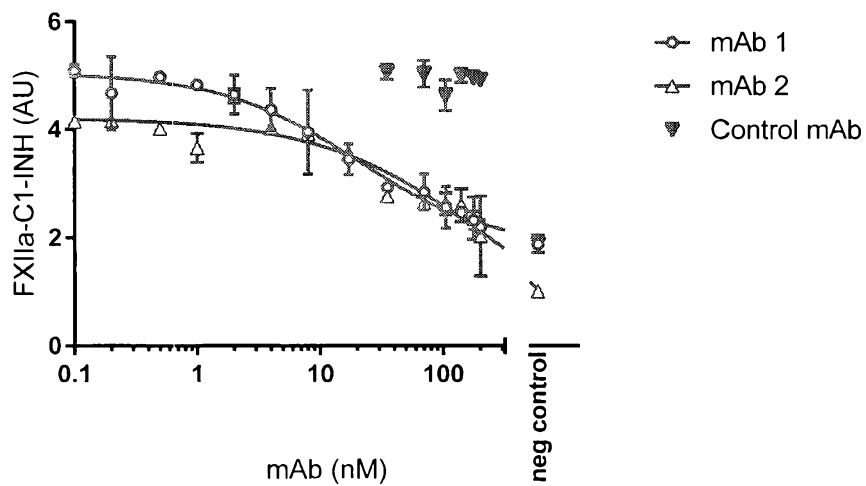


FIG. 12B

SEQUENCE LISTING

<110> Omeros Corporation
5 DEMOPULOS, Gregory A.
DUDLER, Thomas
NILSSON, Bo

<120> Compositions and Methods of Inhibiting MASP-2 for the Treatment
10 of Various Thrombotic Diseases and Disorders

<130> MP.1.0252.PCT

<150> 62/688,611
15 <151> 2018-06-22

<160> 7

<170> PatentIn version 3.5

20 <210> 1
<211> 2460
<212> DNA
<213> homo sapiens

25 <400> 1
ggccagctgg acgggcacac catgaggctg ctgacctcc tgggccttct gtgtggctcg
60

30 gtggccaccc ccttggggcc gaagtggcct gaacctgtgt tcgggcgcct ggcattcccc
120
ggctttccag gggagtatgc caatgaccag gagcggcgct ggacctgac tgcaccccc
180

35 ggctaccgcc tgcgcctcta cttacccac ttgcacctgg agctctccca cctctgcgag
240

40 tacgacttcg tcaagctgag ctcgggggcc aagggtgctgg ccacgctgtg cgggcaggag
300

45 agcacagaca cggagcgggc ccctggcaag gacaatttct actcgtggg ctccagcctg
360

gacattacct tccgctccga ctactccaac gagaagccgt tcacgggggtt cgaggccttc
420

50 tatgcagccg aggacattga cgagtgccag gtggccccgg gagaggcgcc cacctgcgac
480

caccactgcc acaaccacct gggcggtttc tactgtcctt gccgcgcagg ctacgtcctg
540

55 caccgtaaca agcgcacctg ctacagccctg tgctccggcc aggtctttcac ccagaggtct
600

ggggagctca gcagccctga ataccacgg ccgtatccca aactctccag ttgcacttac
660

agcatcagcc tggaggaggg gttcagtgtc attctggact ttgtggagtc cttcgatgtg
720

5 gagacacacc ctgaaaccct gtgtccctac gacttttctca agattcaaac agacagagaa
780

gaacatggcc cattctgtgg gaagacattg cccacacagga ttgaaacaaa aagcaacacg
840

10 gtgaccatca cctttgtcac agatgaatca ggagaccaca caggctggaa gatccactac
900

acgagcacag cgcagccttg cccttatccg atggcgccac ctaatggcca cgtttcacct
960

15 gtgcaagcca aatacatcct gaaagacagc ttctccatct tttgcgagac tggctatgag
1020

cttctgcaag gtcacttgcc cctgaaatcc ttactgcag tttgtcagaa agatggatct
1080

20 tgggaccggc caatgccgc gtgcagcatt gttgactgtg gccctcctga tgatctaccc
1140

25 agtggccgag tggagtacat cacaggctct ggagtgacca cctacaaagc tgtgattcag
1200

tacagctgtg aagagacctt ctacacaatg aaagtgaatg atggtaaata tgtgtgtgag
1260

30 gctgatggat tctggacgag ctccaaagga gaaaaatcac tcccagtctg tgagcctgtt
1320

tgtggactat cagcccgcac aacaggaggg cgtatatatg gagggcaaaa ggcaaacct
1380

35 ggtgattttc cttggcaagt cctgatatta ggtggaacca cagcagcagg tgcactttta
1440

40 tatgacaact gggtcctaac agctgctcat gccgtctatg agcaaaaaca tgatgcatcc
1500

gccctggaca ttogaatggg caccctgaaa agactatcac ctattatac acaagcctgg
1560

45 tctgaagctg tttttataca tgaaggttat actcatgatg ctggctttga caatgacata
1620

gcactgatta aattgaataa caaagttgta atcaatagca acatcacgcc tatttgtctg
1680

50 ccaagaaaag aagctgaatc ctttatgagg acagatgaca ttggaactgc atctggatgg
1740

55 ggattaaccc aaaggggttt tcttgctaga aatctaattg atgtcgacat accgattgtt
1800

gaccatcaaa aatgtactgc tgcatatgaa aagccaccct atccaagggg aagtgttaact
 1860

5 gctaacatgc tttgtgctgg cttagaaaagt gggggcaagg acagctgcag aggtgacagc
 1920

ggagggggcac tgggtgtttct agatagtga acagagaggt ggtttgtggg aggaatagtg
 1980

10 tcctgggggtt ccatgaattg tggggaagca ggtcagtatg gagtctacac aaaagttatt
 2040

aactatatc cctggatcga gaacataatt agtgattttt aacttgctg tctgcagtca
 2100

15 aggattcttc attttttagaa atgcctgtga agaccttggc agcgacgtgg ctcgagaagc
 2160

attcatcatt actgtggaca tggcagttgt tgctccaccc aaaaaaacag actccaggtg
 2220

20 aggetgctgt catttctcca cttgccagtt taattccagc cttaccatt gactcaaggg
 2280

25 gacataaacc acgagagtga cagtcattct tgcccaccca gtgtaatgtc actgctcaaa
 2340

ttacatttca ttaccttaaa aagccagtct cttttcatac tggctgttgg catttctgta
 2400

30 aactgcctgt ccatgctctt tgtttttaaa cttgttctta ttgaaaaaaaa aaaaaaaaaa
 2460

35 <210> 2
 <211> 686
 <212> PRT
 <213> homo sapiens

40 <400> 2

Met Arg Leu Leu Thr Leu Leu Gly Leu Leu Cys Gly Ser Val Ala Thr
 1 5 10 15

45 Pro Leu Gly Pro Lys Trp Pro Glu Pro Val Phe Gly Arg Leu Ala Ser
 20 25 30

50 Pro Gly Phe Pro Gly Glu Tyr Ala Asn Asp Gln Glu Arg Arg Trp Thr
 35 40 45

55 Leu Thr Ala Pro Pro Gly Tyr Arg Leu Arg Leu Tyr Phe Thr His Phe
 50 55 60

Asp Leu Glu Leu Ser His Leu Cys Glu Tyr Asp Phe Val Lys Leu Ser

65

70

75

80

5

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

10

Thr Glu Arg Ala Pro Gly Lys Asp Thr Phe Tyr Ser Leu Gly Ser Ser
100 105 110

15

Leu Asp Ile Thr Phe Arg Ser Asp Tyr Ser Asn Glu Lys Pro Phe Thr
115 120 125

Gly Phe Glu Ala Phe Tyr Ala Ala Glu Asp Ile Asp Glu Cys Gln Val
130 135 140

20

Ala Pro Gly Glu Ala Pro Thr Cys Asp His His Cys His Asn His Leu
145 150 155 160

25

Gly Gly Phe Tyr Cys Ser Cys Arg Ala Gly Tyr Val Leu His Arg Asn
165 170 175

30

Lys Arg Thr Cys Ser Ala Leu Cys Ser Gly Gln Val Phe Thr Gln Arg
180 185 190

Ser Gly Glu Leu Ser Ser Pro Glu Tyr Pro Arg Pro Tyr Pro Lys Leu
195 200 205

35

Ser Ser Cys Thr Tyr Ser Ile Ser Leu Glu Glu Gly Phe Ser Val Ile
210 215 220

40

Leu Asp Phe Val Glu Ser Phe Asp Val Glu Thr His Pro Glu Thr Leu
225 230 235 240

45

Cys Pro Tyr Asp Phe Leu Lys Ile Gln Thr Asp Arg Glu Glu His Gly
245 250 255

50

Pro Phe Cys Gly Lys Thr Leu Pro His Arg Ile Glu Thr Lys Ser Asn
260 265 270

Thr Val Thr Ile Thr Phe Val Thr Asp Glu Ser Gly Asp His Thr Gly
275 280 285

55

Trp Lys Ile His Tyr Thr Ser Thr Ala Gln Pro Cys Pro Tyr Pro Met
290 295 300

Ala Pro Pro Asn Gly His Val Ser Pro Val Gln Ala Lys Tyr Ile Leu
 305 310 315 320

5

Lys Asp Ser Phe Ser Ile Phe Cys Glu Thr Gly Tyr Glu Leu Leu Gln
 325 330 335

10

Gly His Leu Pro Leu Lys Ser Phe Thr Ala Val Cys Gln Lys Asp Gly
 340 345 350

15

Ser Trp Asp Arg Pro Met Pro Ala Cys Ser Ile Val Asp Cys Gly Pro
 355 360 365

20

Pro Asp Asp Leu Pro Ser Gly Arg Val Glu Tyr Ile Thr Gly Pro Gly
 370 375 380

25

Val Thr Thr Tyr Lys Ala Val Ile Gln Tyr Ser Cys Glu Glu Thr Phe
 385 390 395 400

30

Tyr Thr Met Lys Val Asn Asp Gly Lys Tyr Val Cys Glu Ala Asp Gly
 405 410 415

35

Phe Trp Thr Ser Ser Lys Gly Glu Lys Ser Leu Pro Val Cys Glu Pro
 420 425 430

40

Val Cys Gly Leu Ser Ala Arg Thr Thr Gly Gly Arg Ile Tyr Gly Gly
 435 440 445

45

Gln Lys Ala Lys Pro Gly Asp Phe Pro Trp Gln Val Leu Ile Leu Gly
 450 455 460

50

Gly Thr Thr Ala Ala Gly Ala Leu Leu Tyr Asp Asn Trp Val Leu Thr
 465 470 475 480

55

Ala Ala His Ala Val Tyr Glu Gln Lys His Asp Ala Ser Ala Leu Asp
 485 490 495

Ile Arg Met Gly Thr Leu Lys Arg Leu Ser Pro His Tyr Thr Gln Ala
 500 505 510

Trp Ser Glu Ala Val Phe Ile His Glu Gly Tyr Thr His Asp Ala Gly
 515 520 525

Phe Asp Asn Asp Ile Ala Leu Ile Lys Leu Asn Asn Lys Val Val Ile

530 535 540
 5 Asn Ser Asn Ile Thr Pro Ile Cys Leu Pro Arg Lys Glu Ala Glu Ser
 545 550 555 560

 10 Phe Met Arg Thr Asp Asp Ile Gly Thr Ala Ser Gly Trp Gly Leu Thr
 565 570 575

 15 Gln Arg Gly Phe Leu Ala Arg Asn Leu Met Tyr Val Asp Ile Pro Ile
 580 585 590

 20 Val Asp His Gln Lys Cys Thr Ala Ala Tyr Glu Lys Pro Pro Tyr Pro
 595 600 605

 25 Arg Gly Ser Val Thr Ala Asn Met Leu Cys Ala Gly Leu Glu Ser Gly
 610 615 620

 30 Gly Lys Asp Ser Cys Arg Gly Asp Ser Gly Gly Ala Leu Val Phe Leu
 625 630 635 640

 35 Asp Ser Glu Thr Glu Arg Trp Phe Val Gly Gly Ile Val Ser Trp Gly
 645 650 655

 40 Ser Met Asn Cys Gly Glu Ala Gly Gln Tyr Gly Val Tyr Thr Lys Val
 660 665 670

 45 Ile Asn Tyr Ile Pro Trp Ile Glu Asn Ile Ile Ser Asp Phe
 675 680 685

 50 <210> 3
 <211> 671
 <212> PRT
 <213> homo sapiens

 55 <400> 3

 Thr Pro Leu Gly Pro Lys Trp Pro Glu Pro Val Phe Gly Arg Leu Ala
 1 5 10 15

 Ser Pro Gly Phe Pro Gly Glu Tyr Ala Asn Asp Gln Glu Arg Arg Trp
 20 25 30

 Thr Leu Thr Ala Pro Pro Gly Tyr Arg Leu Arg Leu Tyr Phe Thr His
 35 40 45

Phe Asp Leu Glu Leu Ser His Leu Cys Glu Tyr Asp Phe Val Lys Leu
 50 55 60

5 Ser Ser Gly Ala Lys Val Leu Ala Thr Leu Cys Gly Gln Glu Ser Thr
 65 70 75 80

10 Asp Thr Glu Arg Ala Pro Gly Lys Asp Thr Phe Tyr Ser Leu Gly Ser
 85 90 95

15 Ser Leu Asp Ile Thr Phe Arg Ser Asp Tyr Ser Asn Glu Lys Pro Phe
 100 105 110

Thr Gly Phe Glu Ala Phe Tyr Ala Ala Glu Asp Ile Asp Glu Cys Gln
 115 120 125

20 Val Ala Pro Gly Glu Ala Pro Thr Cys Asp His His Cys His Asn His
 130 135 140

25 Leu Gly Gly Phe Tyr Cys Ser Cys Arg Ala Gly Tyr Val Leu His Arg
 145 150 155 160

30 Asn Lys Arg Thr Cys Ser Ala Leu Cys Ser Gly Gln Val Phe Thr Gln
 165 170 175

35 Arg Ser Gly Glu Leu Ser Ser Pro Glu Tyr Pro Arg Pro Tyr Pro Lys
 180 185 190

Leu Ser Ser Cys Thr Tyr Ser Ile Ser Leu Glu Glu Gly Phe Ser Val
 195 200 205

40 Ile Leu Asp Phe Val Glu Ser Phe Asp Val Glu Thr His Pro Glu Thr
 210 215 220

45 Leu Cys Pro Tyr Asp Phe Leu Lys Ile Gln Thr Asp Arg Glu Glu His
 225 230 235 240

50 Gly Pro Phe Cys Gly Lys Thr Leu Pro His Arg Ile Glu Thr Lys Ser
 245 250 255

Asn Thr Val Thr Ile Thr Phe Val Thr Asp Glu Ser Gly Asp His Thr
 260 265 270

55 Gly Trp Lys Ile His Tyr Thr Ser Thr Ala Gln Pro Cys Pro Tyr Pro
 275 280 285

Met Ala Pro Pro Asn Gly His Val Ser Pro Val Gln Ala Lys Tyr Ile
 290 295 300
 5

Leu Lys Asp Ser Phe Ser Ile Phe Cys Glu Thr Gly Tyr Glu Leu Leu
 305 310 315 320

10

Gln Gly His Leu Pro Leu Lys Ser Phe Thr Ala Val Cys Gln Lys Asp
 325 330 335

15

Gly Ser Trp Asp Arg Pro Met Pro Ala Cys Ser Ile Val Asp Cys Gly
 340 345 350

20

Pro Pro Asp Asp Leu Pro Ser Gly Arg Val Glu Tyr Ile Thr Gly Pro
 355 360 365

25

Gly Val Thr Thr Tyr Lys Ala Val Ile Gln Tyr Ser Cys Glu Glu Thr
 370 375 380

30

Phe Tyr Thr Met Lys Val Asn Asp Gly Lys Tyr Val Cys Glu Ala Asp
 385 390 395 400

Gly Phe Trp Thr Ser Ser Lys Gly Glu Lys Ser Leu Pro Val Cys Glu
 405 410 415

35

Pro Val Cys Gly Leu Ser Ala Arg Thr Thr Gly Gly Arg Ile Tyr Gly
 420 425 430

40

Gly Gln Lys Ala Lys Pro Gly Asp Phe Pro Trp Gln Val Leu Ile Leu
 435 440 445

45

Gly Gly Thr Thr Ala Ala Gly Ala Leu Leu Tyr Asp Asn Trp Val Leu
 450 455 460

Thr Ala Ala His Ala Val Tyr Glu Gln Lys His Asp Ala Ser Ala Leu
 465 470 475 480

50

Asp Ile Arg Met Gly Thr Leu Lys Arg Leu Ser Pro His Tyr Thr Gln
 485 490 495

55

Ala Trp Ser Glu Ala Val Phe Ile His Glu Gly Tyr Thr His Asp Ala
 500 505 510

Gly Phe Asp Asn Asp Ile Ala Leu Ile Lys Leu Asn Asn Lys Val Val
 515 520 525

5 Ile Asn Ser Asn Ile Thr Pro Ile Cys Leu Pro Arg Lys Glu Ala Glu
 530 535 540

10 Ser Phe Met Arg Thr Asp Asp Ile Gly Thr Ala Ser Gly Trp Gly Leu
 545 550 555 560

15 Thr Gln Arg Gly Phe Leu Ala Arg Asn Leu Met Tyr Val Asp Ile Pro
 565 570 575

Ile Val Asp His Gln Lys Cys Thr Ala Ala Tyr Glu Lys Pro Pro Tyr
 580 585 590

20 Pro Arg Gly Ser Val Thr Ala Asn Met Leu Cys Ala Gly Leu Glu Ser
 595 600 605

25 Gly Gly Lys Asp Ser Cys Arg Gly Asp Ser Gly Gly Ala Leu Val Phe
 610 615 620

30 Leu Asp Ser Glu Thr Glu Arg Trp Phe Val Gly Gly Ile Val Ser Trp
 625 630 635 640

35 Gly Ser Met Asn Cys Gly Glu Ala Gly Gln Tyr Gly Val Tyr Thr Lys
 645 650 655

Val Ile Asn Tyr Ile Pro Trp Ile Glu Asn Ile Ile Ser Asp Phe
 660 665 670

40 <210> 4
 <211> 2091
 <212> DNA
 <213> rattus

45 <400> 4
 tggcacacaa tgaggctact gatcgctctg ggtctgcttt ggagtttggt ggccacactt
 60

50 ttgggctcca agtggcctga gcctgtattc gggcgccctgg tgtccctggc cttcccagag
 120

aagtatggca accatcagga tcgatcctgg acgctgactg caccocctgg cttccgctg
 180

55 cgccctctact tcaccacatt caacctggaa ctctcttacc gctgcgagta tgactttgtc
 240

aagttgacct cagggaccaa ggtgctagcc acgctgtgtg ggcaggagag tacagatact
300

5 gagcgggcac ctggcaatga caccttctac tcaactgggtc ccagcctaaa ggtcaccttc
360

cactccgact actccaatga gaagccattc acaggatttg aggccttcta tgcagcggag
420

10 gatgtggatg aatgcagaac atccctggga gactcagtcc cttgtgacca ttattgccac
480

aactacctgg gcggctacta ctgctcctgc cgagtgggct acattctgca ccagaacaag
540

15 catacctgct cagccctttg ttcaggccag gtgttcaactg ggaggtctgg ctttctcagt
600

20 agccctgagt acccacagcc ataccccaaa ctctccagct gcgcctacaa catccgcctg
660

gaggaaggct tcagtatcac cctggacttc gtggagtcct ttgatgtgga gatgcacct
720

25 gaagcccagt gccctacga ctccctcaag attcaaacag acaagaggga atacggcccc
780

ttttgtggga agacgctgcc ccccaggatt gaaactgaca gcaacaaggt gaccattacc
840

30 ttaccaccg acgagtcagg gaaccacaca ggctggaaga tacactacac aagcacagca
900

cagccctgcc ctgatccaac ggcgccacct aatggtcaca tttcacctgt gcaagccacg
960

tatgtcctga aggacagctt ttctgtcttc tgcaagactg gcttcgagct tctgcaagg
1020

40 tctgtccccc tgaagtcatt cactgctgtc tgtcagaaag atggatcttg ggaccggccg
1080

ataccagagt gcagcattat tgactgtggc cctcccgatg acctaccaa tggccacgtg
1140

45 gactatatca caggccctga agtgaccacc tacaaagctg tgattcagta cagctgtgaa
1200

gagactttct acacaatgag cagcaatggt aaatatgtgt gtgaggctga tggattctgg
1260

50 acgagctcca aaggagaaaa atccctcccg gtttgcaagc ctgtctgtgg actgtccaca
1320

55 cacacttcag gaggccgtat aattggagga cagcctgcaa agcctggtga ctttccttgg
1380

caagtcttgt tactgggtga aactacagca gcaggtgctc ttatacatga cgactgggtc
 1440
 5 ctaacacggg ctcatgctgt atatgggaaa acagaggcga tgcctccct ggacatccgc
 1500
 atgggcatcc tcaaaaggct ctccctcatt tacactcaag cctggccaga ggctgtcttt
 1560
 10 atccatgaag gctacactca cggagctggt tttgacaatg atatagcact gattaaactc
 1620
 aagaacaaag tcacaatcaa cagaaacatc atgccgattt gtctaccaag aaaagaagct
 1680
 15 gcaccccttaa tgaaaacaga cttcgttga actgtggctg gctgggggtt aaccagaag
 1740
 20 gggtttcttg ctagaaacct aatgtttgtg gacataccaa ttgttgacca ccaaaaatgt
 1800
 gctactgcgt atacaaagca gccctaccca ggagcaaaag tgactgttaa catgctctgt
 1860
 25 gctggcctag accgcggtgg caaggacagc tgcagaggtg acagcggagg ggcattagt
 1920
 tttctagaca atgaaacaca gagatggttt gtgggaggaa tagtttcctg gggttctatt
 1980
 30 aactgtgggg ggtcagaaca gtatggggtc tacacgaaag tcacgaacta tattccctgg
 2040
 attgagaaca taataaataa tttctaattt gcaaaaaaaaa aaaaaaaaaa a
 35 2091
 <210> 5
 <211> 685
 40 <212> PRT
 <213> rattus
 <400> 5
 45 Met Arg Leu Leu Ile Val Leu Gly Leu Leu Trp Ser Leu Val Ala Thr
 1 5 10 15
 Leu Leu Gly Ser Lys Trp Pro Glu Pro Val Phe Gly Arg Leu Val Ser
 50 20 25 30
 Leu Ala Phe Pro Glu Lys Tyr Gly Asn His Gln Asp Arg Ser Trp Thr
 35 40 45
 55 Leu Thr Ala Pro Pro Gly Phe Arg Leu Arg Leu Tyr Phe Thr His Phe
 50 55 60

5	Asn 65	Leu	Glu	Leu	Ser	Tyr 70	Arg	Cys	Glu	Tyr	Asp 75	Phe	Val	Lys	Leu	Thr 80
10	Ser	Gly	Thr	Lys	Val 85	Leu	Ala	Thr	Leu	Cys 90	Gly	Gln	Glu	Ser	Thr 95	Asp
15	Thr	Glu	Arg	Ala 100	Pro	Gly	Asn	Asp	Thr 105	Phe	Tyr	Ser	Leu	Gly 110	Pro	Ser
20	Leu	Lys	Val 115	Thr	Phe	His	Ser	Asp 120	Tyr	Ser	Asn	Glu	Lys 125	Pro	Phe	Thr
25	Gly	Phe 130	Glu	Ala	Phe	Tyr	Ala 135	Ala	Glu	Asp	Val	Asp 140	Glu	Cys	Arg	Thr
30	Ser 145	Leu	Gly	Asp	Ser	Val 150	Pro	Cys	Asp	His	Tyr 155	Cys	His	Asn	Tyr	Leu 160
35	Gly	Gly	Tyr	Tyr	Cys 165	Ser	Cys	Arg	Val	Gly 170	Tyr	Ile	Leu	His 175	Gln	Asn
40	Lys	His	Thr	Cys 180	Ser	Ala	Leu	Cys	Ser 185	Gly	Gln	Val	Phe	Thr 190	Gly	Arg
45	Ser	Gly	Phe 195	Leu	Ser	Ser	Pro	Glu 200	Tyr	Pro	Gln	Pro	Tyr 205	Pro	Lys	Leu
50	Ser	Ser 210	Cys	Ala	Tyr	Asn	Ile 215	Arg	Leu	Glu	Glu	Gly 220	Phe	Ser	Ile	Thr
55	Leu 225	Asp	Phe	Val	Glu	Ser 230	Phe	Asp	Val	Glu	Met 235	His	Pro	Glu	Ala	Gln 240
60	Cys	Pro	Tyr	Asp	Ser 245	Leu	Lys	Ile	Gln	Thr 250	Asp	Lys	Arg	Glu	Tyr 255	Gly
65	Pro	Phe	Cys	Gly 260	Lys	Thr	Leu	Pro	Pro 265	Arg	Ile	Glu	Thr	Asp 270	Ser	Asn
70	Lys	Val	Thr 275	Ile	Thr	Phe	Thr	Thr 280	Asp	Glu	Ser	Gly	Asn 285	His	Thr	Gly

Trp Lys Ile His Tyr Thr Ser Thr Ala Gln Pro Cys Pro Asp Pro Thr
 290 295 300

5 Ala Pro Pro Asn Gly His Ile Ser Pro Val Gln Ala Thr Tyr Val Leu
 305 310 315 320

10 Lys Asp Ser Phe Ser Val Phe Cys Lys Thr Gly Phe Glu Leu Leu Gln
 325 330 335

15 Gly Ser Val Pro Leu Lys Ser Phe Thr Ala Val Cys Gln Lys Asp Gly
 340 345 350

Ser Trp Asp Arg Pro Ile Pro Glu Cys Ser Ile Ile Asp Cys Gly Pro
 355 360 365

20 Pro Asp Asp Leu Pro Asn Gly His Val Asp Tyr Ile Thr Gly Pro Glu
 370 375 380

25 Val Thr Thr Tyr Lys Ala Val Ile Gln Tyr Ser Cys Glu Glu Thr Phe
 385 390 395 400

30 Tyr Thr Met Ser Ser Asn Gly Lys Tyr Val Cys Glu Ala Asp Gly Phe
 405 410 415

35 Trp Thr Ser Ser Lys Gly Glu Lys Ser Leu Pro Val Cys Lys Pro Val
 420 425 430

Cys Gly Leu Ser Thr His Thr Ser Gly Gly Arg Ile Ile Gly Gly Gln
 435 440 445

40 Pro Ala Lys Pro Gly Asp Phe Pro Trp Gln Val Leu Leu Leu Gly Glu
 450 455 460

45 Thr Thr Ala Ala Gly Ala Leu Ile His Asp Asp Trp Val Leu Thr Ala
 465 470 475 480

50 Ala His Ala Val Tyr Gly Lys Thr Glu Ala Met Ser Ser Leu Asp Ile
 485 490 495

55 Arg Met Gly Ile Leu Lys Arg Leu Ser Leu Ile Tyr Thr Gln Ala Trp
 500 505 510

Pro Glu Ala Val Phe Ile His Glu Gly Tyr Thr His Gly Ala Gly Phe
 515 520 525

Asp Asn Asp Ile Ala Leu Ile Lys Leu Lys Asn Lys Val Thr Ile Asn
 530 535 540
 5

Arg Asn Ile Met Pro Ile Cys Leu Pro Arg Lys Glu Ala Ala Ser Leu
 545 550 555 560
 10

Met Lys Thr Asp Phe Val Gly Thr Val Ala Gly Trp Gly Leu Thr Gln
 565 570 575
 15

Lys Gly Phe Leu Ala Arg Asn Leu Met Phe Val Asp Ile Pro Ile Val
 580 585 590
 20

Asp His Gln Lys Cys Ala Thr Ala Tyr Thr Lys Gln Pro Tyr Pro Gly
 595 600 605
 25

Ala Lys Val Thr Val Asn Met Leu Cys Ala Gly Leu Asp Arg Gly Gly
 610 615 620
 30

Lys Asp Ser Cys Arg Gly Asp Ser Gly Gly Ala Leu Val Phe Leu Asp
 625 630 635 640
 35

Asn Glu Thr Gln Arg Trp Phe Val Gly Gly Ile Val Ser Trp Gly Ser
 645 650 655
 40

Ile Asn Cys Gly Gly Ser Glu Gln Tyr Gly Val Tyr Thr Lys Val Thr
 660 665 670
 45

Asn Tyr Ile Pro Trp Ile Glu Asn Ile Ile Asn Asn Phe
 675 680 685
 50

<210> 6
 <211> 118
 <212> PRT
 <213> artificial sequence
 <220>
 <223> Synthetic
 <400> 6
 55

Gln Val Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu
 1 5 10 15
 Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Arg Gly
 20 25 30

5	Lys	Met	Gly	Val	Ser	Trp	Ile	Arg	Gln	Pro	Pro	Gly	Lys	Ala	Leu	Glu
			35					40					45			
	Trp	Leu	Ala	His	Ile	Phe	Ser	Ser	Asp	Glu	Lys	Ser	Tyr	Arg	Thr	Ser
		50					55					60				
10	Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ser	Lys	Asp	Thr	Ser	Lys	Asn	Gln	Val
	65					70					75					80
15	Val	Leu	Thr	Met	Thr	Asn	Met	Asp	Pro	Val	Asp	Thr	Ala	Thr	Tyr	Tyr
					85					90					95	
20	Cys	Ala	Arg	Ile	Arg	Arg	Gly	Gly	Ile	Asp	Tyr	Trp	Gly	Gln	Gly	Thr
				100					105					110		
	Leu	Val	Thr	Val	Ser	Ser										
			115													
25	<210>	7														
	<211>	106														
	<212>	PRT														
30	<213>	artificial	sequence													
	<220>															
	<223>	Synthetic														
35	<400>	7														
	Gln	Pro	Val	Leu	Thr	Gln	Pro	Pro	Ser	Leu	Ser	Val	Ser	Pro	Gly	Gln
	1				5					10					15	
40	Thr	Ala	Ser	Ile	Thr	Cys	Ser	Gly	Glu	Lys	Leu	Gly	Asp	Lys	Tyr	Ala
				20					25					30		
45	Tyr	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Val	Leu	Val	Met	Tyr
			35					40					45			
50	Gln	Asp	Lys	Gln	Arg	Pro	Ser	Gly	Ile	Pro	Glu	Arg	Phe	Ser	Gly	Ser
		50					55					60				
	Asn	Ser	Gly	Asn	Thr	Ala	Thr	Leu	Thr	Ile	Ser	Gly	Thr	Gln	Ala	Met
	65					70					75					80
55	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ala	Trp	Asp	Ser	Ser	Thr	Ala	Val
						85				90					95	

16

Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

5

ABSTRACT

In one aspect, the invention provides compositions and methods for preventing, reducing, and/or treating a disease, disorder or condition associated with fibrin-induced
5 activation of the complement system and the associated activation of the coagulation and/or contact systems comprising administering a therapeutic amount of a MASP-2 inhibitory antibody to a subject in need thereof. In some embodiments, the methods of the invention provide anticoagulation and/or antifibrinolysis and/or antifibrinogenesis without affecting hemostasis. In one embodiment of this aspect of the invention, the
10 compositions and methods are useful for treating a subject is suffering from, or at risk of developing, a disease, disorder or condition associated with complement-related inflammation, excessive coagulation or contact system activation initiated by fibrin or activated platelets.

Figure accompanying abstract

**MASP-2 mAb inhibition of MASP-2-ATIII
in plasma stimulated by fibrin**

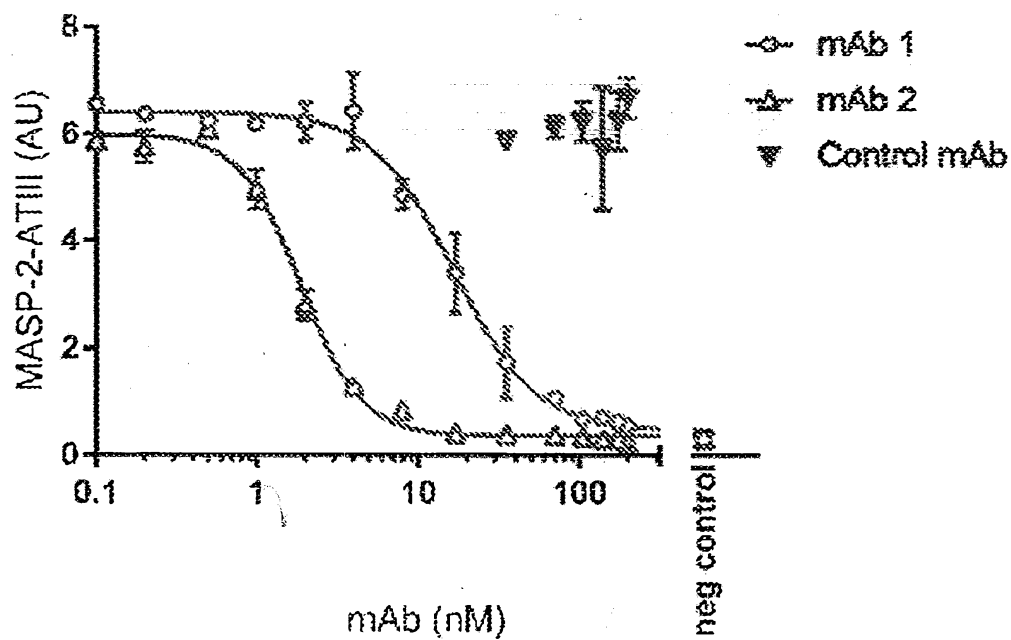


FIG. 10A