BIOMARKER FOR ASSESSING THE RISK OF DEVELOPING ACUTE COVID-19 AND POST-ACUTE COVID-19

STATEMENT REGARDING SEQUENCE LISTING

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_0319_PCT_Sequence_Listing_20220131_ST25.txt. The text file is 147 KB; was created on February 1, 2022; and is being submitted via EFS-Web with the filing of the specification.

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, 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, 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.

All three pathways (i.e., the classical, lectin and alternative) have been thought to converge at C5, which is cleaved to form products with multiple proinflammatory effects. The converged pathway has been referred to as the terminal complement pathway. C5a is the most potent anaphylatoxin, inducing alterations in smooth muscle and vascular tone, as well as vascular permeability. It is also a powerful chemotaxin and activator of both neutrophils and monocytes. C5a-mediated cellular activation can significantly amplify inflammatory responses by inducing the release of multiple additional inflammatory mediators, including cytokines, hydrolytic enzymes, arachidonic acid metabolites, and reactive oxygen species. C5 cleavage leads to the formation of C5b-9, also known as the membrane attack complex (MAC). There is now strong evidence that sublytic MAC deposition may play an important role in inflammation in addition to its role as a lytic pore-forming complex.

In addition to its essential role in immune defense, the complement system contributes to tissue damage in many clinical conditions. Although there is extensive evidence implicating both the classical and alternative complement pathways in the pathogenesis of non-infectious human diseases, the role of the lectin pathway is just beginning to be evaluated. Recent studies provide evidence that activation of the lectin

pathway can be responsible for complement activation and related inflammation in ischemia/reperfusion injury. Collard et al. (2000) reported that cultured endothelial cells subjected to oxidative stress bind MBL and show deposition of C3 upon exposure to human serum (Collard et al., *Am. J. Pathol. 156*:1549-1556, (2000)). In addition, treatment of human sera with blocking anti-MBL monoclonal antibodies inhibited MBL binding and complement activation. These findings were extended to a rat model of myocardial ischemia-reperfusion in which rats treated with a blocking antibody directed against rat MBL showed significantly less myocardial damage upon occlusion of a coronary artery than rats treated with a control antibody (Jordan et al., *Circulation 104*:1413-1418, (2001)). The molecular mechanism of MBL binding to the vascular endothelium after oxidative stress is unclear; a recent study suggests that activation of the lectin pathway after oxidative stress may be mediated by MBL binding to vascular endothelial cytokeratins, and not to glycoconjugates (Collard et al., *Am. J. Pathol. 159*:1045-1054, (2001)). Other studies have implicated the classical and alternative pathways in the pathogenesis of ischemia/reperfusion injury and the role of the lectin pathway in this disease remains controversial (Riedermann, N.C., et al., *Am. J. Pathol. 162*:363-367, 2003).

Fibrosis is the formation of excessive connective tissue in an organ or tissue, commonly in response to damage or injury. A hallmark of fibrosis is the production of excessive extracellular matrix following local trauma. The normal physiological response to injury results in the deposition of connective tissue, but this initially beneficial reparative process may persist and become pathological, altering the architecture and function of the tissue. At the cellular level, epithelial cells and fibroblasts proliferate and differentiate into myofibroblasts, resulting in matrix contraction, increased rigidity, microvascular compression, and hypoxia. An influx of inflammatory cells, including macrophages and lymphocytes, results in cytokine release and amplifies the deposition of collagen, fibronectin and other molecular markers of fibrosis. Conventional therapeutic approaches have largely been targeted towards the inflammatory process of fibrosis, using corticosteroids and immunosuppressive drugs. Unfortunately, these anti-inflammatory agents have had little to no clinical effect. Currently there are no effective treatments or therapeutics for fibrosis, but both animal studies and anecdotal human reports suggest that fibrotic tissue damage may be reversed (Tampe and Zeisberg, *Nat Rev Nephrol*, Vol 10:226-237, 2014).

The kidney has a limited capacity to recover from injury. Various renal pathologies result in local inflammation that causes scarring and fibrosis of renal tissue. The perpetuation of inflammatory stimuli drives tubulointerstitial inflammation and fibrosis and progressive renal functional impairment in chronic kidney disease. Its progression to end-stage renal failure is associated with significant morbidity and mortality. Since tubulointerstitial fibrosis is the common end point of multiple renal pathologies, it represents a key target for therapies aimed at preventing renal failure. Risk factors (e.g., proteinuria) independent of the primary renal disease contribute to the development of renal fibrosis and loss of renal excretory function by driving local inflammation, which in turn enhances disease progression.

In view of the role of fibrosis in many diseases and disorders, such as, for example, tubulointerstitial fibrosis leading to chronic kidney disease, there is a pressing need to develop therapeutically effective agents for treating diseases and conditions caused or exacerbated by fibrosis. In further view of the paucity of new and existing treatments targeting inflammatory pro-fibrotic pathways in renal disease, there is a need to develop therapeutically effective agents to treat, inhibit, prevent and/or reverse renal fibrosis and thereby prevent progressive chronic kidney disease.

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS coronavirus 2 or SARS-CoV-2), a virus that is closely related to the SARS virus (World Health Organization, 2/11/2020, Novel Coronavirus Situation Report 22). Those affected by COVID-19 may develop a fever, dry cough, fatigue and shortness of breath. Cases can progress to respiratory dysfunction, including pneumonia, severe acute respiratory syndrome, and death in the most vulnerable (see e.g., Hui D.S. et al., *Int J Infect Dis* 91:264-266, Jan 14, 2020). There is no vaccine or specific antiviral treatment, with management involving treatment of symptoms and supportive care.

Influenza (also known as "the flu") is an infectious disease caused by an RNA influenza virus. Symptoms of influenza virus infection can be mild to severe, and include high fever, runny nose, sore throat, muscle and joint pain, headache, coughing and feeling tired. These symptoms typically begin two days after exposure to the virus and most last less than a week, however, the cough may last for more than two weeks. (see "Influenza Seasonal, World Health Organization 6 November 2018). Complications of influenza may include viral pneumonia, acute respiratory distress syndrome (ARDS) secondary

bacterial pneumonia, sinus infections and worsening of previous health problems such as asthma or heart failure (see "Key Facts About Influenza (Flu)" Centers for Disease Control and Prevention (CDC), September 9, 2014). Influenza's effects are much more severe and last longer than those of the common cold. Most people will recover completely in about one to two weeks, but others will develop life-threatening complications such as pneumonia. Thus, influenza can be deadly, especially for the weak, young and old, those with compromised immune systems, or the chronically ill. See Hilleman MR, *Vaccine*. **20** (25–26): 3068–87 (2002).

Three of the four types of influenza viruses affect humans: Type A, Type B, and Type C. (*see "Types of Influenza Viruses Seasonal Influenza (Flu), Centers for Disease Control and Prevention (CDC). 27 September 2017).* Type D has not been known to infect humans, but is believed to have the potential to do so (see "Novel Influenza D virus: Epidemiology, pathology, evolution and biological characteristics," *Virulence. **8** (8): 1580–91, 2017).* The serotypes of influenza A that have been confirmed in humans are: H1N1 (caused the "Spanish Flu" in 1918 and "Swine Flu" in 2009); H2N2 (caused the "Asian Flu" in 1957), H3N2 (caused the "Hong Kong Flu" in 1968), H5N1 (caused the "Bird Flu in 2004), H7N7, H1N2, H9N2, H7N2, H7N3, H10N7, H7N9 and H6N1. See World Health Organization (30 June 2006). "Epidemiology of WHO-confirmed human cases of avian influenza A (H5N1) infection, *Wkly Epidemiol Rec*. **81** (26): 249–57.; *Fouchier RA, et al. (2004) PNAS **101** (5): 1356-61; Wkly Epidemiol Rec*. **83** (46): 415–20, Asian Lineage Avian Influenza A(H7N9) Virus, Centers for Disease Control and Prevention (CDC), 7 December 2018).

Common symptoms of the influenza virus (also known as the flu) such as fever, headaches and fatigue are the result of large amounts of proinflammatory cytokines and chemokines (such as interferon or tumor necrosis factor) produced from influenza-infected cells. See Eccles R. et al., Lancet Infect Dis 5(11):718-25 (2005); Schmitz N, et al., *Journal of Virology*. **79** (10): 6441–8 (2005). This massive immune response may result in a life-threatening cytokine storm. This effect has been proposed to be the cause of the unusual lethality of both the H5N1 avian influenza, and the 1918 pandemic strain. Cheung CY, et al., *Lancet*. **360** (9348): 1831–37 (2002); Kash JC, et al., *Nature*. **443** (7111): 578–81 (2006). Influenza also appears to trigger programmed cell death (apoptosis) see Spiro SG, et al., Clinical Respiratory Medicine, Elsevier Health Sciences. p. 311 (2012).

Thus, there is an urgent need to develop therapeutically effective agents to treat, inhibit and/or prevent coronavirus-induced pneumonia and acute respiratory distress syndrome and influenza virus induced pneumonia and acute respiratory distress syndrome.

In addition, to maximize success in treating and protecting people against COVID-19, there is an urgent need for biomarkers and highly accurate tests to identify those persons at risk of developing acute COVID-19 and/or long term disease (post-acute COVID-19, otherwise known as Long-COVID-19 syndrome), or has developed a protective immune response versus a COVID-19 disease response. There is also a need for tests to determine the efficacy of therapeutics to treat and/or prevent COVID-19-related complications in subjects infected with SARS-CoV-2, including those suffering from, or at risk of developing Long-COVID-19.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

In one aspect, the present invention provides a method for treating, inhibiting, alleviating, or preventing acute respiratory distress syndrome, pneumonia or some other pulmonary or other acute manifestation of COVID-19, such as thrombosis, in a mammalian subject infected with SARS-CoV-2, comprising (i) determining the level of MASP-2/C1-INH complex in a biological sample obtained from the subject, wherein an increased level of MASP-2/C1-INH complex as compared to a healthy control sample or other reference standard is indicative of an increased risk of developing one or more acute manifestations of COVID-19; and (ii) administering to the subject having an increased level of MASP-2/C1-INH complex an amount of a MASP-2 inhibitory agent effective to inhibit MASP-2-dependent complement activation. In some embodiments, the subject is suffering from one or more respiratory symptoms and the method comprises administering to the subject an amount of a MASP-2 inhibitory agent effective to improve at least one respiratory symptom (i.e., improve respiratory function). In one embodiment, the MASP-2 inhibitory agent is a MASP-2 antibody or antigen-binding

fragment thereof. In one embodiment, the MASP-2 inhibitory agent is a MASP-2 monoclonal antibody, or fragment thereof that specifically binds to a portion of SEQ ID NO:6. In one embodiment, the MASP-2 inhibitory agent selectively inhibits lectin pathway complement activation without substantially inhibiting C1q-dependent complement activation. In one embodiment, the MASP-2 inhibitory agent is a small molecule, such as a synthetic or semi-synthetic small molecule that inhibits MASP-2-dependent complement activation. In one embodiment, the MASP-2 inhibitory agent is an expression inhibitor of MASP-2. In one embodiment, the MASP-2 inhibitory antibody is a monoclonal antibody, or fragment thereof that specifically binds to human MASP-2. In one embodiment, the MASP-2 inhibitory antibody or fragment thereof is selected from the group consisting of a recombinant antibody, an antibody having reduced effector function, a chimeric antibody, a humanized antibody, and a human antibody. In one embodiment, the MASP-2 inhibitory antibody does not substantially inhibit the classical pathway. In one embodiment, the MASP-2 inhibitory antibody inhibits C3b deposition in 90% human serum with an IC_{50} of 30 nM or less. In one embodiment, the MASP-2 inhibitory antibody or antigen-binding fragment thereof, comprises a heavy chain variable region comprising CDR-H1, CDR-H2 and CDR-H3 of the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising CDR-L1, CDR-L2 and CDR-L3 of the amino acid sequence set forth as SEQ ID NO:69. In one embodiment, the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising the amino acid sequence set forth as SEQ ID NO:69.

In another aspect, the present invention provides a method for treating, ameliorating, preventing or reducing the risk of developing one or more COVID-19-related long-term sequelae in a mammalian subject that has been infected with SARS-CoV-2, comprising (i) determining the level of MASP-2/C1-INH complex in a biological sample obtained from the subject, wherein an increased level of MASP-2/C1-INH complex as compared to a healthy control sample is indicative of an increased risk of developing one or more COVID-19-related long term sequelae; and (ii) administering to the subject having an increased level of MASP-2/C1-INH complex an amount of a MASP-2 inhibitory agent effective to inhibit MASP-2-dependent complement activation.

In one embodiment, the MASP-2 inhibitory agent is a MASP-2 antibody or antigen-binding fragment thereof. In one embodiment, the MASP-2 inhibitory agent is a MASP-2 monoclonal antibody, or fragment thereof that specifically binds to a portion of SEQ ID NO:6. In one embodiment, the MASP-2 inhibitory agent selectively inhibits lectin pathway complement activation without substantially inhibiting C1q-dependent complement activation. In one embodiment, the MASP-2 inhibitory agent is a small molecule, such as a synthetic or semi-synthetic small molecule that inhibits MASP-2-dependent complement activation. In one embodiment, the MASP-2 inhibitory agent is an expression inhibitor of MASP-2. In one embodiment, the MASP-2 inhibitory antibody is a monoclonal antibody, or fragment thereof that specifically binds to human MASP-2. In one embodiment, the MASP-2 inhibitory antibody or fragment thereof is selected from the group consisting of a recombinant antibody, an antibody having reduced effector function, a chimeric antibody, a humanized antibody, and a human antibody. In one embodiment, the MASP-2 inhibitory antibody does not substantially inhibit the classical pathway. In one embodiment, the MASP-2 inhibitory antibody inhibits C3b deposition in 90% human serum with an IC_{50} of 30 nM or less. In one embodiment, the MASP-2 inhibitory antibody or antigen-binding fragment thereof, comprises a heavy chain variable region comprising CDR-H1, CDR-H2 and CDR-H3 of the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising CDR-L1, CDR-L2 and CDR-L3 of the amino acid sequence set forth as SEQ ID NO:69. In one embodiment, the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising the amino acid sequence set forth as SEQ ID NO:69.

In another aspect, the present disclosure provides a monoclonal antibody, or antigen binding fragment thereof, that specifically binds to human MASP-2 in complex with C1-INH, wherein the antibody comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:88, wherein the CDRs are numbered according to the Kabat numbering system. In one embodiment, the MASP-2 specific antibody comprises a heavy chain variable region having at least 95% identify with the amino acid sequence set

forth as SEQ ID NO:87 and a light chain variable region having at least 95% identify with the amino acid sequence set forth as SEQ ID NO:88. In one embodiment, the MASP-2 specific antibody or antigen-binding fragment thereof is labeled with a detectable moiety, for example a detectable moiety suitable for use in an immunoassay as further described herein. In one embodiment, the MASP-2 specific antibody or fragment thereof is immobilized on a substrate, such as a substrate suitable for use in an immunoassay, such as an immunoassay for detecting MASP-2/C1-INH complex.

In another aspect, the present disclosure provides a monoclonal antibody, or antigen binding fragment thereof, that specifically binds to human MASP-2 in complex with C1-INH, wherein the antibody comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system. In one embodiment, the MASP-2 specific antibody comprises a heavy chain variable region having at least 95% identify with the amino acid sequence set forth as SEQ ID NO:97 and a light chain variable region having at least 95% identify with the amino acid sequence set forth as SEQ ID NO:98. In one embodiment, the MASP-2 specific antibody or antigen-binding fragment thereof is labeled with a detectable moiety, for example a detectable moiety suitable for use in an immunoassay as further described herein. In one embodiment, the MASP-2 specific antibody or fragment thereof is immobilized on a substrate, such as a substrate suitable for use in an immunoassay, such as an immunoassay for detecting MASP-2/C1-INH complex.

In another aspect, the present disclosure provides a method of measuring the amount of MASP-2/C1-INH in a biological sample comprising: (a) providing a test biological sample from a human subject; (b) performing an immunoassay comprising capturing and detecting MASP-2/C1-INH complex in the test sample, wherein MASP-2/C1-INH complex is captured with a monoclonal antibody that specifically binds to human MASP-2; and the MASP-2/C1-INH complex is detected directly or indirectly with an antibody that specifically binds to C1-INH; and (c) comparing the level of MASP-2/C1-INH complex detected in accordance with (b) with a predetermined level or control sample wherein the level of MASP-2/C1-INH complex detected in the test sample is indicative of the extent of Lectin Pathway Complement activation. In some

embodiments, the biological sample is a fluid sample from a human subject selected from the group consisting of whole blood, serum, plasma, urine and cerebrospinal fluid. In some embodiments, the human subject is currently infected with SARS-CoV-2, or has previously been infected with SARS-CoV-2. In some embodiments, the antibody that specifically binds to MASP-2 comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:88, wherein the CDRs are numbered according to the Kabat numbering system. In some embodiments, the antibody that specifically binds to MASP-2 comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

In another aspect, the present disclosure provides a method of determining the risk of a subject that is or has been infected with SARS-CoV-2 for developing COVID-19-related ARDS or long-term sequelae associated with COVID-19 comprising: (a) obtaining a biological sample from the subject; (b) measuring the level of MASP-2/C1-INH complex in the sample; (c) comparing the measured level with a predetermined level of MASP-2/C1-INH complex or a reference standard to assess the risk of developing COVID-19-related ARDS and/or long-term sequelae associated with COVID-19; and (d) determining the risk of the subject for developing COVID-19-related ARDS and/or long-term sequelae associated with COVID-19 and reporting the results to the patient, physician or database; (e) optionally, administering a treatment to the subject determined to be likely to develop acute disease and/or long-term sequelae associated with COVID-19 infection. In some embodiments, the level of MASP-2/C1-INH complex is measured in an immunoassay. In some embodiments, step (b) comprises performing an immunoassay such as an ELISA assay to measure the level of MASP-2/C1-INH complex in the biological sample. In some embodiments, the immunoassay comprises the use of an antibody that specifically binds to MASP-2 comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:88, wherein the CDRs are numbered according to the Kabat numbering system. In some embodiments, the immunoassay comprises the use of

an antibody that specifically binds to MASP-2 comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

In another aspect, the present disclosure provides a method for monitoring the efficacy of treatment with a MASP-2 inhibitory antibody, or antigen-binding fragment thereof, in a mammalian subject in need thereof, the method comprising:(a) administering a dose of a MASP-2 inhibitory antibody, or antigen-binding fragment thereof, to a mammalian subject at a first point in time;(b) assessing a first level of MASP-2/C1-INH complex in a biological sample obtained from the subject after step (a);(c) treating the subject with the MASP-2 inhibitory antibody, or antigen-binding fragment thereof, at a second point in time;(d) assessing a second level of MASP-2/C1-INH complex in a biological sample obtained from the subject after step (c); and (e) comparing the level of MASP-2/C1-INH complex assessed in step (b) with the level of MASP-2/C1-INH complex assessed in step (d) to determine the efficacy of the MASP-2 inhibitory antibody or antigen-binding fragment thereof in the mammalian subject. In some embodiments, the subject is a human subject suffering from, or at risk of developing COVID-19 or long-term sequelae associated with COVID-19. In some embodiments, the subject is a human subject suffering from, or at risk of developing a disease or disorder selected from the group consisting of HSCT-TMA, IgAN, Lupus Nephritis and Graft-versus-Host Disease or some other lectin pathway disease or disorder. In some embodiments, the level of MASP-2/C1-INH complex is measured in an immunoassay. In some embodiments, step (b) comprises performing an immunoassay such as an ELISA assay to measure the level of MASP-2/C1-INH complex in the biological sample. In some embodiments, the immunoassay comprises the use of an antibody that specifically binds to MASP-2 comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:88, wherein the CDRs are numbered according to the Kabat numbering system. In some embodiments, the immunoassay comprises the use of an antibody that specifically binds to MASP-2 comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2

and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

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:

FIGURE 1 is a diagram illustrating the genomic structure of human MASP-2;

FIGURE 2A is a schematic diagram illustrating the domain structure of human MASP-2 protein;

FIGURE 2B is a schematic diagram illustrating the domain structure of human MAp19 protein;

FIGURE 3 is a diagram illustrating the murine MASP-2 knockout strategy;

FIGURE 4 is a diagram illustrating the human MASP-2 minigene construct;

FIGURE 5A presents results demonstrating that MASP-2-deficiency leads to the loss of lectin-pathway-mediated C4 activation as measured by lack of C4b deposition on mannan, as described in Example 2;

FIGURE 5B presents results demonstrating that MASP-2-deficiency leads to the loss of lectin-pathway-mediated C4 activation as measured by lack of C4b deposition on zymosan, as described in Example 2;

FIGURE 5C presents results demonstrating the relative C4 activation levels of serum samples obtained from MASP-2+/-; MASP-2-/- and wild-type strains as measure by C4b deposition on mannan and on zymosan, as described in Example 2;

FIGURE 6 presents results demonstrating that the addition of murine recombinant MASP-2 to MASP-2-/- serum samples recovers lectin-pathway-mediated C4 activation in a protein concentration dependent manner, as measured by C4b deposition on mannan, as described in Example 2;

FIGURE 7 presents results demonstrating that the classical pathway is functional in the MASP-2-/- strain, as described in Example 8;

FIGURE 8A presents results demonstrating that anti-MASP-2 Fab2 antibody #11 inhibits C3 convertase formation, as described in Example 10;

FIGURE 8B presents results demonstrating that anti-MASP-2 Fab2 antibody #11 binds to native rat MASP-2, as described in Example 10;

FIGURE 8C presents results demonstrating that anti-MASP-2 Fab2 antibody #41 inhibits C4 cleavage, as described in Example 10;

FIGURE 9 presents results demonstrating that all of the anti-MASP-2 Fab2 antibodies tested that inhibited C3 convertase formation also were found to inhibit C4 cleavage, as described in Example 10;

FIGURE 10 is a diagram illustrating the recombinant polypeptides derived from rat MASP-2 that were used for epitope mapping of the MASP-2 blocking Fab2 antibodies, as described in Example 11;

FIGURE 11 presents results demonstrating the binding of anti-MASP-2 Fab2 #40 and #60 to rat MASP-2 polypeptides, as described in Example 11;

FIGURE 12A graphically illustrates the level of MAC deposition in the presence or absence of human MASP-2 monoclonal antibody (OMS646) under lectin pathway-specific assay conditions, demonstrating that OMS646 inhibits lectin-mediated MAC deposition with an IC_{50} value of approximately 1 nM, as described in Example 12;

FIGURE 12B graphically illustrates the level of MAC deposition in the presence or absence of human MASP-2 monoclonal antibody (OMS646) under classical pathway-specific assay conditions, demonstrating that OMS646 does not inhibit classical pathway-mediated MAC deposition, as described in Example 12;

FIGURE 12C graphically illustrates the level of MAC deposition in the presence or absence of human MASP-2 monoclonal antibody (OMS646) under alternative pathway-specific assay conditions, demonstrating that OMS646 does not inhibit alternative pathway-mediated MAC deposition, as described in Example 12;

FIGURE 13 graphically illustrates the pharmacokinetic (PK) profile of human MASP-2 monoclonal antibody (OMS646) in mice, showing the OMS646 concentration (mean of n=3 animals/groups) as a function of time after administration at the indicated dose, as described in Example 12;

FIGURE 14A graphically illustrates the pharmacodynamic (PD) response of human MASP-2 monoclonal antibody (OMS646), measured as a drop in systemic lectin pathway activity, in mice following intravenous administration, as described in Example 12;

FIGURE 14B graphically illustrates the pharmacodynamic (PD) response of human MASP-2 monoclonal antibody (OMS646), measured as a drop in systemic lectin pathway activity, in mice following subcutaneous administration, as described in Example 12;

FIGURE 15 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with Sirius red, wherein the tissue sections were obtained from wild-type and MASP-2-/- mice following 7 days of unilateral ureteric obstruction (UUO) and sham-operated wild-type and MASP-2-/- mice, as described in Example 14;

FIGURE 16 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with the F4/80 macrophage-specific antibody, wherein the tissue sections were obtained from wild-type and MASP-2-/- mice following 7 days of unilateral ureteric obstruction (UUO) and sham-operated wild-type and MASP-2-/- mice, as described in Example 14.

FIGURE 17 graphically illustrates the relative mRNA expression levels of collagen-4, as measured by quantitative PCR (qPCR), in kidney tissue sections obtained from wild-type and MASP-2-/- mice following 7 days of unilateral ureteric obstruction (UUO) and sham-operated wild-type and MASP-2-/- mice, as described in Example 14.

FIGURE 18 graphically illustrates the relative mRNA expression levels of Transforming Growth Factor Beta-1 (TGFβ-1), as measured by qPCR, in kidney tissue sections obtained from wild-type and MASP-2-/- mice following 7 days of unilateral ureteric obstruction (UUO) and sham-operated wild-type and MASP-2-/- mice, as described in Example 14.

FIGURE 19 graphically illustrates the relative mRNA expression levels of Interleukin-6 (IL-6), as measured by qPCR, in kidney tissue sections obtained from wild-type and MASP-2-/- mice following 7 days of unilateral ureteric obstruction (UUO) and sham-operated wild-type and MASP-2-/- mice, as described in Example 14.

FIGURE 20 graphically illustrates the relative mRNA expression levels of Interferon-γ, as measured by qPCR, in kidney tissue sections obtained from wild-type and MASP-2-/- mice following 7 days of unilateral ureteric obstruction (UUO) and sham-operated wild-type and MASP-2-/- mice, as described in Example 14.

FIGURE 21 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with Siruis red, wherein the tissue sections were obtained following 7 days of unilateral ureteric obstruction (UUO) from wild-type mice treated

with a MASP-2 inhibitory antibody and an isotype control antibody, as described in Example 15.

FIGURE 22 graphically illustrates the hydroxyl proline content from kidneys harvested 7 days after unilateral ureteric obstruction (UUO) obtained from wild-type mice treated with MASP-2 inhibitory antibody as compared with the level of hydroxyl proline in tissue from obstructed kidneys obtained from wild-type mice treated with an IgG4 isotype control, as described in Example 15.

FIGURE 23 graphically illustrates the total amount of serum proteins (mg/ml) measured on day 15 of the protein overload study in wild-type control mice (n=2) that received saline only, wild-type mice that received BSA (n=6) and MASP-2-/- mice that received BSA (n=6), as described in Example 16.

FIGURE 24 graphically illustrates the total amount of excreted protein (mg) in urine collected over a 24 hour period on day 15 of the protein overload study from wild-type control mice (n=2) that received saline only, wild-type that received BSA (n=6) and MASP-2-/- mice that received BSA (n=6), as described in Example 16.

FIGURE 25 shows representative hematoxylin and eosin (H&E) stained renal tissue sections from the following groups of mice on day 15 of the protein overload study as follows: (panel A) wild-type control mice; (panel B) MASP-2-/- control mice, (panel C) wild-type mice treated with BSA; and (panel D) MASP-2-/- mice treated with bovine serum albumin (BSA), as described in Example 16.

FIGURE 26 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with macrophage-specific antibody F4/80, showing the macrophage mean stained area (%), wherein the tissue sections were obtained on day 15 of the protein overload study from wild-type control mice (n=2), wild-type mice treated with BSA (n=6), and MASP-2-/- mice treated with BSA (n=5), as described in Example 16.

FIGURE 27A graphically illustrates the analysis for the presence of a macrophage-proteinuria correlation in each wild-type mouse (n=6) treated with BSA by plotting the total excreted proteins measured in urine from a 24-hour sample versus the macrophage infiltration (mean stained area %), as described in Example 16.

FIGURE 27B graphically illustrates the analysis for the presence of a macrophage-proteinuria correlation in each MASP-2-/- mouse (n=5) treated with BSA by

plotting the total excreted proteins in urine in a 24-hour sample versus the macrophage infiltration (mean stained area %), as described in Example 16.

FIGURE 28 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-TGFβ antibody (measured as % TGFβ antibody-stained area) in wild-type mice treated with BSA (n=4) and MASP-2-/- mice treated with BSA (n=5), as described in Example 16.

FIGURE 29 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-TNFα antibody (measured as % TNFα antibody-stained area) in wild-type mice treated with BSA (n=4) and MASP-2-/- mice treated with BSA (n=5), as described in Example 16.

FIGURE 30 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-IL-6 antibody (measured as % IL-6 antibody-stained area) in wild-type control mice, MASP-2-/- control mice, wild-type mice treated with BSA (n=7) and MASP-2-/- mice treated with BSA (n=7), as described in Example 16.

FIGURE 31 graphically illustrates the frequency of TUNEL apoptotic cells counted in serially selected 20 high power fields (HPFs) from tissue sections from the renal cortex in wild-type control mice (n=1), MASP-2-/- control mice (n=1), wild-type mice treated with BSA (n=6) and MASP-2-/- mice treated with BSA (n=7), as described in Example 16.

FIGURE 32 shows representative H&E stained tissue sections from the following groups of mice at day 15 after treatment with BSA: (panel A) wild-type control mice treated with saline, (panel B) isotype antibody treated control mice and (panel C) wild-type mice treated with a MASP-2 inhibitory antibody, as described in Example 17.

FIGURE 33 graphically illustrates the frequency of TUNEL apoptotic cells counted in serially selected 20 high power fields (HPFs) from tissue sections from the renal cortex in wild-type mice treated with saline control and BSA (n=8), wild-type mice treated with the isotype control antibody and BSA (n=8) and wild-type mice treated with a MASP-2 inhibitory antibody and BSA (n=7), as described in Example 17.

FIGURE 34 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-TGFβ antibody (measured as % TGFβ antibody-stained area) in wild-type mice treated with BSA and saline (n=8), wild-type mice treated with BSA and isotype control antibody (n=7) and wild-type mice treated with BSA and MASP-2 inhibitory antibody (n=8), as described in Example 17.

FIGURE 35 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-TNFα antibody (measured as % TNFα antibody-stained area) in wild-type mice treated with BSA and saline (n=8), BSA and isotype control antibody (n=7) and wild-type mice treated with BSA and MASP-2 inhibitory antibody (n=8), as described in Example 17.

FIGURE 36 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-IL-6 antibody (measured as % IL-6 antibody-stained area) in in wild-type mice treated with BSA and saline (n=8), BSA and isotype control antibody (n=7) and wild-type mice treated with BSA and MASP-2 inhibitory antibody (n=8), as described in Example 17.

FIGURE 37 shows representative H&E stained tissue sections from the following groups of mice at day 14 after treatment with Adriamycin or saline only (control): (panels A-1, A-2, A-3) wild-type control mice treated with only saline; (panels B-1, B-2, B-3) wild-type mice treated with Adriamycin; and (panels C-1, C-2, C-3) MASP-2-/- mice treated with Adriamycin, as described in Example 18;

FIGURE 38 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with macrophage-specific antibody F4/80 showing the macrophage mean stained area (%) from the following groups of mice at day 14 after treatment with Adriamycin or saline only (wild-type control): wild-type control mice treated with only saline; wild-type mice treated with Adriamycin; MASP-2-/- mice treated with saline only, and MASP-2 -/- mice treated with Adriamycin, wherein $^{**}p=0.007$, as described in Example 18;

FIGURE 39 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with Sirius Red, showing the collagen deposition stained area (%) from the following groups of mice at day 14 after treatment with Adriamycin or saline only (wild-type control): wild-type control mice treated with only saline; wild-type mice treated with Adriamycin; MASP-2-/- mice treated with saline only, and MASP-2 -/- mice treated with Adriamycin, wherein $^{**}p=0.005$, as described in Example 18; and

FIGURE 40 graphically illustrates the urine albumin/creatinine ratio (uACR) in two IgA patients during the course of a twelve-week study with weekly treatment with a MASP-2 inhibitory antibody (OMS646), as described in Example 19.

FIGURE 41A shows a representative image of the immunohistochemistry analysis of tissue sections of septal blood vessels from the lung of a COVID-19 patient (H&E, 400x), as described in Example 21.

FIGURE 41B shows a representative image of the immunohistochemistry analysis of tissue sections of septal blood vessels from the lung of a COVID-19 patient (H&E, 400x), as described in Example 21.

FIGURE 41C shows a representative image of the immunohistochemistry analysis of tissue sections of medium diameter lung septal blood vessels from a COVID-19 patient, as described in Example 21.

FIGURE 41D shows a representative image of the immunohistochemistry analysis of tissue sections of liver parenchyma from a COVID-19 patient (H&E, 400x), as described in Example 21.

FIGURE 42A graphically illustrates the circulating endothelial cell (CEC)/ml counts in the peripheral blood of normal healthy controls (n-6) as compared to the CEC/ml counts in COVID-19 patients that were not part of this study (n=33), as described in Example 21.

FIGURE 42B graphically illustrates the CEC/ml counts in the 6 patients selected for this study before (baseline) and after treatment with narsoplimab, boxes represent values from the first to the third quartile, horizontal line shows the median value and the whiskers indicate the min and max value, as described in Example 21.

FIGURE 43 graphically illustrates the serum level of C Reactive Protein (CRP) (median; interquartile range (IQR)) in 6 patients with COVID-19 at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab, as described in Example 21.

FIGURE 44 graphically illustrates the serum level of Lactate Dehydrogenase (LDH) (median; IQR) in 6 patients with COVID-19 at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab, as described in Example 21.

FIGURE 45 graphically illustrates the serum level of Interleukin 6 (IL-6) (median; interquartile range (IQR)) in 6 patients with COVID-19 at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab, as described in Example 21.

FIGURE 46 graphically illustrates the serum level of Interleukin 8 (IL-8) (median; interquartile range (IQR)) in 6 patients with COVID-19 at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab, as described in Example 21.

FIGURE 47A shows the CT-scan of patient #4 on Day 5 since enrollment (i.e., after treatment with narsoplimab) wherein the patient is observed to have severe interstitial pneumonia with diffuse ground-glass opacity involving both the peripheral and central regions, consolidation in lower lobes, especially in the left lung, and massive bilateral pulmonary embolism with filling defects in interlobar and segmental arteries (not shown), as described in Example 21.

FIGURE 47B shows the CT-scan of patient #4 on Day 16 since enrollment (i.e., after treatment with narsoplimab) in which the ground-glass opacity is significantly reduced and almost complete resolution of parenchymal consolidation, as described in Example 21.

FIGURE 48 graphically illustrates the serum levels of IL-6 (pg/mL) at baseline and at different time points after narsoplimab treatment (after 2 doses, after four doses) in the patients treated with narsoplimab, wherein boxes represent values from the first to the third quartile, horizontal line shows the median value, and dots show all patient values, as described in Example 21.

FIGURE 49 graphically illustrates the serum levels of IL-8 (pg/mL) at baseline and at different time points after narsoplimab treatment (after two doses, after 4 doses) in the patients treated with narsoplimab, wherein boxes represent values from the first to the third quartile, horizontal line shows the median value, and dots show all patient values, as described in Example 21.

FIGURE 50 graphically illustrates the clinical outcome of six COVID-19 infected patients treated with narsoplimab, as described in Example 21.

FIGURE 51A graphically illustrates the serum levels of Aspartate aminotransferase (AST) (Units/Liter, U/L) values before and after narsoplimab treatment. Black lines represent median and interquartile range (IQR). The red line represents normality level and dots show all patient values, as described in Example 21.

FIGURE 51B graphically illustrates the serum levels of D-Dimer values (ng/ml), in the four patients in whom base line values were available before treatment with narsoplimab started. Black circles indicate when steroid treatment was initiated. The red line represents normality level, as described in Example 21.

FIGURE 52A graphically illustrates the serum level of D-Dimer values (ng/ml), in the seventh COVID-19 infected patient treated with narsoplimab (patient #7) at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab, wherein dosing with narsoplimab is indicated by the vertical arrows and wherein the horizonal line represents normality level, as described in Example 22.

FIGURE 52B graphically illustrates the serum level of C Reactive Protein (CRP) in patient #7 infected with COVID-19 at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab, wherein dosing with narsoplimab is indicated by the vertical arrows and wherein the horizontal line represents normality level, as described in Example 22.

FIGURE 52C graphically illustrates the serum level of Aspartate aminotransferase (AST) (Units/Liter, U/L) in patient #7 infected with COVID-19 at baseline prior to treatment (day 0) and at different time points after narsoplimab treatment, wherein dosing with narsoplimab is indicated by the vertical arrows and wherein the horizonal line represents normality level, as described in Example 22.

FIGURE 52D graphically illustrates the serum level of Alanine transaminase (ALT) (Units/Liter, U/L) in patient #7 infected with COVID-19 at baseline prior to treatment (day 0) and at different time points after narsoplimab treatment, wherein dosing

with narsoplimab is indicated by the vertical arrows and wherein the horizontal line represents normality level, as described in Example 22.

FIGURE 52E graphically illustrates the serum level of Lactate Dehydrogenase (LDH) in patient #7 with COVID-19 at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab, wherein dosing with narsoplimab is indicated by the vertical arrows and wherein the horizontal line represents normality level, as described in Example 22.

FIGURE 53 graphically illustrates the titer of anti-SARS-CoV-2 antibodies in patient #7 over time, indicating that treatment with narsoplimab does not impede effector function of the adaptive immune response, as described in Example 22.

FIGURE 54 graphically illustrates concentration-dependent binding of recombinant MASP-2 to SARS-Cov-2 nucleocapsid protein (NP2) as compared to the BSA control, as described in Example 23.

FIGURE 55 depicts an SDS-PAGE Western blot gel showing that MASP-2 directly binds to NP and cleaves C4 and the addition of a MASP-2 inhibitory antibody HG4 inhibits the NP/MASP-2-mediated C4 cleavage, as described in Example 23.

FIGURE 56 graphically illustrates the CH50 values in various populations of subjects in the longitudinal study, where each “x” symbol on the graph represents an individual subject, as described in Example 24.

FIGURE 57 graphically illustrates the C5a levels (ng/ml) in plasma samples obtained from various populations of subjects in the longitudinal study, where each “x” symbol on the graph represents an individual subject, as described in Example 24.

FIGURE 58 graphically illustrates the level of Bb (μg/mL) in plasma obtained from various populations of subjects in the longitudinal study, where each “x” symbol on the graph represents an individual subject, as described in Example 24.

FIGURE 59 graphically illustrates the amount of MASP-2/C1-INH complex detected, based on OD_{450} values, with each of the four candidate anti-MASP-2 mAbs (clone C1, C7, D8 and H1) at various concentrations of activated serum, as described in Example 25.

FIGURE 60 graphically illustrates the results of the ELISA assay measuring MASP-2/C1-INH complex in 5% serum from acute COVID patients (16 samples from 3

patients <14 days after hospitalization), convalescent patients (n=15), seropositive staff (n=15) and seronegative staff (n=34), as described in Example 25.

FIGURE 61 graphically illustrates the amount of MASP-2/C1-INH complex present in the 3 acute COVID-19 patients (#2, #3 and #4) upon admission to the hospital and over time up to 14 days after admission, wherein the line at the bottom of the graph shows the amount of MASP-2/C1-INH detected in pooled normal sero-negative health care workers, as described in Example 25.

FIGURE 62 is a schematic diagram illustrating the steps involved in a bead-based immunofluorescence assay which uses anti-C1s antibodies or anti-MASP-2 antibodies immobilised on polystyrene microspheres, or magnetic polystyrene microspheres (i.e., beads), to capture serine protease/C1-INH complexes (i.e., the analyte) from human serum or plasma, and anti-C1INH antibodies as a detection antibody to detect the captured complexes, as described in Example 26.

FIGURE 63 graphically illustrates the detection of MASP-2/C1-INH complexes in pooled human serum from acute COVID-19 patients in a bead-based assay using anti-MASP-2 mAb #C8 as a capture antibody as compared to BSA coated control beads, as described in Example 26.

FIGURE 64 is a photograph of a non-reducing gel loaded with 6 μg of samples obtained during SEC purification of recombinant MASP-2/C1-INH complexes as described in Example 27.

FIGURE 65 graphically illustrates the levels of MASP-2/C1-INH complex in acute COVID-19 patients, as determined in a duplexed bead-based assay, as described in Example 28.

FIGURE 66 graphically illustrates the levels of C1s/C1-INH complex in acute COVID-19 patients, as determined in a duplexed bead-based assay, as described in Example 28.

FIGURE 67 graphically illustrates the CH_{50} values in acute COVID-19 patients, convalescent patients, sero-positive staff and sero-negative staff in the longitudinal study as described in Example 28.

FIGURE 68 graphically illustrates the C5a values in acute COVID-19 patients, convalescent patients, sero-positive staff and sero-negative staff in the longitudinal study as described in Example 28.

FIGURE 69 graphically illustrates the levels MASP-2/C1-INH complex in samples from 8 acute COVID-19 patients at admission (prior to narsoplimab treatment) and after narsoplimab treatment (day 3-4 after starting treatment; day 7-8, day 9 to discharge) as compared to 16 healthy controls, as described in Example 29.

FIGURE 70A graphically illustrates the CH_{50} values in samples from 8 acute COVID-19 patients at admission (prior to narsoplimab treatment) and after narsoplimab treatment (day 3-4 after starting treatment; day 7-8, day 9 to discharge) as compared to 16 healthy controls, as described in Example 29.

FIGURE 70B graphically illustrates the C5a values in samples from 8 acute COVID-19 patients at admission (prior to narsoplimab treatment) and after narsoplimab treatment (day 3-4 after starting treatment; day 7-8, day 9 to discharge) as compared to 16 healthy controls, as described in Example 29.

FIGURE 71 graphically illustrates the levels MASP-2/C1-INH complex in samples from 7 COVID-19 patients at admission (day 0, prior to narsoplimab treatment) and after narsoplimab treatment (day 2-4 after starting treatment; day 6-8 and day 9 to discharge) as compared to samples obtained from 9 COVID-19 patients that were not treated with narsoplimab (untreated controls) during the same time period and a pool of healthy control subjects (healthy controls), as described in Example 30.

FIGURE 72A graphically illustrates the CH_{50} values in samples from 7 COVID-19 patients at admission (day 0, prior to narsoplimab treatment) and after narsoplimab treatment (day 2-4 after starting treatment; day 6-8 and day 9 to discharge) as compared to samples obtained from 9 COVID-19 patients that were not treated with narsoplimab (untreated controls) during the same time period and a pool of healthy control subjects (healthy controls), as described in Example 30.

FIGURE 72B graphically illustrates the C5a values in samples from 7 COVID-19 patients at admission (day 0, prior to narsoplimab treatment) and after narsoplimab treatment (day 2-4 after starting treatment; day 6-8 andday 9 to discharge) as compared to samples obtained from 9 COVID-19 patients that were not treated with narsoplimab (untreated controls) during the same time period and a pool of healthy control subjects (healthy controls), as described in Example 30.

FIGURE 73 graphically illustrates the viable bacterial count of *K. pneumoniae* after incubation of sera from COVID-19 patients prior to treatment with narsoplimab (pre-treatment) and in COVID-19 patients after treatment with narsoplimab as compared

to sera from COVID-19 patients not treated with narsoplimab as compared to normal healthy serum (NHS) and heat-inactivated normal healthy serum (HI-NHS), as described in Example 30.

DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1 human MAp19 cDNA

SEQ ID NO:2 human MAp19 protein (with leader)

SEQ ID NO:3 human MAp19 protein (mature)

SEQ ID NO:4 human MASP-2 cDNA

SEQ ID NO:5 human MASP-2 protein (with leader)

SEQ ID NO:6 human MASP-2 protein (mature)

SEQ ID NO:7 human MASP-2 gDNA (exons 1-6)

ANTIGENS: (IN REFERENCE TO THE MASP-2 MATURE PROTEIN)

SEQ ID NO:8 CUBI sequence (aa 1-121)

SEQ ID NO:9 CUBEGF sequence (aa 1-166)

SEQ ID NO:10 CUBEGFCUBII (aa 1-293)

SEQ ID NO:11 EGF region (aa 122-166)

SEQ ID NO:12 serine protease domain (aa 429 – 671)

SEQ ID NO:13 serine protease domain inactive (aa 610-625 with Ser618 to Ala mutation)

SEQ ID NO:14 TPLGPKWPEPVFGRL (CUBI peptide)

SEQ ID NO:15 TAPPGYRLRLYFTHFDLELSHLCEYDFVKLSSGAKVLATLC GQ (CUBI peptide)

SEQ ID NO:16 TFRSDYSN (MBL binding region core)

SEQ ID NO:17 FYSLGSSLDITFRSDYSNEKPFTGF (MBL binding region)

SEQ ID NO:18 IDECQVAPG (EGF PEPTIDE)

SEQ ID NO:19 ANMLCAGLESGGKDSCRGDSGGALV (serine protease binding core)Detailed Description

PEPTIDE INHIBITORS:

SEQ ID NO:20 MBL full length cDNA

SEQ ID NO:21 MBL full length protein

SEQ ID NO:22 OGK-X-GP (consensus binding)

SEQ ID NO:23 OGKLG

SEQ ID NO:24 GLR GLQ GPO GKL GPO G

SEQ ID NO:25 GPO GPO GLR GLQ GPO GKL GPO GPO GPO

SEQ ID NO:26 GKDGRDGTKGEKGEPGQGLRGLQGPOGKLGPOG

SEQ ID NO:27 GAOGSOGEKGAOGPQGPOGPOGKMGPKGEOGDO (human h-ficolin)

SEQ ID NO:28 GCOGLOGAOGDKGEAGTNGKRGERGPOGPOGKAGPOGPN GAOGEO (human ficolin p35)

SEQ ID NO:29 LQRALEILPNRVTIKANRPFLVFI (C4 cleavage site)

EXPRESSION INHIBITORS:

SEQ ID NO:30 cDNA of CUBI-EGF domain (nucleotides 22-680 of SEQ ID NO:4)

SEQ ID NO:31 5' CGGGCACACCATGAGGCTGCTGACCCTCCTGGGC 3' Nucleotides 12-45 of SEQ ID NO:4 including the MASP-2 translation start site (sense)

SEQ ID NO:32 5'GACATTACCTTCCGCTCCGACTCCAACGAGAAG3' Nucleotides 361-396 of SEQ ID NO:4 encoding a region comprising the MASP-2 MBL binding site (sense)

SEQ ID NO:33 5'AGCAGCCCTGAATACCCACGGCCGTATCCCAAA3' Nucleotides 610-642 of SEQ ID NO:4 encoding a region comprising the CUBII domain

CLONING PRIMERS:

SEQ ID NO:34 CGGGATCCATGAGGCTGCTGACCCTC (5' PCR for CUB)

SEQ ID NO:35 GGAATTCCTAGGCTGCATA (3' PCR FOR CUB)

SEQ ID NO:36 GGAATTCCTACAGGGCGCT (3' PCR FOR CUBIEGF)

SEQ ID NO:37 GGAATTCCTAGTAGTGGAT (3' PCR FOR CUBIEGFCUBII)

SEQ ID NOS:38-47 are cloning primers for humanized antibody

SEQ ID NO:48 is 9 aa peptide bond

EXPRESSION VECTOR:

SEQ ID NO:49 is the MASP-2 minigene insert

SEQ ID NO: 50 is the murine MASP-2 cDNA

SEQ ID NO: 51 is the murine MASP-2 protein (w/leader)

SEQ ID NO: 52 is the mature murine MASP-2 protein

SEQ ID NO: 53 the rat MASP-2 cDNA

SEQ ID NO: 54 is the rat MASP-2 protein (w/ leader)

SEQ ID NO: 55 is the mature rat MASP-2 protein

SEQ ID NO: 56-59 are the oligonucleotides for site-directed mutagenesis of human MASP-2 used to generate human MASP-2A

SEQ ID NO: 60-63 are the oligonucleotides for site-directed mutagenesis of murine MASP-2 used to generate murine MASP-2A

SEQ ID NO: 64-65 are the oligonucleotides for site-directed mutagenesis of rat MASP-2 used to generate rat MASP-2A

SEQ ID NO: 66 DNA encoding 17D20_dc35VH21N11VL (OMS646) heavy chain variable region (VH) (without signal peptide)

SEQ ID NO: 67 17D20_dc35VH21N11VL (OMS646) heavy chain variable region (VH) polypeptide

SEQ ID NO: 68 17N16mc heavy chain variable region (VH) polypeptide

SEQ ID NO: 69 17D20_dc35VH21N11VL (OMS646) light chain variable region (VL) polypeptide

SEQ ID NO: 70 DNA encoding 17D20_dc35VH21N11VL (OMS646) light chain variable region (VL)

SEQ ID NO: 71 17N16_dc17N9 light chain variable region (VL) polypeptide

SEQ ID NO:72: SGMI-2L(full-length)

SEQ ID NO: 73: SGMI-2M (medium truncated version)

SEQ ID NO:74: SGMI-2S (short truncated version)

SEQ ID NO:75: mature polypeptide comprising the VH-M2ab6-SGMI-2-N and the human IgG4 constant region with hinge mutation

SEQ ID NO:76: mature polypeptide comprising the VH-M2ab6-SGMI-2-C and the human IgG4 constant region with hinge mutation

SEQ ID NO:77: mature polypeptide comprising the VL-M2ab6-SGMI-2-N and the human Ig lambda constant region

SEQ ID NO:78: mature polypeptide comprising the VL-M2ab6-SGMI-2-C and the human Ig lambda constant region

SEQ ID NO:79: peptide linker (10aa)

SEQ ID NO:80: peptide linker (6aa)

SEQ ID NO:81: peptide linker (4aa)

SEQ ID NO:82: polynucleotide encoding the polypeptide comprising the VH-M2ab6-SGMI-2-N and the human IgG4 constant region with hinge mutation

SEQ ID NO:83: polynucleotide encoding the polypeptide comprising the VH-M2ab 6-SGMI-2-C and the human IgG4 constant region with hinge mutation

SEQ ID NO:84: polynucleotide encoding the polypeptide comprising the VL-M2ab6-SGMI-2-N and the human Ig lambda constant region

SEQ ID NO:85: polynucleotide encoding the polypeptide comprising the VL-M2ab6-SGMI-2-C and the human Ig lambda constant region

SEQ ID NO:86: C1 inhibitor (C1-INH) homo sapiens

SEQ ID NO:87: MASP-2 mAb C7 heavy chain variable region

SEQ ID NO:88: MASP-2 mAb C7 light chain variable region

SEQ ID NO:89: MASP-2 mAb C7 HC-CDR1

SEQ ID NO:90: MASP-2 mAb C7 HC-CDR2

SEQ ID NO:91: MASP-2 mAb C7 HC-CDR3

SEQ ID NO:92: MASP-2 mAb C7 LC-CDR1

SEQ ID NO :93: MASP-2 mAb C7 LC-CDR2

SEQ ID NO:94: MASP-2 mAb C7 LC-CDR3

SEQ ID NO:95: MASP-2 mAb C7 cDNA encoding the heavy chain variable region

SEQ ID NO:96: MASP-2 mAb C7 cDNA encoding the light chain variable region

SEQ ID NO:97: MASP-2 mAb C8 heavy chain variable region

SEQ ID NO:98: MASP-2 mAb C8 light chain variable region

DETAILED DESCRIPTION

As described herein, the inventors have observed that the concentrations of the MASP-2/C1-INH in the blood (e.g., serum and/or plasma) are abnormally high in patients with severe COVID-19 and also in subjects previously infected with COVID-19 and suffering from long-term sequelae. The inventors have also observed that, following recovery, the concentration of the MASP-2/C1-INH complex decreases to normal levels in most instances. The inventors believe that monitoring a patient infected with SARS-CoV-2 for an increase in the concentration of MASP-2/C1-INH complex is useful for diagnosing a patient as having, or at risk for developing acute COVID-19, and also for diagnosing a subject as having, or at risk for developing post-acute COVID-19 (also referred to as Long-COVID-19) and optionally treating a subject identified as having such risk with a complement inhibitor, such as a MASP-2 inhibitor. As further described herein, the use of a MASP-2 inhibitory agent is also useful to treat, inhibit, alleviate or prevent acute respiratory distress syndrome in a subject infected with coronavirus, such as COVID-19 and is also useful to treat, inhibit, alleviate, or prevent acute respiratory distress in a subject infected with influenza virus. Therefore, monitoring the status of the MASP-2/C1-INH complex can also be useful for determining whether a COVID-19 patient is responding to therapy with a complement inhibitor such as a MASP-2 inhibitor and optionally adjusting the dosage of the MASP-2 inhibitor as needed to bring the level of MASP-2/C1-INH into the normal range.

The disclosure also provides assay methods for measuring fluid-phase MASP-2/C1-INH complex in a biological sample. Also provided are compositions, kits and methods for interrogating the concentration of the fluid-phase MASP-2/C1-INH complex in a biological fluid, such as a biological fluid obtained from a subject infected with SARS-CoV-2.

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- dependent activation of the lectin pathway, which occurs under physiological conditions *(i.e.*, in the presence of Ca^{++}) 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, which has been determined to primarily cause opsonization.

As used herein, the term "alternative pathway" refers to complement activation that is triggered, for example, by zymosan from fungal and yeast cell walls, lipopolysaccharide (LPS) from Gram negative outer membranes, and rabbit erythrocytes, as well as from many pure polysaccharides, rabbit erythrocytes, viruses, bacteria, animal tumor cells, parasites and damaged cells, and which has traditionally been thought to arise from spontaneous proteolytic generation of C3b from complement factor C3.

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 including mannan-binding lectin (MBL), CL-11 and the ficolins (H-ficolin, M-ficolin, or L-ficolin).

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 recognition molecule C1q.

As used herein, the term "MASP-2 inhibitory agent" refers to any agent that binds to or directly interacts with MASP-2 and effectively inhibits MASP-2-dependent complement activation, including anti-MASP-2 antibodies and MASP-2 binding fragments thereof, natural and synthetic peptides, small molecules, soluble MASP-2 receptors, expression inhibitors and isolated natural inhibitors, and also encompasses peptides that compete with MASP-2 for binding to another recognition molecule (e.g., MBL, H-ficolin, M-ficolin, or L-ficolin) in the lectin pathway, but does not encompass antibodies that bind to such other recognition molecules. MASP-2 inhibitory agents useful in the method of the invention may reduce MASP-2-dependent complement activation by greater than 20%, such as greater than 50%, such as greater than 90%. In one embodiment, the MASP-2 inhibitory agent reduces MASP-2-dependent complement activation by greater than 90% (i.e., resulting in MASP-2 complement activation of only 10% or less).

As used herein, the term “fibrosis” refers to the formation or presence of excessive connective tissue in an organ or tissue. Fibrosis may occur as a repair or replacement response to a stimulus such as tissue injury or inflammation. A hallmark of fibrosis is the production of excessive extracellular matrix. The normal physiological response to injury results in the deposition of connective tissue as part of the healing process, but this connective tissue deposition may persist and become pathological, altering the architecture and function of the tissue. At the cellular level, epithelial cells and fibroblasts proliferate and differentiate into myofibroblasts, resulting in matrix contraction, increased rigidity, microvascular compression, and hypoxia.

As used herein, the term "treating fibrosis in a mammalian subject suffering from or at risk of developing a disease or disorder caused or exacerbated by fibrosis and/or inflammation" refers to reversing, alleviating, ameliorating, or inhibiting fibrosis in said mammalian subject.

As used herein, the term “proteinuria” refers to the presence of urinary protein in an abnormal amount, such as in amounts exceeding 0.3g protein in a 24-hour urine collection from a human subject, or in concentrations of more than 1g per liter in a human subject.

As used herein, the term “improving proteinuria” or “reducing proteinuria’ refers to reducing the 24-hour urine protein excretion in a subject suffering from proteinuria by at least 20%, such as at least 30%, such as at least 40%, such at least 50% or more in comparison to baseline 24-hour urine protein excretion in the subject prior to treatment with a MASP-2 inhibitory agent. In one embodiment, treatment with a MASP-2 inhibitory agent in accordance with the methods of the invention is effective to reduce proteinuria in a human subject such as to achieve greater than 20 percent reduction in 24-hour urine protein excretion, or such as greater than 30 percent reduction in 24-hour urine protein excretion, or such as greater than 40 percent reduction in 24-hour urine protein excretion, or such as greater than 50 percent reduction in 24-hour urine protein excretion).

As used herein, the terms "small molecule," "small organic molecule," and "small inorganic molecule" refer to molecules (either organic, organometallic, or inorganic), organic molecules, and inorganic molecules, respectively, which are either naturally occurring or synthetic and that have a molecular weight of more than about 50 Da and

less than about 2500 Da. Small organic (for example) molecules may be less than about 2000 Da, between about 100 Da to about 1000 Da, or between about 100 to about 600 Da, or between about 200 to 500 Da.

As used herein, the term "antibody" encompasses antibodies and antibody fragments thereof, derived from any antibody-producing mammal (e.g., mouse, rat, 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, for example, MASP-2, 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-idiotype 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, an anti-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.

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.

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 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.

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 C-9) that inserts into and disrupts membranes (also referred to as C5b-9).

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), 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.

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: 5.

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 of SEQ ID NO: 5). In some embodiments, an antigenic peptide fragment of 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 any one of SEQ ID NOS: 5).

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.

II. Overview of the Invention

As described herein, the inventors have identified the central role of the lectin pathway in the initiation and disease progression of tubular renal pathology, thereby implicating a key role of the lectin pathway activation in the pathophysiology of a diverse range of renal diseases including IgA nephropathy, C3 glomerulopathy and other glomerulonephritides. As further described herein, the inventors discovered that inhibition of mannan-binding lectin-associated serine protease-2 (MASP-2), the key regulator of the lectin pathway of the complement system, significantly reduces inflammation and fibrosis in various animal models of fibrotic disease including the unilateral ureteral obstruction (UUO) model, the protein overload model and the adriamycin-induced nephrology model of renal fibrosis. Therefore, the inventors have demonstrated that inhibition of MASP-2-mediated lectin pathway activation provides an effective therapeutic approach to ameliorate, treat or prevent renal fibrosis, e.g., tubulointerstitial fibrosis, regardless of the underlying cause. As further described herein, the use of a MASP-2 inhibitory agent is also useful to treat, inhibit, alleviate or prevent acute respiratory distress syndrome in a subject infected with coronavirus, such as COVID-19.

Lectins (MBL, M-ficolin, H-ficolin, L-ficolin and CL-11) are the specific recognition molecules that trigger the innate complement system and the system includes the lectin initiation pathway and the associated terminal pathway amplification loop that amplifies lectin-initiated activation of terminal complement effector molecules. C1q is the specific recognition molecule that triggers the acquired complement system and the system includes the classical initiation pathway and associated terminal pathway amplification loop that amplifies C1q-initiated activation of terminal complement effector molecules. We refer to these two major complement activation systems as the lectin-dependent complement system and the C1q-dependent complement system, respectively.

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. With the recognition that it is possible to inhibit the lectin mediated MASP-2 pathway while leaving the classical pathway intact comes the realization that it would be

highly desirable to specifically inhibit only the complement activation system causing a particular pathology without completely shutting down the immune defense capabilities of complement. For example, in disease states in which complement activation is mediated predominantly by the lectin-dependent complement system, it would be advantageous to specifically inhibit only this system. This would leave the C1q-dependent complement activation system intact to handle immune complex processing and to aid in host defense against infection.

The preferred protein component to target in the development of therapeutic agents to specifically inhibit the lectin-dependent complement system is MASP-2. Of all the known protein components of the lectin-dependent complement system (MBL, H-ficolin, M-ficolin, L-ficolin, MASP-2, C2-C9, Factor B, Factor D, and properdin), only MASP-2 is both unique to the lectin-dependent complement system and required for the system to function. The lectins (MBL, H-ficolin, M-ficolin, L-ficolin and CL-11) are also unique components in the lectin-dependent complement system. However, loss of any one of the lectin components would not necessarily inhibit activation of the system due to lectin redundancy. It would be necessary to inhibit all five lectins in order to guarantee inhibition of the lectin-dependent complement activation system. Furthermore, since MBL and the ficolins are also known to have opsonic activity independent of complement, inhibition of lectin function would result in the loss of this beneficial host defense mechanism against infection. In contrast, this complement-independent lectin opsonic activity would remain intact if MASP-2 was the inhibitory target. An added benefit of MASP-2 as the therapeutic target to inhibit the lectin-dependent complement activation system is that the plasma concentration of MASP-2 is among the lowest of any complement protein (≈ 500 ng/ml); therefore, correspondingly low concentrations of high-affinity inhibitors of MASP-2 may be sufficient to obtain full inhibition (Moller-Kristensen, M., et al., *J. Immunol Methods* 282:159-167, 2003).

As described herein in Example 14, it was determined in an animal model of fibrotic kidney disease (unilateral ureteral obstruction UUO) that mice without the MASP-2 gene (MASP-2-/-) exhibited significantly less kidney disease compared to wild-type control animals, as shown by inflammatory cell infiltrates (75% reduction) and histological markers of fibrosis such as collagen deposition (one third reduction). As further shown in Example 15, wild-type mice systemically treated with an anti-MASP-2 monoclonal

antibody that selectively blocks the lectin pathway while leaving the classical pathway intact, were protected from renal fibrosis, as compared to wild-type mice treated with an isotype control antibody. These results demonstrate that the lectin pathway is a key contributor to kidney disease and further demonstrate that a MASP-2 inhibitor that blocks the lectin pathway, such as a MASP-2 antibody, is effective as an antifibrotic agent. As further shown in Example 16, in the protein overload model, wild-type mice treated with bovine-serum albumin (BSA) developed proteinuric nephropathy, whereas MASP-2-/- mice treated with the same level of BSA had reduced renal injury. As shown in Example 17, wild-type mice systemically treated with an anti-MASP-2 monoclonal antibody that selectively blocks the lectin pathway while leaving the classical pathway intact, were protected from renal injury in the protein overload model. As described in Example 18, MASP-2-/- mice exhibited less renal inflammation and tubulointerstitial injury in an Adriamycin-induced nephrology model of renal fibrosis as compared to wild-type mice. As described in Example 19, in an ongoing Phase 2 open-label renal trial, patients with IgA nephropathy that were treated with an anti-MASP-2 antibody demonstrated a clinically meaningful and statistically significant decrease in urine albumin-to-creatinine ratios (uACRs) throughout the trial and reduction in 24-hour urine protein levels from baseline to the end of treatment. As further described in Example 19, in the same Phase 2 renal trial, patients with membranous nephropathy that were treated with an anti-MASP-2 antibody also demonstrated reductions in uACR during treatment.

In accordance with the foregoing, the present invention relates to the use of MASP-2 inhibitory agents, such as MASP-2 inhibitory antibodies, as antifibrotic agents, the use of MASP-2 inhibitory agents for the manufacture of a medicament for the treatment of a fibrotic condition, and methods of preventing, treating, alleviating or reversing a fibrotic condition in a human subject in need thereof, said method comprising administering to said patient an efficient amount of a MASP-2 inhibitory agent (e.g., an anti-MASP-2 antibody).

As described in Examples 20, 21, and 22, clinical improvement was observed in patients suffering from COVID-19-related respiratory failure following treatment with narsoplimab, which inhibits MASP-2 and lectin pathway activation. As described in Example 21, all six COVID-19 patients treated with narsoplimab demonstrated clinical improvement. In each case, COVID-19 lung injury had progressed to ARDS prior to narsoplimab treatment and all patients were receiving non-invasive mechanical

ventilation at the time treatment was initiated. Narsoplimab-treated COVID-19 patients for whom follow-up (5-6 month) data are available show no observed clinical or laboratory evidence of longer-term sequelae. As further described in Example 22, additional COVID-19 patients treated with narsoplimab also demonstrated clinical improvement. As further demonstrated in Example 22, narsoplimab-treated patients developed appropriately high titers of anti-SARS-Cov-2 antibodies, indicating that treatment with narsoplimab does not impede effector function of the adaptive immune response.

As further described herein in Examples 24 to 30, the inventors have observed that the concentrations of the MASP-2/C1-INH in the blood (e.g., serum and/or plasma) are abnormally high in patients with severe COVID-19 and also in subjects previously infected with COVID-19 and suffering from long-term sequelae. The inventors have also observed that, following recovery, the concentration of the MASP-2/C1-INH complex decreases to normal levels in most instances. The inventors believe that monitoring a patient infected with SARS-CoV-2 for an increase in the concentration of MASP-2/C1-INH complex is useful for diagnosing a patient as having, or at risk for developing acute COVID-19, and also for diagnosing a subject as having, or at risk for developing post-acute COVID-19 (also referred to as Long-COVID-19) and optionally treating a subject identified as having such risk with a complement inhibitor, such as a MASP-2 inhibitor. As further described herein, the use of a MASP-2 inhibitory agent is also useful to treat, inhibit, alleviate or prevent acute respiratory distress syndrome in a subject infected with coronavirus, such as COVID-19 and is also useful to treat, inhibit, alleviate, or prevent acute respiratory distress in a subject infected with influenza virus. Therefore, monitoring the status of the MASP-2/C1-INH complex can also be useful for determining whether a COVID-19 patient is responding to therapy with a complement inhibitor such as a MASP-2 inhibitor and optionally adjusting the dosage of the MASP-2 inhibitor as needed to bring the level of MASP-2/C1-INH into the normal range.

The disclosure also provides assay methods for measuring fluid-phase MASP-2/C1-INH complex in a biological sample. Also provided are compositions, kits and methods for interrogating the concentration of the fluid-phase MASP-2/C1-INH complex in a biological fluid, such as a biological fluid obtained from a subject infected with SARS-CoV-2.

Accordingly, the methods of the invention can be used to treat, inhibit, alleviate, prevent, or reverse coronavirus-induced pneumonia or acute respiratory distress syndrome in a human subject suffering from coronavirus, such as COVID-19, SARS or MERS, as further described herein. The methods of the invention can also be used to treat, inhibit, alleviate, prevent, or reverse influenza virus-induced pneumonia or acute respiratory distress syndrome in a human subject suffering from influenza virus, such as influenza Type A virus serotypes (H1N1 (caused the "Spanish Flu" in 1918 and "Swine Flu" in 2009); H2N2 (caused the "Asian Flu" in 1957), H3N2 (caused the "Hong Kong Flu" in 1968), H5N1 (caused the "Bird Flu in 2004), H7N7, H1N2, H9N2, H7N2, H7N3, H10N7, H7N9 and H6N1); or influenza Type B virus, or influenza Type C virus.

III. THE ROLE OF MASP-2 IN DISEASES AND CONDITIONS CAUSED OR EXACERBATED BY FIBROSIS

Fibrosis is the formation or presence of excessive connective tissue in an organ or tissue, commonly in response to damage or injury. A hallmark of fibrosis is the production of excessive extracellular matrix following an injury. In the kidney, fibrosis is characterized as a progressive detrimental connective tissue deposition on the kidney parenchyma which inevitably leads to a decline in renal function independently of the primary renal disease which causes the original kidney injury. So called epithelial to mesenchymal transition (EMT), a change in cellular characteristics in which tubular epithelial cells are transformed to mesenchymal fibroblasts, constitutes the principal mechanism of renal fibrosis. Fibrosis affects nearly all tissues and organ systems and may occur as a repair or replacement response to a stimulus such as tissue injury or inflammation. The normal physiological response to injury results in the deposition of connective tissue but, if this process becomes pathological, the replacement of highly differentiated cells by scarring connective tissue alters the architecture and function of the tissue. At the cellular level, epithelial cells and fibroblasts proliferate and differentiate into myofibroblasts, resulting in matrix contraction, increased rigidity, microvascular compression, and hypoxia. Currently there are no effective treatments or therapeutics for fibrosis, but both animal studies and anecdotal human reports suggest that fibrotic tissue damage may be reversed (Tampe and Zeisberg, *Nat Rev Nephrol,* vol 10:226-237, 2014).

Many diseases result in fibrosis that causes progressive organ failure, including diseases of the kidney (e.g., chronic kidney disease, IgA nephropathy, C3 glomerulopathy

and other glomerulonephritides), lung (e.g., idiopathic pulmonary fibrosis, cystic fibrosis, bronchiectasis), liver (e.g., cirrhosis, nonalcoholic fatty liver disease), heart (e.g., myocardial infarction, atrial fibrosis, valvular fibrosis, endomyocardial fibrosis), brain (e.g., stroke), skin (e.g., excessive wound healing, scleroderma, systemic sclerosis, keloids), vasculature (e.g., atherosclerotic vascular disease), intestine (e.g., Crohn's disease), eye (e.g., anterior subcapsular cataract, posterior capsule opacification), musculoskeletal soft-tissue structures (e.g., adhesive capsulitis, Dupuytren's contracture, myelofibrosis), reproductive organs (e.g., endometriosis, Peyronie's disease), and some infectious diseases (e.g., coronoavirus, alpha virus, Hepatitis C, Hepatitis B, etc.).

While fibrosis occurs in many tissues and diseases, there are common molecular and cellular mechanisms to its pathology. The deposition of extracellular matrix by fibroblasts is accompanied by immune cell infiltrates, predominately mononuclear cells (see Wynn T., *Nat Rev Immunol* 4(8):583-594, 2004, hereby incorporated herein by reference). A robust inflammatory response results in the expression of growth factors (TGF-beta, VEGF, Hepatocyte Growth Factor, connective tissue growth factor), cytokines and hormones (endothelin, IL-4, IL-6, IL-13, chemokines), degradative enzymes (elastase, matrix metaloproteinases, cathepsins), and extracellular matrix proteins (collagens, fibronectin, integrins).

In addition, the complement system becomes activated in numerous fibrotic diseases. Complement components, including the membrane attack complex, have been identified in numerous fibrotic tissue specimens. For example, components of the lectin pathway have been found in fibrotic lesions of kidney disease (Satomura et al., *Nephron.* 92(3):702-4 (2002); Sato et al., *Lupus* 20(13):1378-86 (2011); Liu et al., *Clin Exp Immunol, 174(1):152-60 (*2013)); liver disease (Rensen et al., *Hepatology* 50(6): 1809-17 (2009)); and lung disease (Olesen et al., *Clin Immunol* 121(3):324-31 (2006)).

Overshooting complement activation has been established as a key contributor to immune complex-mediated as well as antibody independent glomerulonephritides. There is, however, a strong line of evidence demonstrating that uncontrolled activation of complement *in situ* is intrinsically involved in the pathophysiological progression of TI fibrosis in non-glomerular disease (Quigg R.J, *J Immunol* 171:3319-3324, 2003, Naik A. et al., *Semin Nephrol* 33:575-585, 2013, Mathern D.R. et al., *Clin J Am Soc Nephrol* 10:P1636-1650, 2015). The strong proinflammatory signals that are triggered by local complement activation may be initiated by complement components filtered into the

proximal tubule and subsequently entering the interstitial space, or abnormal synthesis of complement components by tubular or other resident and infiltrating cells, or by altered expression of complement regulatory proteins on kidney cells, or absence or loss or gain for function mutations in complement regulatory components (Mathern D.R. et al., *Clin J Am Soc Nephrol* 10:P1636-1650, 2015, Sheerin N.S., et al., *FASEB J* 22: 1065-1072, 2008). In mice for example, deficiency of the complement regulatory protein CR1-related gene/protein y (Crry), results in tubulointerstitial (TI) complement activation with consequent inflammation and fibrosis typical of the injury seen in human TI diseases (Naik A. et al., *Semin Nephrol* 33:575-585, 2013, Bao L. et al., *J Am Soc Nephrol* 18:811-822, 2007). Exposure of tubular epithelial cells to the anaphylatoxin C3a results in epithelial to mesenchymal transition (Tsang Z. et al., *J Am Soc Nephrol* 20:593-603, 2009). Blocking C3a signaling via the C3a receptor alone has recently been shown to lessen renal TI fibrosis in proteinuric and non-proteinuric animals (Tsang Z. et al., *J Am Soc Nephrol* 20:593-603, 2009, Bao L. et al., *Kidney Int.* 80: 524-534, 2011).

As described herein, the inventors have identified the central role of the lectin pathway in the initiation and disease progression of tubular renal pathology, thereby implicating a key role of the lectin pathway activation in the pathophysiology of a diverse range of renal diseases including IgA nephropathy, C3 glomerulopathy and other glomerulonephritides (Endo M. et al., *Nephrol Dialysis Transplant* 13: 1984-1990, 1998; Hisano S. et al., *Am J Kidney Dis* 45:295-302, 2005; Roos A. et al., *J Am Soc Nephrol* 17: 1724-1734, 2006; Liu L.L. et al., *Clin Exp. Immunol* 174:152-160, 2013; Lhotta K. et al., *Nephrol Dialysis Transplant* 14:881-886, 1999; Pickering et al., *Kidney International* 84:1079-1089, 2013), diabetic nephropathy (Hovind P. et al., *Diabetes* 54:1523-1527, 2005), ischaemic reperfusion injury (Asgari E. et al., *FASEB* J 28:3996-4003, 2014) and transplant rejection (Berger S.P. et al., *Am J Transplant* 5:1361-1366, 2005).

As further described herein, the inventors have demonstrated that MASP-2 inhibition reduces inflammation and fibrosis in mouse models of tubulointerstitial disease. Therefore, MASP-2 inhibitory agents are expected to be useful in the treatment of renal fibrosis, including tubulointerstitial inflammation and fibrosis, proteinuria, IgA nephropathy, C3 glomerulopathy and other glomerulonephritides and renal ischaemia reperfusion injury.

Lung Disease

Pulmonary fibrosis is the formation or development of excess fibrous connective tissue in the lungs, wherein normal lung tissue is replaced with fibrotic tissue. This scarring leads to stiffness of the lungs and impaired lung structure and function. In humans, pulmonary fibrosis is thought to result from repeated injury to the tissue within and between the tiny air sacs (alveoli) in the lungs. In an experimental setting, a variety of animal models have replicated aspects of the human disease. For example, a foreign agent such as bleomycin, fluorescein isothiocyanate, silica, or asbestos may be instilled into the trachea of an animal (Gharaee-Kermani et al., *Animal Models of Pulmonary Fibrosis. Methods Mol. Med.*, 2005, 117:251-259).

Accordingly, in certain embodiments, the disclosure provides a method of inhibiting pulmonary fibrosis in a subject suffering from a lung disease or disorder caused or exacerbated by fibrosis and/or inflammation such as coronaviruas-induced ARDS, comprising administering a MASP-2 inhibitory agent, such as a MASP-2 inhibitory antibody, to a subject in need thereof. This method includes administering a composition comprising an amount of a MASP-2 inhibitor effective to inhibit pulmonary fibrosis, decrease lung fibrosis, and/or improve lung function. Improvements in symptoms of lung function include improvement of lung function and/or capacity, decreased fatigue, and improvement in oxygen saturation.

The MASP-2 inhibitory composition may be administered locally to the region of fibrosis, such as by local application of the composition during surgery or local injection, either directly or remotely, for example, by catheter. Alternately, the MASP-2 inhibitory agent 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.

In certain embodiments, the MASP-2 inhibitory agents (e.g., MASP-2 inhibitory antibodies) are administered in combination with one or more agents or treatment modalities appropriate for the underlying lung disease or condition.

Infectious Diseases

Infectious diseases such as coronavirus and chronic infectious diseases such as Hepatitis C and Hepatitis B cause tissue inflammation and fibrosis, and high lectin pathway activity may be detrimental. In such diseases, inhibitors of MASP-2 may be

beneficial. For example, MBL and MASP-1 levels are found to be a significant predictor of the severity of liver fibrosis in hepatitis C virus (HCV) infection (Brown et al., *Clin Exp Immunol.* 147(1):90-8, 2007; Saadanay et al., *Arab J Gastroenterol.* 12(2):68-73, 2011; Saeed et al., *Clin Exp Immunol.* 174(2):265-73, 2013). MASP-1 has previously been shown to be a potent activator of MASP-2 and the lectin pathway (Megyeri et al., *J Biol Chem.* 29: 288(13):8922-34, 2013). Alphaviruses such as chikungunya virus and Ross River virus induce a strong host inflammatory response resulting in arthritis and myositis, and this pathology is mediated by MBL and the lectin pathway (Gunn et al., *PLoS Pathog.* 8(3):e1002586, 2012).

Accordingly, in certain embodiments, the disclosure provides a method of preventing, treating, reverting, inhibiting and/or reducing fibrosis and/or inflammation in a subject suffering from, or having previously suffered from, an infectious disease such as coronavirus or influenza virus that causes inflammation and/or fibrosis, comprising administering a MASP-2 inhibitory agent, such as a MASP-2 inhibitory antibody, to a subject in need thereof.

The MASP-2 inhibitory composition may be administered locally to the region of fibrosis, such as by local application of the composition during surgery or local injection, either directly or remotely, for example, by catheter. Alternately, the MASP-2 inhibitory agent 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.

In certain embodiments, the MASP-2 inhibitory agents (e.g., MASP-2 inhibitory antibodies) are administered in combination with one or more agents or treatment modalities appropriate for the underlying infectious disease.

In some embodiments, the infectious disease that causes inflammation and/or fibrosis is selected from the group consisting of: coronavirus, alpha virus, Hepatitis A, Hepatitis B, Hepatitis C, tuberculosis, HIV and influenza.

In certain embodiments, the MASP-2 inhibitory agents (e.g., MASP-2 inhibitory antibodies or MASP-2 inhibitory small molecule compounds) are administered in

combination with one or more agents or treatment modalities appropriate for the underlying disease or disorder.

In certain embodiments of any of the various methods and pharmaceutical compositions described herein, the MASP-2 inhibitory antibody or small molecule compound selectively blocks the lectin pathway while leaving intact the classical pathway.

IV. MASP-2 INHIBITORY AGENTS

In various aspects, the present invention provides methods of inhibiting the adverse effects of fibrosis and/or inflammation comprising administering a MASP-2 inhibitory agent to a subject in need thereof. MASP-2 inhibitory agents are administered in an amount effective to inhibit MASP-2-dependent complement activation in a living subject. In the practice of this aspect of the invention, representative MASP-2 inhibitory agents include: molecules that inhibit the biological activity of MASP-2 (such as small molecule inhibitors, anti-MASP-2 antibodies (e.g., MASP-2 inhibitory antibodies) or blocking peptides which interact with MASP-2 or interfere with a protein-protein interaction), and molecules that decrease the expression of MASP-2 (such as MASP-2 antisense nucleic acid molecules, MASP-2 specific RNAi molecules and MASP-2 ribozymes), thereby preventing MASP-2 from activating the lectin complement pathway. The MASP-2 inhibitory agents 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.

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 agent 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), the reduction of C4 cleavage and C4b deposition (measured, for example as described in Example 2), or the reduction of C3 cleavage and C3b deposition (measured, for example, as described in Example 2).

According to the present invention, MASP-2 inhibitory agents are utilized that are effective in inhibiting respiratory distress (or stated another way, improving respiratory function) in a subject infected with coronavirus.

The assessment of respiratory function may be carried out periodically, e.g., each hour, each day, each week, or each month. This assessment is preferably carried out at several time points for a given subject or at one or several time points for a given subject and a healthy control. The assessment may be carried out at regular time intervals, e.g. each hour, each day, each week, or each month. When one assessment has led to the finding of a decrease of respiratory distress (i.e., an increase in respiratory function) , a MASP-2 inhibitory agent, such as a MASP-2 inhibitory antibody, is said to be effective to treat a subject suffering from coronavirus-induced acute respiratory distress syndrome.

MASP-2 inhibitory agents useful in the practice of this aspect of the invention include, for example, MASP-2 antibodies and fragments thereof, MASP-2 inhibitory peptides, small molecules, MASP-2 soluble receptors and expression inhibitors. MASP-2 inhibitory agents may inhibit the MASP-2-dependent complement activation system by blocking the biological function of MASP-2. For example, an inhibitory agent may effectively block MASP-2 protein-to-protein interactions, interfere with MASP-2 dimerization or assembly, block Ca^{2+} binding, interfere with the MASP-2 serine protease active site, or may reduce MASP-2 protein expression.

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

In one embodiment, a MASP-2 inhibitory agent useful in the methods of the invention is a specific MASP-2 inhibitory agent that specifically binds to a polypeptide comprising SEQ ID NO:6 with an affinity of at least ten times greater than to other antigens in the complement system. In another embodiment, a MASP-2 inhibitory agent specifically binds to a polypeptide comprising SEQ ID NO:6 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 agent specifically binds to at least one of (i) the CCP1-CCP2 domain (aa 300-431 of SEQ ID NO:6) or the serine protease domain of MASP-2 (aa 445-682 of SEQ ID NO:6) and inhibits MASP-2-dependent complement activation. In one embodiment, the MASP-2 inhibitory agent is a MASP-2 monoclonal

antibody, or fragment thereof that specifically binds to MASP-2. The binding affinity of the MASP-2 inhibitory agent can be determined using a suitable binding assay.

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 cDNA molecule set forth in SEQ ID NO:4 encodes a representative example of MASP-2 (consisting of the amino acid sequence set forth in SEQ ID NO:5) and provides the human MASP-2 polypeptide with a leader sequence (aa 1-15) that is cleaved after secretion, resulting in the mature form of human MASP-2 (SEQ ID NO:6). As shown in FIGURE 2, the human *MASP 2* gene encompasses twelve exons. The human MASP-2 cDNA is encoded by exons B, C, D, F, G, H, I, J, K AND L. An alternative splice results in a 20 kDa protein termed MBL-associated protein 19 ("MAp19", also referred to as "sMAP") (SEQ ID NO:2), encoded by (SEQ ID NO:1) arising from exons B, C, D and E as shown in FIGURE 2. The cDNA molecule set forth in SEQ ID NO:50 encodes the murine MASP-2 (consisting of the amino acid sequence set forth in SEQ ID NO:51) and provides the murine MASP-2 polypeptide with a leader sequence that is cleaved after secretion, resulting in the mature form of murine MASP-2 (SEQ ID NO:52). The cDNA molecule set forth in SEQ ID NO:53 encodes the rat MASP-2 (consisting of the amino acid sequence set forth in SEQ ID NO:54) and provides the rat MASP-2 polypeptide with a leader sequence that is cleaved after secretion, resulting in the mature form of rat MASP-2 (SEQ ID NO:55).

Those skilled in the art will recognize that the sequences disclosed in SEQ ID NO:4, SEQ ID NO:50 and SEQ ID NO:53 represent single alleles of human, murine and rat MASP-2 respectively, and that allelic variation and alternative splicing are expected to occur. Allelic variants of the nucleotide sequences shown in SEQ ID NO:4, SEQ ID NO:50 and SEQ ID NO:53, 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.

The domains of the human MASP-2 protein (SEQ ID NO:6) are shown in FIGURE 1 and 2A and include an N-terminal C1r/C1s/sea urchin Vegf/bone morphogenic protein (CUBI) domain (aa 1-121 of SEQ ID NO:6), an epidermal growth factor-like domain (aa 122-166), a second CUBI domain (aa 167-293), as well as a tandem of complement control protein domains and a serine protease domain.

Alternative splicing of the *MASP 2* gene results in MAp19 shown in FIGURE 1. MAp19 is a nonenzymatic protein containing the N-terminal CUBI-EGF region of MASP-2 with four additional residues (EQSL) derived from exon E as shown in FIGURE 1.

Several proteins have been shown to bind to, or interact with MASP-2 through protein-to-protein interactions. For example, MASP-2 is known to bind to, and form Ca^{2+} dependent complexes with, the lectin proteins MBL, H-ficolin and L-ficolin. Each MASP-2/lectin complex has been shown to activate complement through the MASP-2-dependent cleavage of proteins C4 and C2 (Ikeda, K., et al., *J. Biol. Chem. 262*:7451-7454, 1987; Matsushita, M., et al., *J. Exp. Med. 176*:1497-2284, 2000; Matsushita, M., et al., *J. Immunol. 168*:3502-3506, 2002). Studies have shown that the CUBI-EGF domains of MASP-2 are essential for the association of MASP-2 with MBL (Thielens, N.M., et al., *J. Immunol. 166*:5068, 2001). It has also been shown that the CUBIEGFCUBII domains mediate dimerization of MASP-2, which is required for formation of an active MBL complex (Wallis, R., et al., *J. Biol. Chem. 275*:30962-30969, 2000). Therefore, MASP-2 inhibitory agents can be identified that bind to or interfere with MASP-2 target regions known to be important for MASP-2-dependent complement activation.

ANTI-MASP-2 INHIBITORY ANTIBODIES

In some embodiments of this aspect of the invention, the MASP-2 inhibitory agent comprises an anti-MASP-2 antibody that inhibits the MASP-2-dependent complement activation system. The anti-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, chimeric, humanized, anti-idiotype, 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.

MASP-2 antibodies can be screened for the ability to inhibit MASP-2-dependent complement activation system and for antifibrotic activity and/or the ability to inhibit renal damage associated with proteinuria or Adriamycin-induced nephropathy using the assays described herein. Several MASP-2 antibodies have been described in the literature and some have been newly generated, some of which are listed below in TABLE 1. For example, as described in Examples 10 and 11 herein, anti-MASP-2 Fab2 antibodies have been identified that block MASP-2-dependent complement activation. As described in

Example 12, and also described in WO2012/151481, which is hereby incorporated herein by reference, fully human MASP-2 scFv antibodies (e.g., OMS646) have been identified that block MASP-2-dependent complement activation. As described in Example 13, and also described in WO2014/144542, which is hereby incorporated herein by reference, SGMI-2 peptide-bearing MASP-2 antibodies and fragments thereof with MASP-2 inhibitory activity were generated by fusing the SGMI-2 peptide amino acid sequence (SEQ ID NO:72, 73 or 74) onto the amino or carboxy termini of the heavy and/or light chains of a human MASP-2 antibody (e.g., OMS646-SGMI-2).

Accordingly, in one embodiment, the MASP-2 inhibitory agent for use in the methods of the invention comprises a human antibody such as, for example OMS646. 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:6), wherein the antibody comprises: (I) (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:67; and ii) a heavy-chain CDR-H2 comprising the amino acid sequence from 50-65 of SEQ ID NO:67; and iii) a heavy-chain CDR-H3 comprising the amino acid sequence from 95-107 of SEQ ID NO:67 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:69; and ii) a light-chain CDR-L2 comprising the amino acid sequence from 50-56 of SEQ ID NO:69; and iii) a light-chain CDR-L3 comprising the amino acid sequence from 89-97 of SEQ ID NO:69, or (II) a variant thereof comprising a heavy-chain variable region with at least 90% identity to SEQ ID NO:67 (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity to SEQ ID NO:67) and a light-chain variable region with at least 90% identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity to SEQ ID NO:69.

In some embodiments, the method comprises administering to the subject a composition comprising an amount of a MASP-2 inhibitory antibody, or antigen binding fragment thereof, comprising a heavy-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:67 and a light-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:69.

In some embodiments, the method comprises administering to the subject a composition comprising a MASP-2 inhibitory antibody, or antigen binding fragment thereof, that specifically recognizes at least part of an epitope on human MASP-2 recognized by reference antibody OMS646 comprising a heavy-chain variable region as set forth in SEQ ID NO:67 and a light-chain variable region as set forth in SEQ ID NO:69. In one embodiment, the MASP-2 inhibitory agent for use in the methods of the invention comprises the human antibody OMS646.

TABLE 1: EXEMPLARY MASP-2 SPECIFIC ANTIBODIES

ANTIGEN	**ANTIBODY TYPE**	**REFERENCE**
Recombinant MASP-2	Rat Polyclonal	Peterson, S.V., et al., *Mol. Immunol.* 37:803-811, 2000
Recombinant human CCP1/2-SP fragment (MoAb 8B5)	Rat MoAb (subclass IgG1)	Moller-Kristensen, M., et al., *J. of Immunol. Methods* 282:159-167, 2003
Recombinant human MAp19 (MoAb 6G12) (cross reacts with MASP-2)	Rat MoAb (subclass IgG1)	Moller-Kristensen, M., et al., *J. of Immunol. Methods* 282:159-167, 2003
hMASP-2	Mouse MoAb (S/P) Mouse MoAb (N-term)	Peterson, S.V., et al., *Mol. Immunol.* 35:409, April 1998
hMASP-2 (CCP1-CCP2-SP domain	rat MoAb: Nimoab101, produced by hybridoma cell line 03050904 (ECACC)	WO 2004/106384
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

ANTIGEN	ANTIBODY TYPE	REFERENCE
Rat MASP-2 (full-length)	MASP-2 Fab2 antibody fragments	Example 10
hMASP-2 (full-length)	Fully human scFv clones	Example 12 and WO2012/151481
hMASP-2 (full-length)	SGMI-2 peptide bearing MASP-2 antibodies	Example 13 and WO2014/144542

ANTI-MASP-2 ANTIBODIES WITH REDUCED EFFECTOR FUNCTION

In some embodiments of this aspect of the invention, the anti-MASP-2 antibodies 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 IgG_2 or IgG_4 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 herein and also 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-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.

PRODUCTION OF ANTI-MASP-2 ANTIBODIES

Anti-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:6 may be used to induce anti-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 CUBIEGF domains, as well as the region encompassing the serine-protease active site, may be expressed as recombinant polypeptides as described in Example 3 and used as antigens. In addition, peptides comprising a portion of at least 6 amino acids of the MASP-2 polypeptide (SEQ ID NO:6) are also useful to induce MASP-2 antibodies. Additional examples of MASP-2 derived antigens useful to induce MASP-2 antibodies are provided below in TABLE 2. 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, such as MASP-2A, as further described herein. In some embodiments of this aspect of the invention, anti-MASP-2 antibodies are obtained using a transgenic mouse strain as described herein.

Antigens useful for producing anti-MASP-2 antibodies also include fusion polypeptides, such as fusions of MASP-2 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.

TABLE 2: MASP-2 DERIVED ANTIGENS

SEQ ID NO:	**Amino Acid Sequence**
SEQ ID NO:6	Human MASP-2 protein
SEQ ID NO:51	Murine MASP-2 protein
SEQ ID NO:8	CUBI domain of human MASP-2 (aa 1-121 of SEQ ID NO:6)
SEQ ID NO:9	CUBIEGF domains of human MASP-2 (aa 1-166 of SEQ ID NO:6)
SEQ ID NO:10	CUBIEGFCUBII domains of human MASP-2 (aa 1-293 of SEQ ID NO:6)

SEQ ID NO:	Amino Acid Sequence
SEQ ID NO:11	EGF domain of human MASP-2 (aa 122-166 of SEQ ID NO:6)
SEQ ID NO:12	Serine-Protease domain of human MASP-2 (aa 429-671 of SEQ ID NO:6)
SEQ ID NO:13 GKDSCRGDAGGALVFL	Serine-Protease inactivated mutant form (aa 610-625 of SEQ ID NO:6 with mutated Ser 618)
SEQ ID NO:14 TPLGPKWPEPVFGRL	Human CUBI peptide
SEQ ID NO:15: TAPPGYRLRLYFTHFDLEL SHLCEYDFVKLSSGAKVL ATLCGQ	Human CUBI peptide
SEQ ID NO:16: TFRSDYSN	MBL binding region in human CUBI domain
SEQ ID NO:17: FYSLGSSLDITFRSDYSNEK PFTGF	MBL binding region in human CUBI domain
SEQ ID NO:18 IDECQVAPG	EGF peptide
SEQ ID NO:19 ANMLCAGLESGGKDSCRG DSGGALV	Peptide from serine-protease active site

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.), page 105. The immunogenicity 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, keyhole limpet hemocyanin and dinitrophenol. Polyclonal antibodies are typically raised in animals such as horses, cows, dogs, chicken, rats, mice, rabbits, guinea pigs, goats, or sheep. Alternatively, an anti-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 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 agent is an anti-MASP-2 monoclonal antibody. Anti-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 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, 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 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. Examples further describing the production of anti-MASP-2 monoclonal antibodies are provided herein (see also *Current Protocols in Immunology*, Vol. 1., John Wiley & Sons, pages 2.5.1-2.6.7, 1991.)

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

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 as further described herein. Transgenic mice with a human immunoglobulin genome are commercially available (e.g., from Abgenix, Inc., Fremont, CA, and Medarex, Inc., Annandale, N.J.). Methods for obtaining human antibodies from transgenic mice are described, for 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 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. A variety of assays known to those skilled in the art may be utilized to detect antibodies which specifically bind to MASP-2. Exemplary assays include Western blot or immunoprecipitation analysis by standard methods (e.g., as described in Ausubel et al.), immunoelectrophoresis, enzyme-linked immuno-sorbent assays, dot blots, inhibition or competition assays and sandwich assays (as described in Harlow and Land, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1988). Once antibodies are identified that specifically bind to MASP-2, the anti-MASP-2 antibodies are tested for the ability to function as a MASP-2 inhibitory agent in one of several assays such as, for example, a lectin-specific C4 cleavage assay (described in Example 2), a C3b deposition assay (described in Example 2) or a C4b deposition assay (described in Example 2).

The affinity of anti-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 anti-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.

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 anti-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 CDRH3 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.

An example of the generation of a humanized anti-MASP-2 antibody from a murine anti-MASP-2 monoclonal antibody is provided herein in Example 6. 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

Anti-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, Fd, 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-IDIOTYPE ANTIBODIES

Once anti-MASP-2 antibodies are identified with the desired inhibitory activity, these antibodies can be used to generate anti-idiotype 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 agents 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 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 sulfhydryl 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 MoAb 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 γ4 IgG isotype, which does not bind Fcγ 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. (Ipswich, Mass.), and Pharmacia LKB Biotechnology Inc. (Piscataway, N.J.).

Another form of an anti-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. CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (see, for example, Larrick et al., *Methods: A Companion to Methods* in *Enzymology 2*:106, 1991; Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Ritter et al. (eds.), page 166, 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 MASP-2 inhibitory agent is a high-affinity human or humanized monoclonal anti-MASP-2 antibody with reduced effector function.

PEPTIDE INHIBITORS

In some embodiments of this aspect of the invention, the MASP-2 inhibitory agent comprises isolated MASP-2 peptide inhibitors, including isolated natural peptide inhibitors and synthetic peptide inhibitors that inhibit the MASP-2-dependent complement activation system. As used herein, the term "isolated MASP-2 peptide inhibitors" refers to peptides that inhibit MASP-2 dependent complement activation by binding to, competing with MASP-2 for binding to another recognition molecule (e.g., MBL, H-ficolin, M-ficolin, or L-ficolin) in the lectin pathway, and/or directly interacting with MASP-2 to inhibit MASP-2-dependent complement activation that are substantially pure and are essentially free of other substances with which they may be found in nature to an extent practical and appropriate for their intended use.

Peptide inhibitors have been used successfully *in vivo* to interfere with protein-protein interactions and catalytic sites. For example, peptide inhibitors to adhesion molecules structurally related to LFA-1 have recently been approved for clinical use in coagulopathies (Ohman, E.M., et al., *European Heart J. 16*:50-55, 1995). Short linear peptides (<30 amino acids) have been described that prevent or interfere with

integrin-dependent adhesion (Murayama, O., et al., *J. Biochem. 120*:445-51, 1996). Longer peptides, ranging in length from 25 to 200 amino acid residues, have also been used successfully to block integrin-dependent adhesion (Zhang, L., et al., *J. Biol. Chem. 271*(47):29953-57, 1996). In general, longer peptide inhibitors have higher affinities and/or slower off-rates than short peptides and may therefore be more potent inhibitors. Cyclic peptide inhibitors have also been shown to be effective inhibitors of integrins *in vivo* for the treatment of human inflammatory disease (Jackson, D.Y., et al., *J. Med. Chem. 40*:3359-68, 1997). One method of producing cyclic peptides involves the synthesis of peptides in which the terminal amino acids of the peptide are cysteines, thereby allowing the peptide to exist in a cyclic form by disulfide bonding between the terminal amino acids, which has been shown to improve affinity and half-life *in vivo* for the treatment of hematopoietic neoplasms (e.g., U.S. Patent No. 6,649,592, to Larson).

SYNTHETIC MASP-2 PEPTIDE INHIBITORS

MASP-2 inhibitory peptides useful in the methods of this aspect of the invention are exemplified by amino acid sequences that mimic the target regions important for MASP-2 function. The inhibitory peptides useful in the practice of the methods of the invention range in size from about 5 amino acids to about 300 amino acids. TABLE 3 provides a list of exemplary inhibitory peptides that may be useful in the practice of this aspect of the present invention. A candidate MASP-2 inhibitory peptide may be tested for the ability to function as a MASP-2 inhibitory agent in one of several assays including, for example, a lectin specific C4 cleavage assay (described in Example 2), and a C3b deposition assay (described in Example 2).

In some embodiments, the MASP-2 inhibitory peptides are derived from MASP-2 polypeptides and are selected from the full length mature MASP-2 protein (SEQ ID NO:6), or from a particular domain of the MASP-2 protein such as, for example, the CUBI domain (SEQ ID NO:8), the CUBIEGF domain (SEQ ID NO:9), the EGF domain (SEQ ID NO:11), and the serine protease domain (SEQ ID NO:12). As previously described, the CUBEGFCUBII regions have been shown to be required for dimerization and binding with MBL (Thielens et al., *supra*). In particular, the peptide sequence TFRSDYN (SEQ ID NO:16) in the CUBI domain of MASP-2 has been shown to be involved in binding to MBL in a study that identified a human carrying a homozygous mutation at Asp105 to Gly105, resulting in the loss of MASP-2 from the MBL complex (Stengaard-Pedersen, K., et al., *New England J. Med. 349*:554-560, 2003).

In some embodiments, MASP-2 inhibitory peptides are derived from the lectin proteins that bind to MASP-2 and are involved in the lectin complement pathway. Several different lectins have been identified that are involved in this pathway, including mannan-binding lectin (MBL), L-ficolin, M-ficolin and H-ficolin. (Ikeda, K., et al., *J. Biol. Chem. 262*:7451-7454, 1987; Matsushita, M., et al., *J. Exp. Med. 176*:1497-2284, 2000; Matsushita, M., et al., *J. Immunol. 168*:3502-3506, 2002). These lectins are present in serum as oligomers of homotrimeric subunits, each having N-terminal collagen-like fibers with carbohydrate recognition domains. These different lectins have been shown to bind to MASP-2, and the lectin/MASP-2 complex activates complement through cleavage of proteins C4 and C2. H-ficolin has an amino-terminal region of 24 amino acids, a collagen-like domain with 11 Gly-Xaa-Yaa repeats, a neck domain of 12 amino acids, and a fibrinogen-like domain of 207 amino acids (Matsushita, M., et al., *J. Immunol. 168*:3502-3506, 2002). H-ficolin binds to GlcNAc and agglutinates human erythrocytes coated with LPS derived from *S. typhimurium*, *S. minnesota* and *E. coli*. H-ficolin has been shown to be associated with MASP-2 and MAp19 and activates the lectin pathway. *Id.* L-ficolin/P35 also binds to GlcNAc and has been shown to be associated with MASP-2 and MAp19 in human serum and this complex has been shown to activate the lectin pathway (Matsushita, M., et al., *J. Immunol. 164*:2281, 2000). Accordingly, MASP-2 inhibitory peptides useful in the present invention may comprise a region of at least 5 amino acids selected from the MBL protein (SEQ ID NO:21), the H-ficolin protein (Genbank accession number NM_173452), the M-ficolin protein (Genbank accession number O00602) and the L-ficolin protein (Genbank accession number NM_015838).

More specifically, scientists have identified the MASP-2 binding site on MBL to be within the 12 Gly-X-Y triplets "GKD GRD GTK GEK GEP GQG LRG LQG POG KLG POG NOG PSG SOG PKG QKG DOG KS" (SEQ ID NO:26) that lie between the hinge and the neck in the C-terminal portion of the collagen-like domain of MBP (Wallis, R., et al., *J. Biol. Chem. 279*:14065, 2004). This MASP-2 binding site region is also highly conserved in human H-ficolin and human L-ficolin. A consensus binding site has been described that is present in all three lectin proteins comprising the amino acid sequence "OGK-X-GP" (SEQ ID NO:22) where the letter "O" represents hydroxyproline and the letter "X" is a hydrophobic residue (Wallis et al., 2004, *supra*). Accordingly, in some embodiments, MASP-2 inhibitory peptides useful in this aspect of the invention are

at least 6 amino acids in length and comprise SEQ ID NO:22. Peptides derived from MBL that include the amino acid sequence "GLR GLQ GPO GKL GPO G" (SEQ ID NO:24) have been shown to bind MASP-2 *in vitro* (Wallis, et al., 2004, *supra*). To enhance binding to MASP-2, peptides can be synthesized that are flanked by two GPO triplets at each end ("GPO GPO GLR GLQ GPO GKL GPO GGP OGP O" SEQ ID NO:25) to enhance the formation of triple helices as found in the native MBL protein (as further described in Wallis, R., et al., *J. Biol. Chem. 279*:14065, 2004).

MASP-2 inhibitory peptides may also be derived from human H-ficolin that include the sequence "GAO GSO GEK GAO GPQ GPO GPO GKM GPK GEO GDO" (SEQ ID NO:27) from the consensus MASP-2 binding region in H-ficolin. Also included are peptides derived from human L-ficolin that include the sequence "GCO GLO GAO GDK GEA GTN GKR GER GPO GPO GKA GPO GPN GAO GEO" (SEQ ID NO:28) from the consensus MASP-2 binding region in L-ficolin.

MASP-2 inhibitory peptides may also be derived from the C4 cleavage site such as "LQRALEILPNRVTIKANRPFLVFI" (SEQ ID NO:29) which is the C4 cleavage site linked to the C-terminal portion of antithrombin III (Glover, G.I., et al., *Mol. Immunol. 25*:1261 (1988)).

TABLE 3: EXEMPLARY MASP-2 INHIBITORY PEPTIDES

SEQ ID NO	**Source**
SEQ ID NO:6	Human MASP-2 protein
SEQ ID NO:8	CUBI domain of MASP-2 (aa 1-121 of SEQ ID NO:6)
SEQ ID NO:9	CUBIEGF domains of MASP-2 (aa 1-166 of SEQ ID NO:6)
SEQ ID NO:10	CUBIEGFCUBII domains of MASP-2 (aa 1-293 of SEQ ID NO:6)
SEQ ID NO:11	EGF domain of MASP-2 (aa 122-166)
SEQ ID NO:12	Serine-protease domain of MASP-2 (aa 429-671)
SEQ ID NO:16	MBL binding region in MASP-2
SEQ ID NO:3	Human MAp19
SEQ ID NO:21	Human MBL protein

SEQ ID NO	Source
SEQ ID NO:22 OGK-X-GP, Where "O" = hydroxyproline and "X" is a hydrophobic amino acid residue	Synthetic peptide Consensus binding site from Human MBL and Human ficolins
SEQ ID NO:23 OGKLG	Human MBL core binding site
SEQ ID NO:24 GLR GLQ GPO GKL GPO G	Human MBP Triplets 6-10- demonstrated binding to MASP-2
SEQ ID NO:25 GPOGPOGLRGLQGPO GKLGPOGGPOGPO	Human MBP Triplets with GPO added to enhance formation of triple helices
SEQ ID NO:26 GKDGRDGTKGEKGEP GQGLRGLQGPOGKLG POGNOGPSGSOGPKG QKGDOGKS	Human MBP Triplets 1-17
SEQ ID NO:27 GAOGSOGEKGAOGPQ GPOGPOGKMGPKGEO GDO	Human H-Ficolin (Hataka)
SEQ ID NO:28 GCOGLOGAOGDKGE AGTNGKRGERGPOGP OGKAGPOGPNGAOGE O	Human L-Ficolin P35
SEQ ID NO:29 LQRALEILPNRVTIKA NRPFLVFI	Human C4 cleavage site
SEQ ID NO:72 LEVTCEPGTTFKDKCNT CRCGSDGKSAVCTKLW CNQ	SGMI-2L (full-length)
SEQ ID NO:73 TCEPGTTFKDKCNTCRC GSDGKSAVCTKLWCNQ	SGMI-2M (medium truncated version)

SEQ ID NO	Source
SEQ ID NO:74 TCRCGSDGKSAVCTKL WCNQ	SGMI-2S (short truncated version)

Note: The letter "O" represents hydroxyproline. The letter "X" is a hydrophobic residue.

Peptides derived from the C4 cleavage site as well as other peptides that inhibit the MASP-2 serine protease site can be chemically modified so that they are irreversible protease inhibitors. For example, appropriate modifications may include, but are not necessarily limited to, halomethyl ketones (Br, Cl, I, F) at the C-terminus, Asp or Glu, or appended to functional side chains; haloacetyl (or other α-haloacetyl) groups on amino groups or other functional side chains; epoxide or imine-containing groups on the amino or carboxy termini or on functional side chains; or imidate esters on the amino or carboxy termini or on functional side chains. Such modifications would afford the advantage of permanently inhibiting the enzyme by covalent attachment of the peptide. This could result in lower effective doses and/or the need for less frequent administration of the peptide inhibitor.

In addition to the inhibitory peptides described above, MASP-2 inhibitory peptides useful in the method of the invention include peptides containing the MASP-2-binding CDRH3 region of anti-MASP-2 MoAb obtained as described herein. The sequence of the CDR regions for use in synthesizing the peptides may be determined by methods known in the art. The heavy chain variable region is a peptide that generally ranges from 100 to 150 amino acids in length. The light chain variable region is a peptide that generally ranges from 80 to 130 amino acids in length. The CDR sequences within the heavy and light chain variable regions include only approximately 3-25 amino acid sequences that may be easily sequenced by one of ordinary skill in the art.

Those skilled in the art will recognize that substantially homologous variations of the MASP-2 inhibitory peptides described above will also exhibit MASP-2 inhibitory activity. Exemplary variations include, but are not necessarily limited to, peptides having insertions, deletions, replacements, and/or additional amino acids on the carboxy-terminus or amino-terminus portions of the subject peptides and mixtures thereof. Accordingly, those homologous peptides having MASP-2 inhibitory activity are considered to be useful in the methods of this invention. The peptides described may also

include duplicating motifs and other modifications with conservative substitutions. Conservative variants are described elsewhere herein, and include the exchange of an amino acid for another of like charge, size or hydrophobicity and the like.

MASP-2 inhibitory peptides may be modified to increase solubility and/or to maximize the positive or negative charge in order to more closely resemble the segment in the intact protein. The derivative may or may not have the exact primary amino acid structure of a peptide disclosed herein so long as the derivative functionally retains the desired property of MASP-2 inhibition. The modifications can include amino acid substitution with one of the commonly known twenty amino acids or with another amino acid, with a derivatized or substituted amino acid with ancillary desirable characteristics, such as resistance to enzymatic degradation or with a D-amino acid or substitution with another molecule or compound, such as a carbohydrate, which mimics the natural confirmation and function of the amino acid, amino acids or peptide; amino acid deletion; amino acid insertion with one of the commonly known twenty amino acids or with another amino acid, with a derivatized or substituted amino acid with ancillary desirable characteristics, such as resistance to enzymatic degradation or with a D-amino acid or substitution with another molecule or compound, such as a carbohydrate, which mimics the natural confirmation and function of the amino acid, amino acids or peptide; or substitution with another molecule or compound, such as a carbohydrate or nucleic acid monomer, which mimics the natural conformation, charge distribution and function of the parent peptide. Peptides may also be modified by acetylation or amidation.

The synthesis of derivative inhibitory peptides can rely on known techniques of peptide biosynthesis, carbohydrate biosynthesis and the like. As a starting point, the artisan may rely on a suitable computer program to determine the conformation of a peptide of interest. Once the conformation of peptide disclosed herein is known, then the artisan can determine in a rational design fashion what sort of substitutions can be made at one or more sites to fashion a derivative that retains the basic conformation and charge distribution of the parent peptide but which may possess characteristics which are not present or are enhanced over those found in the parent peptide. Once candidate derivative molecules are identified, the derivatives can be tested to determine if they function as MASP-2 inhibitory agents using the assays described herein.

SCREENING FOR MASP-2 INHIBITORY PEPTIDES

One may also use molecular modeling and rational molecular design to generate and screen for peptides that mimic the molecular structures of key binding regions of MASP-2 and inhibit the complement activities of MASP-2. The molecular structures used for modeling include the CDR regions of anti-MASP-2 monoclonal antibodies, as well as the target regions known to be important for MASP-2 function including the region required for dimerization, the region involved in binding to MBL, and the serine protease active site as previously described. Methods for identifying peptides that bind to a particular target are well known in the art. For example, molecular imprinting may be used for the *de novo* construction of macromolecular structures such as peptides that bind to a particular molecule. See, for example, Shea, K.J., "Molecular Imprinting of Synthetic Network Polymers: The De Novo synthesis of Macromolecular Binding and Catalytic Sties," *TRIP 2*(5) 1994.

As an illustrative example, one method of preparing mimics of MASP-2 binding peptides is as follows. Functional monomers of a known MASP-2 binding peptide or the binding region of an anti-MASP-2 antibody that exhibits MASP-2 inhibition (the template) are polymerized. The template is then removed, followed by polymerization of a second class of monomers in the void left by the template, to provide a new molecule that exhibits one or more desired properties that are similar to the template. In addition to preparing peptides in this manner, other MASP-2 binding molecules that are MASP-2 inhibitory agents such as polysaccharides, nucleosides, drugs, nucleoproteins, lipoproteins, carbohydrates, glycoproteins, steroid, lipids and other biologically active materials can also be prepared. This method is useful for designing a wide variety of biological mimics that are more stable than their natural counterparts because they are typically prepared by free radical polymerization of function monomers, resulting in a compound with a nonbiodegradable backbone.

PEPTIDE SYNTHESIS

The MASP-2 inhibitory peptides can be prepared using techniques well known in the art, such as the solid-phase synthetic technique initially described by Merrifield, in *J. Amer. Chem. Soc. 85*:2149-2154, 1963. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Foster City, Calif.) in accordance with the instructions provided by the manufacturer. Other techniques may be

found, for example, in Bodanszky, M., et al., *Peptide Synthesis*, second edition, John Wiley & Sons, 1976, as well as in other reference works known to those skilled in the art.

The peptides can also be prepared using standard genetic engineering techniques known to those skilled in the art. For example, the peptide can be produced enzymatically by inserting nucleic acid encoding the peptide into an expression vector, expressing the DNA, and translating the DNA into the peptide in the presence of the required amino acids. The peptide is then purified using chromatographic or electrophoretic techniques, or by means of a carrier protein that can be fused to, and subsequently cleaved from, the peptide by inserting into the expression vector in phase with the peptide encoding sequence a nucleic acid sequence encoding the carrier protein. The fusion protein-peptide may be isolated using chromatographic, electrophoretic or immunological techniques (such as binding to a resin via an antibody to the carrier protein). The peptide can be cleaved using chemical methodology or enzymatically, as by, for example, hydrolases.

The MASP-2 inhibitory peptides that are useful in the method of the invention can also be produced in recombinant host cells following conventional techniques. To express a MASP-2 inhibitory peptide encoding sequence, a nucleic acid molecule encoding the peptide must be operably linked to regulatory sequences that control transcriptional expression in an expression vector and then introduced into a host cell. In addition to transcriptional regulatory sequences, such as promoters and enhancers, expression vectors can include translational regulatory sequences and a marker gene, which are suitable for selection of cells that carry the expression vector.

Nucleic acid molecules that encode a MASP-2 inhibitory peptide can be synthesized with "gene machines" using protocols such as the phosphoramidite method. If chemically synthesized double-stranded DNA is required for an application such as the synthesis of a gene or a gene fragment, then each complementary strand is made separately. The production of short genes (60 to 80 base pairs) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. For the production of longer genes, synthetic genes (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length. For reviews on polynucleotide synthesis, see, for example, Glick and Pasternak, "*Molecular Biotechnology, Principles and Applications of*

Recombinant DNA", ASM Press, 1994; Itakura, K., et al., *Annu. Rev. Biochem. 53*:323, 1984; and Climie, S., et al., *Proc. Nat'l Acad. Sci. USA 87*:633, 1990.

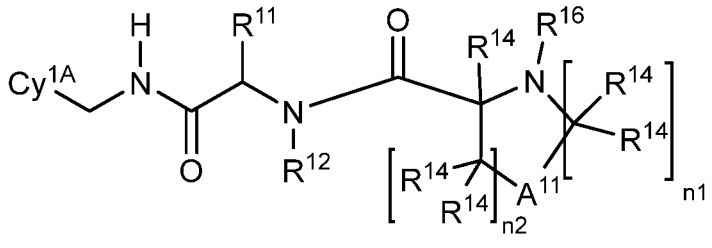
SMALL MOLECULE INHIBITORS OF MASP-2

In some embodiments, MASP-2 inhibitory agents are small molecule inhibitors including natural, semi-synthetic, and synthetic substances that have a low molecular weight (*e.g.*, between 50 and 1000 Da), such as for example, peptides, peptidomimetics, and non-peptide inhibitors (*e.g.*, oligonucleotides and organic compounds). Small molecule inhibitors of MASP-2 can be generated based on the molecular structure of the variable regions of the anti-MASP-2 antibodies.

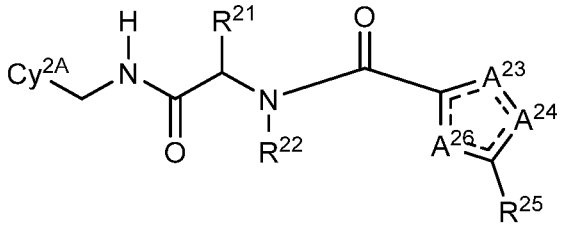
Small molecule inhibitors may also be designed and generated based on the MASP-2 crystal structure using computational drug design (Kuntz I.D., et al., *Science 257*:1078, 1992). The crystal structure of rat MASP-2 has been described (Feinberg, H., et al., *EMBO J. 22*:2348-2359, 2003). Using the method described by Kuntz et al., the MASP-2 crystal structure coordinates are used as an input for a computer program such as DOCK, which outputs a list of small molecule structures that are expected to bind to MASP-2. Use of such computer programs is well known to one of skill in the art. For example, the crystal structure of the HIV-1 protease inhibitor was used to identify unique nonpeptide ligands that are HIV-1 protease inhibitors by evaluating the fit of compounds found in the Cambridge Crystallographic database to the binding site of the enzyme using the program DOCK (Kuntz, I.D., et al., *J. Mol. Biol. 161*:269-288, 1982; DesJarlais, R.L., et al., *PNAS 87*:6644-6648, 1990).

Exemplary MASP-2 inhibitors include, but are not limited to, compounds disclosed in U.S. Patent Application Nos. 62/943,629, 62/943,622, 62/943,611, 62/943,599, 16/425,791 and PCT Application No. PCT/US19/34220, each of which are hereby incorporated by reference in their entirety.

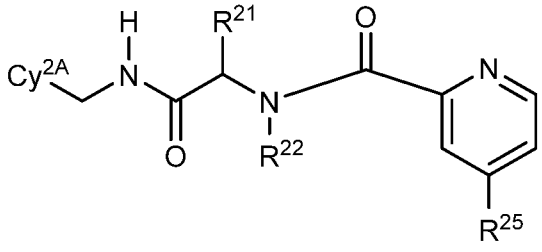
In some embodiments, the small molecule is a compound of Formula (I-1), (IIA), (IIB), (III) or (IV):



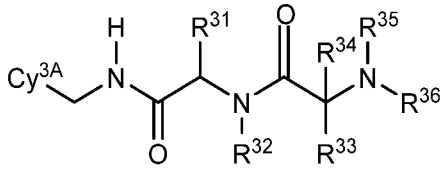
(I-1)



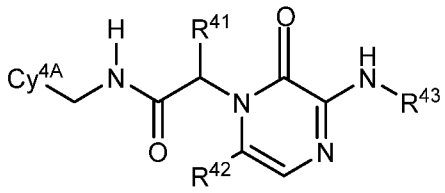
(IIA)



(IIB)



(III)



(IV)

or a salt thereof, wherein:

Cy^{1A} is unsubstituted or substituted C_{6-10} aryl or unsubstituted or substituted 5-10 membered heteroaryl; wherein the ring atoms of the 5-10 membered heteroaryl forming Cy^{1A} consist of carbon atoms and 1, 2, or 3 heteroatoms selected from O, N and S; wherein the substituted C_{6-10} aryl or substituted 5-10 membered heteroaryl forming Cy^{1A} are substituted with 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy1A}, halogen, C_{1-6} haloalkyl, CN, OR^{a11}, S^{Ra11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $C(=NOR^{a11})NR^{c11}R^{d11}$,

$C(=NOC(O)R^{b11})NR^{c11}R^{d11}$, $C(=NR^{e11})NR^{c11}C(O)OR^{a11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo;

each R^{Cy1A} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10-membered heterocycloalkyl forming R^{Cy1A} consist of carbon atoms and 1, 2, 3 or 4 heteroatoms selected from O, N and S, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{Cy1A} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo, and wherein each C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl forming R^{Cy1A} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo;

R^{11} is H or C_{1-6} alkyl, C_{6-10} aryl-C_{1-6} alkyl or 5-10 membered heteroaryl-C_{1-6} alkyl, wherein the C_{1-6} alkyl forming R^{11} is unsubstituted or substituted by 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo, and wherein the C_{6-10} aryl-C_{1-6} alkyl or 5-10 membered heteroaryl-C_{1-6} alkyl forming R^{11} is unsubstituted or substituted by 1, 2 or 3 substituents independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$,

$C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo;

R^{12} is H or C_{1-6} alkyl; or

R^{11} and R^{12}, together with the groups to which they are attached, form a 4-6 membered heterocycloalkyl ring;

A^{11} is $CR^{13}R^{15}$ or N;

each R^{13} is independently Cy^{1B}, $(CR^{13A}R^{13B})_{n3}Cy^{1B}$, ($C_{1-6}$ alkylene)Cy^{1B}, (C_{2-6} alkenylene)Cy^{1B}, (C_{2-6} alkynylene)Cy^{1B} or OCy^{1B}, wherein the C_{1-6} alkylene, C_{2-6} alkenylene, or C_{2-6} alkynylene component of R^{13} is unsubstituted or substituted by 1, 2, 3, 4 or 5 substituents each independently selected from the group consisting of halogen, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo;

each R^{14} is independently selected from H and C_{1-6} alkyl;

R^{15} is selected from H, R^{13}, C_{1-6} alkyl and OH;

a pair of R^{14} groups attached to adjacent carbon atoms, or a pairing of R^{14} and R^{15} groups attached to adjacent carbon atoms, may, independently of other occurrences of R^{14}, together be replaced a bond connecting the adjacent carbon atoms to which the pair of R^{14} groups or pairing of R^{14} and R^{15} groups is attached, such that the adjacent carbon atoms are connected by a double bond; or

a pair of R^{14} groups attached to the same carbon atom, or a pairing of R^{13} and R^{15} groups attached to the same carbon atom, may, independently of other occurrences of R^{14}, and together with the carbon atom to which the pair of R^{14} groups or pairing of R^{13} and R^{15} groups is attached together form a spiro-fused C_{3-10} cycloalkyl or 4-10 membered

heterocycloalkyl ring, wherein the ring atoms of the 4-10 membered heterocycloalkyl ring formed consist of carbon atoms and 1, 2, or 3 heteroatoms selected from O, N and S, wherein the spiro-fused C_{3-10} cycloalkyl or 4-10 membered heterocycloalkyl ring formed is optionally further substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, haloalkyl, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo; or

pairs of R^{14} groups attached to adjacent carbon atoms, or a pairing of R^{14} and R^{15} groups attached to adjacent carbon atoms, may, independently of other occurrences of R^{14}, together with the adjacent carbon atoms to which the pair of R^{14} groups or pairing of R^{14} and R^{15} groups is attached, form a fused C_{3-10} cycloalkyl or 4-10 membered heterocycloalkyl ring, wherein the ring atoms of the 4-10 membered heterocycloalkyl ring formed consist of carbon atoms and 1, 2, or 3 heteroatoms selected from O, N and S, wherein the fused C_{3-10} cycloalkyl or 4-10 membered heterocycloalkyl ring formed is optionally further substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, haloalkyl, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo; or

a grouping of four R^{14} groups attached to two adjacent carbon atoms, or a grouping of two R^{14} , one R^{13} and one R^{15} groups attached to two adjacent carbon atoms, may, independently of other occurrences of R^{14}, together with the two adjacent carbon atoms to which the grouping of four R^{14} groups or grouping of two R^{14} , one R^{13} and one R^{15} groups are attached, form a fused C_{6-10} aryl or 5-10 membered heteroaryl, C_{3-10} cycloalkyl or 4-10 membered heterocycloalkyl ring, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10 membered heterocycloalkyl ring formed consist of carbon atoms and 1, 2, or 3 heteroatoms selected from O, N and S, and wherein the fused C_{6-10}

aryl or 5-10 membered heteroaryl, C_{3-10} cycloalkyl or 4-10 membered heterocycloalkyl ring formed is optionally further substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, haloalkyl, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo;

n1 is 1 or 2;

n2 is 0, 1 or 2;

provided that the sum of n1 and n2 is 1, 2 or 3;

provided that if n1 is 1 or n2 is 0, then A^{11} is $CR^{13}R^{15}$;

n3 is 0, 1 or 2;

each R^{13A} is independently H or C_{1-6} alkyl;

each R^{13B} is independently H or C_{1-6} alkyl; or

or R^{13A} and R^{13B} attached to the same carbon atom, independently of any other R^{13A} and R^{13B} groups, together may form $-(CH_2)_{2-5}-$, thereby forming a 3-6 membered cycloalkyl ring;

Cy^{1B} is unsubstituted or substituted C_{6-10} aryl, unsubstituted or substituted 5-10 membered heteroaryl, unsubstituted or substituted C_{3-10} cycloalkyl, or unsubstituted or substituted 4-10 membered heterocycloalkyl; wherein the ring atoms of the 5-10 membered heteroaryl or 4-10 membered heterocycloalkyl forming Cy^{1B} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; and

wherein the substituted C_{6-10} aryl, substituted 5-10 membered heteroaryl, substituted C_{3-10} cycloalkyl or substituted 4-10 membered heterocycloalkyl forming Cy^{1B} are substituted with 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy1B}, halogen, C_{1-6} haloalkyl, CN, OR^{a11}, S^{Ra11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$,

$OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $C(=NOR^{a11})NR^{c11}R^{d11}$, $C(=NOC(O)R^{b11})NR^{c11}R^{d11}$, $C(=NR^{e11})NR^{c11}C(O)OR^{a11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo;

wherein each R^{Cy1B} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10 membered heterocycloalkyl forming R^{Cy1B} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; wherein each C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{Cy1B} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)^{Rb11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo; and wherein each C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl forming R^{Cy1B} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)^{Rb11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo;

R^{16} is H, Cy^{1C}, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, wherein the C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{16} is unsubstituted or substituted by 1, 2, 3, 4 or 5 substituents selected from the group consisting of Cy^{1C}, halogen, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$,

$S(O)_2NR^{c11}R^{d11}$ and oxo, provided that no more than one of the substituents of R^{16} is Cy^{1C};

Cy^{1C} is unsubstituted or substituted C_{6-10} aryl, unsubstituted or substituted 5-10 membered heteroaryl, unsubstituted or substituted C_{3-10} cycloalkyl, or unsubstituted or substituted 4-10 membered heterocycloalkyl; wherein the ring atoms of the 5-10 membered heteroaryl or 4-10 membered heterocycloalkyl forming Cy^{1C} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; and

wherein the substituted C_{6-10} aryl, substituted 5-10 membered heteroaryl, substituted C_{3-10} cycloalkyl or substituted 4-10 membered heterocycloalkyl forming Cy^{1C} are substituted with 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy1C}, halogen, C_{1-6} haloalkyl, CN, OR^{a11}, S^{Ra11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)R^{b11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $C(=NOR^{a11})NR^{c11}R^{d11}$, $C(=NOC(O)R^{b11})NR^{c11}R^{d11}$, $C(=NR^{e11})NR^{c11}C(O)OR^{a11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo;

wherein each R^{Cy1C} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10 membered heterocycloalkyl forming R^{Cy1C} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; wherein each C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{Cy1C} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)^{Rb11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo; and wherein each C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl forming R^{Cy1C} is independently unsubstituted or substituted with 1, 2 or

3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a11}, SR^{a11}, $C(O)R^{b11}$, $C(O)NR^{c11}R^{d11}$, $C(O)OR^{a11}$, $OC(O)R^{b11}$, $OC(O)NR^{c11}R^{d11}$, $NR^{c11}R^{d11}$, $NR^{c11}C(O)^{Rb11}$, $NR^{c11}C(O)NR^{c11}R^{d11}$, $NR^{c11}C(O)OR^{a11}$, $C(=NR^{e11})NR^{c11}R^{d11}$, $NR^{c11}C(=NR^{e11})NR^{c11}R^{d11}$, $S(O)R^{b11}$, $S(O)NR^{c11}R^{d11}$, $S(O)_2R^{b11}$, $NR^{c11}S(O)_2R^{b11}$, $S(O)_2NR^{c11}R^{d11}$ and oxo;

R^{a11}, R^{b11}, R^{c11} and R^{d11} are each independently selected from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, C_{3-7} cycloalkyl, 5-10 membered heteroaryl, 4-10 membered heterocycloalkyl, C_{6-10} aryl-C_{1-3} alkyl, 5-10 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-10 membered heterocycloalkyl-C_{1-3} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, C_{3-7} cycloalkyl, 5-10 membered heteroaryl, 4-10 membered heterocycloalkyl, C_{6-10} aryl-C_{1-3} alkyl, 5-10 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-10 membered heterocycloalkyl-C_{1-3} alkyl forming R^{a11}, R^{b11}, R^{c11} and R^{d11} are each optionally substituted with 1, 2, 3, 4 or 5 substituents independently selected from C_{1-6} alkyl, halo, CN, OR^{a12}, SR^{a12}, $C(O)R^{b12}$, $C(O)NR^{c12}R^{d12}$, $C(O)OR^{a12}$, $OC(O)R^{b12}$, $OC(O)NR^{c12}R^{d12}$, $NR^{c12}R^{d12}$, $NR^{c12}C(O)R^{b12}$, $NR^{c12}C(O)NR^{c12}R^{d12}$, $NR^{c12}C(O)OR^{a12}$, $C(=NR^{e12})NR^{c12}R^{d12}$, $NR^{c12}C(=NR^{e12})NR^{c12}R^{d12}$, $S(O)R^{b12}$, $S(O)NR^{c12}R^{d12}$, $S(O)_2R^{b12}$, $NR^{c12}S(O)_2R^{b12}$, $S(O)_2NR^{c12}R^{d12}$ and oxo;

or R^{c11} and R^{d11} attached to the same N atom, together with the N atom to which they are both attached, form a 4-, 5-, 6- or 7-membered heterocycloalkyl group or 5-membered heteroaryl group, each optionally substituted with 1, 2 or 3 substituents independently selected from C_{1-6} alkyl, halo, CN, OR^{a12}, SR^{a12}, $C(O)R^{b12}$, $C(O)NR^{c12}R^{d12}$, $C(O)OR^{a12}$, $OC(O)R^{b12}$, $OC(O)NR^{c12}R^{d12}$, $NR^{c12}R^{d12}$, $NR^{c12}C(O)R^{b12}$, $NR^{c12}C(O)NR^{c12}R^{d12}$, $NR^{c12}C(O)OR^{a12}$, $C(=NR^{e12})NR^{c12}R^{d12}$, $NR^{c12}C(=NR^{e12})NR^{c12}R^{d12}$, $S(O)R^{b12}$, $S(O)NR^{c12}R^{d12}$, $S(O)_2R^{b12}$, $NR^{c12}S(O)_2R^{b12}$, $S(O)_2NR^{c12}R^{d12}$ and oxo;

R^{a12}, R^{b12}, R^{c12} and R^{d12} are each independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, phenyl, C_{3-7} cycloalkyl, 5-6 membered heteroaryl, 4-7 membered heterocycloalkyl, phenyl-C_{1-3} alkyl, 5-6 membered heteroaryl-C_{1-3} alkyl, C_{3-7}

cycloalkyl-C_{1-3} alkyl and 4-7 membered heterocycloalkyl-C_{1-3} alkyl, wherein said C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, phenyl, C_{3-7} cycloalkyl, 5-6 membered heteroaryl, 4-7 membered heterocycloalkyl, phenyl-C_{1-3} alkyl, 5-6 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-7 membered heterocycloalkyl-C_{1-3} alkyl forming R^{a12}, R^{b12}, R^{c12} and R^{d12} are each optionally substituted with 1, 2 or 3 substituents independently selected from OH, CN, amino, NH(C_{1-6} alkyl), N(C_{1-6} alkyl)$_2$, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy and oxo;

or R^{c12} and R^{d12} attached to the same N atom, together with the N atom to which they are both attached, form a 4-, 5-, 6- or 7-membered heterocycloalkyl group or 5-membered heteroaryl group, each of which is unsubstituted or substituted with 1, 2 or 3 substituents independently selected from OH, CN, amino, NH(C_{1-6} alkyl), N(C_{1-6} alkyl)$_2$, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy and oxo;

R^{e11} and Re^{12} are each, independently, H, CN or NO_2;

Cy^{2A} is unsubstituted or substituted C_{6-10} aryl or unsubstituted or substituted 5-10 membered heteroaryl; wherein the ring atoms of the 5-10 membered heteroaryl forming Cy^{2A} consist of carbon atoms and 1, 2, or 3 heteroatoms selected from O, N and S; wherein the substituted C_{6-10} aryl or substituted 5-10 membered heteroaryl forming Cy^{2A} are substituted with 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy2A}, halogen, C_{1-6} haloalkyl, CN, OR^{a21}, S^{Ra21}, $C(O)R^{b21}$, $C(O)NR^{c21}R^{d21}$, $C(O)OR^{a21}$, $OC(O)R^{b21}$, $OC(O)NR^{c21}R^{d21}$, $NR^{c21}R^{d21}$, $NR^{c21}C(O)R^{b21}$, $NR^{c21}C(O)NR^{c21}R^{d21}$, $NR^{c21}C(O)OR^{a21}$, $C(=NR^{e21})NR^{c21}R^{d21}$, $C(=NOR^{a21})NR^{c21}R^{d21}$, $C(=NOC(O)R^{b21})NR^{c21}R^{d21}$, $C(=NR^{e21})NR^{c21}C(O)OR^{a21}$, $NR^{c21}C(=NR^{e21})NR^{c21}R^{d21}$, $S(O)R^{b21}$, $S(O)NR^{c21}R^{d21}$, $S(O)_2R^{b21}$, $NR^{c21}S(O)_2R^{b21}$, $S(O)_2NR^{c21}R^{d21}$ and oxo;

each R^{Cy2A} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10-membered heterocycloalkyl forming R^{Cy2A} consist of carbon atoms and 1, 2, 3 or 4

heteroatoms selected from O, N and S, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{Cy2A} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a21}, SR^{a21}, $C(O)R^{b21}$, $C(O)NR^{c21}R^{d21}$, $C(O)OR^{a21}$, $OC(O)R^{b21}$, $OC(O)NR^{c21}R^{d21}$, $NR^{c21}R^{d21}$, $NR^{c21}C(O)R^{b21}$, $NR^{c21}C(O)NR^{c21}R^{d21}$, $NR^{c21}C(O)OR^{a21}$, $C(=NR^{e21})NR^{c21}R^{d21}$, $NR^{c21}C(=NR^{e21})NR^{c21}R^{d21}$, $S(O)R^{b21}$, $S(O)NR^{c21}R^{d21}$, $S(O)_2R^{b21}$, $NR^{c21}S(O)_2R^{b21}$, $S(O)_2NR^{c21}R^{d21}$ and oxo, and wherein each C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl forming R^{Cy2A} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a21}, SR^{a21}, $C(O)R^{b21}$, $C(O)NR^{c21}R^{d21}$, $C(O)OR^{a21}$, $OC(O)R^{b21}$, $OC(O)NR^{c21}R^{d21}$, $NR^{c21}R^{d21}$, $NR^{c21}C(O)R^{b21}$, $NR^{c21}C(O)NR^{c21}R^{d21}$, $NR^{c21}C(O)OR^{a21}$, $C(=NR^{e21})NR^{c21}R^{d21}$, $NR^{c21}C(=NR^{e21})NR^{c21}R^{d21}$, $S(O)R^{b21}$, $S(O)NR^{c21}R^{d21}$, $S(O)_2R^{b21}$, $NR^{c21}S(O)_2R^{b21}$, $S(O)_2NR^{c21}R^{d21}$ and oxo;

R^{21} is H or C_{1-6} alkyl, C_{6-10} aryl-C_{1-6} alkyl or 5-10 membered heteroaryl-C_{1-6} alkyl, wherein the C_{1-6} alkyl forming R^{21} is unsubstituted or substituted by 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a21}, SR^{a21}, $C(O)R^{b21}$, $C(O)NR^{c21}R^{d21}$, $C(O)OR^{a21}$, $OC(O)R^{b21}$, $OC(O)NR^{c21}R^{d21}$, $NR^{c21}R^{d21}$, $NR^{c21}C(O)R^{b21}$, $NR^{c21}C(O)NR^{c21}R^{d21}$, $NR^{c21}C(O)OR^{a21}$, $C(=NR^{e21})NR^{c21}R^{d21}$, $NR^{c21}C(=NR^{e21})NR^{c21}R^{d21}$, $S(O)R^{b21}$, $S(O)NR^{c21}R^{d21}$, $S(O)_2R^{b21}$, $NR^{c21}S(O)_2R^{b21}$, $S(O)_2NR^{c21}R^{d21}$ and oxo, and wherein the C_{6-10} aryl-C_{1-6} alkyl or 5-10 membered heteroaryl-C_{1-6} alkyl forming R^{21} is unsubstituted or substituted by 1, 2 or 3 substituents independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a21}, SR^{a21}, $C(O)R^{b21}$, $C(O)NR^{c21}R^{d21}$, $C(O)OR^{a21}$, $OC(O)R^{b21}$, $OC(O)NR^{c21}R^{d21}$, $NR^{c21}R^{d21}$, $NR^{c21}C(O)R^{b21}$, $NR^{c21}C(O)NR^{c21}R^{d21}$, $NR^{c21}C(O)OR^{a21}$, $C(=NR^{e21})NR^{c21}R^{d21}$, $NR^{c21}C(=NR^{e21})NR^{c21}R^{d21}$, $S(O)R^{b21}$, $S(O)NR^{c21}R^{d21}$, $S(O)_2R^{b21}$, $NR^{c21}S(O)_2R^{b21}$, $S(O)_2NR^{c21}R^{d21}$ and oxo;

R^{22} is H or C_{1-6} alkyl; or

R^{21} and R^{22}, together with the groups to which they are attached, form a 4-6 membered heterocycloalkyl ring;

A^{23} is N or NR^{23};

A^{24} is CR^{24}; N or NR^{24};

A^{26} is CR^{26} or S;

provided that

A^{23}, A^{24} and A^{26} in Formula (IIA) are selected such that the ring comprising A^{23}, A^{24} and A^{26} is a heteroaryl ring and the symbol ⎓ represents an aromatic ring (normalized) bond;

R^{23} is H or C_{1-6} alkyl;

R^{24} is H; C_{1-6} alkyl or phenyl;

R^{25} is Cy^{2B}, $(CR^{25A}R^{25B})_{n25}Cy^{2B}$, ($C_{1-6}$ alkylene)Cy^{2B}, (C_{2-6} alkenylene)Cy^{2B}, or (C_{2-6} alkynylene)Cy^{2B}, wherein the C_{1-6} alkylene, C_{2-6} alkenylene, or C_{2-6} alkynylene component of R^{25} is unsubstituted or substituted by 1, 2, 3, 4 or 5 substituents each independently selected from the group consisting of halogen, CN, OR^{a21}, SR^{a21}, $C(O)R^{b21}$, $C(O)NR^{c21}R^{d21}$, $C(O)OR^{a21}$, $OC(O)R^{b21}$, $OC(O)NR^{c21}R^{d21}$, $NR^{c21}R^{d21}$, $NR^{c21}C(O)R^{b21}$, $NR^{c21}C(O)NR^{c21}R^{d21}$, $NR^{c21}C(O)OR^{a21}$, $C(=NR^{e21})NR^{c21}R^{d21}$, $NR^{c21}C(=NR^{e21})NR^{c21}R^{d21}$, $S(O)R^{b21}$, $S(O)NR^{c21}R^{d21}$, $S(O)_2R^{b21}$, $NR^{c21}S(O)_2R^{b21}$, $S(O)_2NR^{c21}R^{d21}$ and oxo;

R^{26} is H or C_{1-6} alkyl;

each R^{25A} is H or C_{1-6} alkyl;

each R^{25B} is H or C_{1-6} alkyl;

n25 is 0, 1 or 2;

Cy^{2B} is unsubstituted or substituted C_{6-10} aryl, unsubstituted or substituted 5-10 membered heteroaryl, unsubstituted or substituted C_{3-10} cycloalkyl, or unsubstituted or

substituted 4-10 membered heterocycloalkyl; wherein the ring atoms of the 5-10 membered heteroaryl or 4-10 membered heterocycloalkyl forming Cy^{2B} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; and

wherein the substituted C_{6-10} aryl, substituted 5-10 membered heteroaryl, substituted C_{3-10} cycloalkyl or substituted 4-10 membered heterocycloalkyl forming Cy^{2B} are substituted with 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy2B}, halogen, C_{1-6} haloalkyl, CN, OR^{a21}, S^{Ra21}, $C(O)R^{b21}$, $C(O)NR^{c21}R^{d21}$, $C(O)OR^{a21}$, $OC(O)R^{b21}$, $OC(O)NR^{c21}R^{d21}$, $NR^{c21}R^{d21}$, $NR^{c21}C(O)R^{b21}$, $NR^{c21}C(O)NR^{c21}R^{d21}$, $NR^{c21}C(O)OR^{a21}$, $C(=NR^{e21})NR^{c21}R^{d21}$, $C(=NOR^{a21})NR^{c21}R^{d21}$, $C(=NOC(O)R^{b21})NR^{c21}R^{d21}$, $C(=NR^{e21})NR^{c21}C(O)OR^{a21}$, $NR^{c21}C(=NR^{e21})NR^{c21}R^{d21}$, $S(O)R^{b21}$, $S(O)NR^{c21}R^{d21}$, $S(O)_2R^{b21}$, $NR^{c21}S(O)_2R^{b21}$, $S(O)_2NR^{c21}R^{d21}$ and oxo;

wherein each R^{Cy2B} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10 membered heterocycloalkyl forming R^{Cy2B} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; wherein each C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{Cy2B} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a21}, SR^{a21}, $C(O)R^{b21}$, $C(O)NR^{c21}R^{d21}$, $C(O)OR^{a21}$, $OC(O)R^{b21}$, $OC(O)NR^{c21}R^{d21}$, $NR^{c21}R^{d21}$, $NR^{c21}C(O)^{Rb21}$, $NR^{c21}C(O)NR^{c21}R^{d21}$, $NR^{c21}C(O)OR^{a21}$, $C(=NR^{e21})NR^{c21}R^{d21}$, $NR^{c21}C(=NR^{e21})NR^{c21}R^{d21}$, $S(O)R^{b21}$, $S(O)NR^{c21}R^{d21}$, $S(O)_2R^{b21}$, $NR^{c21}S(O)_2R^{b21}$, $S(O)_2NR^{c21}R^{d21}$ and oxo; and wherein each C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl forming R^{Cy2B} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a21}, SR^{a21}, $C(O)R^{b21}$, $C(O)NR^{c21}R^{d21}$, $C(O)OR^{a21}$, $OC(O)R^{b21}$, $OC(O)NR^{c21}R^{d21}$, $NR^{c21}R^{d21}$, $NR^{c21}C(O)^{Rb21}$, $NR^{c21}C(O)NR^{c21}R^{d21}$, $NR^{c21}C(O)OR^{a21}$, $C(=NR^{e21})NR^{c21}R^{d21}$, $NR^{c21}C(=NR^{e21})NR^{c21}R^{d21}$, $S(O)R^{b21}$, $S(O)NR^{c21}R^{d21}$, $S(O)_2R^{b21}$, $NR^{c21}S(O)_2R^{b21}$, $S(O)_2NR^{c21}R^{d21}$ and oxo;

are each independently selected from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, C_{3-7} cycloalkyl, 5-10 membered heteroaryl, 4-10 membered heterocycloalkyl, C_{6-10} aryl-C_{1-3} alkyl, 5-10 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-10 membered heterocycloalkyl-C_{1-3} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, C_{3-7} cycloalkyl, 5-10 membered heteroaryl, 4-10 membered heterocycloalkyl, C_{6-10} aryl-C_{1-3} alkyl, 5-10 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-10 membered heterocycloalkyl-C_{1-3} alkyl forming R^{a21}, R^{b21}, R^{c21} and R^{d21} are each optionally substituted with 1, 2, 3, 4 or 5 substituents independently selected from C_{1-6} alkyl, halo, CN, OR^{a22}, SR^{a22}, $C(O)R^{b22}$, $C(O)NR^{c22}R^{d22}$, $C(O)OR^{a22}$, $OC(O)R^{b22}$, $OC(O)NR^{c22}R^{d22}$, $NR^{c22}R^{d22}$, $NR^{c22}C(O)R^{b22}$, $NR^{c22}C(O)NR^{c22}R^{d22}$, $NR^{c22}C(O)OR^{a22}$, $C(=NR^{e22})NR^{c22}R^{d22}$, $NR^{c22}C(=NR^{e22})NR^{c22}R^{d22}$, $S(O)R^{b22}$, $S(O)NR^{c22}R^{d22}$, $S(O)_2R^{b22}$, $NR^{c22}S(O)_2R^{b22}$, $S(O)_2NR^{c22}R^{d22}$ and oxo;

or R^{c21} and R^{d21} attached to the same N atom, together with the N atom to which they are both attached, form a 4-, 5-, 6- or 7-membered heterocycloalkyl group or 5-membered heteroaryl group, each optionally substituted with 1, 2 or 3 substituents independently selected from C_{1-6} alkyl, halo, CN, OR^{a22}, SR^{a22}, $C(O)R^{b22}$, $C(O)NR^{c22}R^{d22}$, $C(O)OR^{a22}$, $OC(O)R^{b22}$, $OC(O)NR^{c22}R^{d22}$, $NR^{c22}R^{d22}$, $NR^{c22}C(O)R^{b22}$, $NR^{c22}C(O)NR^{c22}R^{d22}$, $NR^{c22}C(O)OR^{a22}$, $C(=NR^{e22})NR^{c22}R^{d22}$, $NR^{c22}C(=NR^{e22})NR^{c22}R^{d22}$, $S(O)R^{b22}$, $S(O)NR^{c22}R^{d22}$, $S(O)_2R^{b22}$, $NR^{c22}S(O)_2R^{b22}$, $S(O)_2NR^{c22}R^{d22}$ and oxo;

R^{a22}, R^{b22}, R^{c22} and R^{d22} are each independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, phenyl, C_{3-7} cycloalkyl, 5-6 membered heteroaryl, 4-7 membered heterocycloalkyl, phenyl-C_{1-3} alkyl, 5-6 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-7 membered heterocycloalkyl-C_{1-3} alkyl, wherein said C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, phenyl, C_{3-7} cycloalkyl, 5-6 membered heteroaryl, 4-7 membered heterocycloalkyl, phenyl-C_{1-3} alkyl, 5-6 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-7 membered heterocycloalkyl-C_{1-3} alkyl forming R^{a22}, R^{b22}, R^{c22} and R^{d22} are each optionally substituted with 1, 2 or 3 substituents

independently selected from OH, CN, amino, NH(C_{1-6} alkyl), N(C_{1-6} alkyl)$_2$, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy and oxo;

or R^{c22} and R^{d22} attached to the same N atom, together with the N atom to which they are both attached, form a 4-, 5-, 6- or 7-membered heterocycloalkyl group or 5-membered heteroaryl group, each of which is unsubstituted or substituted with 1, 2 or 3 substituents independently selected from OH, CN, amino, NH(C_{1-6} alkyl), N(C_{1-6} alkyl)$_2$, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy and oxo;

R^{e21} and Re^{22} are each, independently, H, CN or NO_2;

Cy^{3A} is unsubstituted or substituted C_{6-10} aryl or unsubstituted or substituted 5-10 membered heteroaryl; wherein the ring atoms of the 5-10 membered heteroaryl forming Cy^{3A} consist of carbon atoms and 1, 2, or 3 heteroatoms selected from O, N and S; wherein the substituted C_{6-10} aryl or substituted 5-10 membered heteroaryl forming Cy^{3A} are substituted with 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy3A}, halogen, C_{1-6} haloalkyl, CN, OR^{a31}, S^{Ra31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)R^{b31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $C(=NOR^{a31})NR^{c31}R^{d31}$, $C(=NOC(O)R^{b31})NR^{c31}R^{d31}$, $C(=NR^{e31})NR^{c31}C(O)OR^{a31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo;

each R^{Cy3A} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10-membered heterocycloalkyl forming R^{Cy3A} consist of carbon atoms and 1, 2, 3 or 4 heteroatoms selected from O, N and S, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{Cy3A} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a31}, SR^{a31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)R^{b31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$,

$S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo, and wherein each C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl forming R^{Cy3A} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a31}, SR^{a31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)R^{b31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo;

R^{31} is H or C_{1-6} alkyl, C_{6-10} aryl-C_{1-6} alkyl or 5-10 membered heteroaryl-C_{1-6} alkyl, wherein the C_{1-6} alkyl forming R^{31} is unsubstituted or substituted by 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a31}, SR^{a31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)R^{b31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo, and wherein the C_{6-10} aryl-C_{1-6} alkyl or 5-10 membered heteroaryl-C_{1-6} alkyl forming R^{31} is unsubstituted or substituted by 1, 2 or 3 substituents independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a31}, SR^{a31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)R^{b31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo;

R^{32} is H or C_{1-6} alkyl; or

R^{31} and R^{32}, together with the groups to which they are attached, form a 4-6 membered heterocycloalkyl ring;

R^{33} is Cy^{3B}, $(CR^{33A}R^{33B})_{n33}Cy^{3B}$, ($C_{1-6}$ alkylene)Cy^{3B}, (C_{2-6} alkenylene)Cy^{3B}, or (C_{2-6} alkynylene)Cy^{3B}, wherein the C_{1-6} alkylene, C_{2-6} alkenylene, or C_{2-6} alkynylene component of R^{35} is unsubstituted or substituted by 1, 2, 3, 4 or 5 substituents each independently selected from the group consisting of halogen, CN, OR^{a31}, SR^{a31},

$C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)R^{b31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo;

each R^{33A} is independently H or C_{1-6} alkyl;

each R^{33B} is independently H or C_{1-6} alkyl; or

or R^{33A} and R^{33B} attached to the same carbon atom, independently of any other R^{33A} and R^{33B} groups, together may form $–(CH_2)_{2-5}$-, thereby forming a 3-6 membered cycloalkyl ring;

n33 is 0, 1, 2 or 3;

Cy^{3B} is unsubstituted or substituted C_{6-10} aryl, unsubstituted or substituted 5-10 membered heteroaryl, unsubstituted or substituted C_{3-10} cycloalkyl, or unsubstituted or substituted 4-10 membered heterocycloalkyl; wherein the ring atoms of the 5-10 membered heteroaryl or 4-10 membered heterocycloalkyl forming Cy^{3B} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; and

wherein the substituted C_{6-10} aryl, substituted 5-10 membered heteroaryl, substituted C_{3-10} cycloalkyl or substituted 4-10 membered heterocycloalkyl forming Cy^{3B} are substituted with 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy3B}, halogen, C_{1-6} haloalkyl, CN, OR^{a31}, S^{Ra31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)R^{b31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $C(=NOR^{a31})NR^{c31}R^{d31}$, $C(=NOC(O)R^{b31})NR^{c31}R^{d31}$, $C(=NR^{e31})NR^{c31}C(O)OR^{a31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo;

wherein each R^{Cy3B} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10

membered heterocycloalkyl forming R^{Cy3B} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; wherein each C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{Cy3B} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a31}, SR^{a31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)^{Rb31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo; and wherein each C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl forming R^{Cy3B} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a31}, SR^{a31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)^{Rb31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo;

R^{34} is selected from H and C_{1-6} alkyl;

R^{35} is selected from H, unsubstituted or substituted C_{1-6} alkyl and Cy^{3C}, wherein the substituted C_{1-6} alkyl forming R^{35} is substituted by 1, 2, 3, 4 or 5 substituents selected from the group consisting of Cy^{3C}, halogen, CN, OR^{a31}, SR^{a31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)R^{b31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo; provided that no more than one of the substituents of R^{35} is Cy^{3C};

Cy^{3C} is unsubstituted or substituted C_{6-10} aryl, unsubstituted or substituted 5-10 membered heteroaryl, unsubstituted or substituted C_{3-10} cycloalkyl, or unsubstituted or substituted 4-10 membered heterocycloalkyl; wherein the ring atoms of the 5-10 membered heteroaryl or 4-10 membered heterocycloalkyl forming Cy^{3C} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; and

wherein the substituted C_{6-10} aryl, substituted 5-10 membered heteroaryl, substituted C_{3-10} cycloalkyl or substituted 4-10 membered heterocycloalkyl forming Cy^{3C} are substituted with 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy3C}, halogen, C_{1-6} haloalkyl, CN, OR^{a31}, S^{Ra31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)R^{b31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $C(=NOR^{a31})NR^{c31}R^{d31}$, $C(=NOC(O)R^{b31})NR^{c31}R^{d31}$, $C(=NR^{e31})NR^{c31}C(O)OR^{a31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo;

wherein each R^{Cy3C} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10 membered heterocycloalkyl forming R^{Cy3C} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; wherein each C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{Cy3C} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a31}, SR^{a31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)^{Rb31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo; and wherein each C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl forming R^{Cy3C} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a31}, SR^{a31}, $C(O)R^{b31}$, $C(O)NR^{c31}R^{d31}$, $C(O)OR^{a31}$, $OC(O)R^{b31}$, $OC(O)NR^{c31}R^{d31}$, $NR^{c31}R^{d31}$, $NR^{c31}C(O)^{Rb31}$, $NR^{c31}C(O)NR^{c31}R^{d31}$, $NR^{c31}C(O)OR^{a31}$, $C(=NR^{e31})NR^{c31}R^{d31}$, $NR^{c31}C(=NR^{e31})NR^{c31}R^{d31}$, $S(O)R^{b31}$, $S(O)NR^{c31}R^{d31}$, $S(O)_2R^{b31}$, $NR^{c31}S(O)_2R^{b31}$, $S(O)_2NR^{c31}R^{d31}$ and oxo;

R^{36} is selected from H and C_{1-6} alkyl;

R^{a31}, R^{b31}, R^{c31} and R^{d31} are each independently selected from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, C_{3-7} cycloalkyl, 5-10 membered heteroaryl, 4-10 membered heterocycloalkyl, C_{6-10} aryl-C_{1-3} alkyl, 5-10 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-10 membered heterocycloalkyl-C_{1-3} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, C_{3-7} cycloalkyl, 5-10 membered heteroaryl, 4-10 membered heterocycloalkyl, C_{6-10} aryl-C_{1-3} alkyl, 5-10 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-10 membered heterocycloalkyl-C_{1-3} alkyl forming R^{a31}, R^{b31}, R^{c31} and R^{d31} are each optionally substituted with 1, 2, 3, 4 or 5 substituents independently selected from C_{1-6} alkyl, halo, CN, OR^{a32}, SR^{a32}, $C(O)R^{b32}$, $C(O)NR^{c32}R^{d32}$, $C(O)OR^{a32}$, $OC(O)R^{b32}$, $OC(O)NR^{c32}R^{d32}$, $NR^{c32}R^{d32}$, $NR^{c32}C(O)R^{b32}$, $NR^{c32}C(O)NR^{c32}R^{d32}$, $NR^{c32}C(O)OR^{a32}$, $C(=NR^{e32})NR^{c32}R^{d32}$, $NR^{c32}C(=NR^{e32})NR^{c32}R^{d32}$, $S(O)R^{b32}$, $S(O)NR^{c32}R^{d32}$, $S(O)_2R^{b32}$, $NR^{c32}S(O)_2R^{b32}$, $S(O)_2NR^{c32}R^{d32}$ and oxo;

or R^{c31} and R^{d31} attached to the same N atom, together with the N atom to which they are both attached, form a 4-, 5-, 6- or 7-membered heterocycloalkyl group or 5-membered heteroaryl group, each optionally substituted with 1, 2 or 3 substituents independently selected from C_{1-6} alkyl, halo, CN, OR^{a32}, SR^{a32}, $C(O)R^{b32}$, $C(O)NR^{c32}R^{d32}$, $C(O)OR^{a32}$, $OC(O)R^{b32}$, $OC(O)NR^{c32}R^{d32}$, $NR^{c32}R^{d32}$, $NR^{c32}C(O)R^{b32}$, $NR^{c32}C(O)NR^{c32}R^{d32}$, $NR^{c32}C(O)OR^{a32}$, $C(=NR^{e32})NR^{c32}R^{d32}$, $NR^{c32}C(=NR^{e32})NR^{c32}R^{d32}$, $S(O)R^{b32}$, $S(O)NR^{c32}R^{d32}$, $S(O)_2R^{b32}$, $NR^{c32}S(O)_2R^{b32}$, $S(O)_2NR^{c32}R^{d32}$ and oxo;

R^{a32}, R^{b32}, R^{c32} and R^{d32} are each independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, phenyl, C_{3-7} cycloalkyl, 5-6 membered heteroaryl, 4-7 membered heterocycloalkyl, phenyl-C_{1-3} alkyl, 5-6 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-7 membered heterocycloalkyl-C_{1-3} alkyl, wherein said C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, phenyl, C_{3-7} cycloalkyl, 5-6 membered heteroaryl, 4-7 membered heterocycloalkyl, phenyl-C_{1-3} alkyl, 5-6 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-7 membered heterocycloalkyl-C_{1-3} alkyl forming R^{a32}, R^{b32}, R^{c32} and R^{d32} are each optionally substituted with 1, 2 or 3 substituents

independently selected from OH, CN, amino, $NH(C_{1-6}$ alkyl), $N(C_{1-6}$ alkyl)$_2$, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy and oxo;

or R^{c32} and R^{d32} attached to the same N atom, together with the N atom to which they are both attached, form a 4-, 5-, 6- or 7-membered heterocycloalkyl group or 5-membered heteroaryl group, each of which is unsubstituted or substituted with 1, 2 or 3 substituents independently selected from OH, CN, amino, $NH(C_{1-6}$ alkyl), $N(C_{1-6}$ alkyl)$_2$, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy and oxo; and

R^{e31} and Re^{32} are each, independently, H, CN or NO_2;

Cy^{4A} is unsubstituted or substituted C_{6-10} aryl or unsubstituted or substituted 5-10 membered heteroaryl; wherein the ring atoms of the 5-10 membered heteroaryl forming Cy^{4A} consist of carbon atoms and 1, 2, or 3 heteroatoms selected from O, N and S; wherein the substituted C_{6-10} aryl or substituted 5-10 membered heteroaryl forming Cy^{4A} are substituted with 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy4A}, halogen, C_{1-6} haloalkyl, CN, OR^{a41}, S^{Ra41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $C(=NOR^{a41})NR^{c41}R^{d41}$, $C(=NOC(O)R^{b41})NR^{c41}R^{d41}$, $C(=NR^{e41})NR^{c41}C(O)OR^{a41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo;

each R^{Cy4A} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10-membered heterocycloalkyl forming R^{Cy4A} consist of carbon atoms and 1, 2, 3 or 4 heteroatoms selected from O, N and S, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{Cy4A} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a41}, SR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$,

$S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo, and wherein each C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl forming R^{Cy4A} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a41}, SR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo;

R^{41} is H or C_{1-6} alkyl, C_{6-10} aryl-C_{1-6} alkyl or 5-10 membered heteroaryl-C_{1-6} alkyl, wherein the C_{1-6} alkyl forming R^{41} is unsubstituted or substituted by 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a41}, SR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo, and wherein the C_{6-10} aryl-C_{1-6} alkyl or 5-10 membered heteroaryl-C_{1-6} alkyl forming R^{41} is unsubstituted or substituted by 1, 2 or 3 substituents independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a41}, SR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo;

R^{42} is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, or Cy^{4B}; wherein each of the C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, forming R^{42} is unsubstituted or substituted by 1, 2, 3, 4 or 5 substituents selected from the group consisting of Cy^{4B}, halogen, CN, OR^{a41}, SR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo; provided that no more than one of the substituents is Cy^{4B};

Cy^{4B} is unsubstituted or substituted C_{6-10} aryl, unsubstituted or substituted 5-10 membered heteroaryl, unsubstituted or substituted C_{3-10} cycloalkyl, or unsubstituted or substituted 4-10 membered heterocycloalkyl; wherein the ring atoms of the 5-10 membered heteroaryl or unsubstituted or substituted 4-10 membered heterocycloalkyl forming Cy^{4B} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; and wherein the substituted C_{6-10} aryl, substituted 5-10 membered heteroaryl substituted C_{3-10} cycloalkyl, or 4-10 membered heterocycloalkyl forming Cy^{4B} is substituted with 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy4B}, halogen, C_{1-6} haloalkyl, CN, OR^{a41}, S^{Ra41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $C(=NOR^{a41})NR^{c41}R^{d41}$, $C(=NOC(O)R^{b41})NR^{c41}R^{d41}$, $C(=NR^{e41})NR^{c41}C(O)OR^{a41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo;

wherein each R^{Cy4B} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10-membered heterocycloalkyl forming R^{Cy4B} consist of carbon atoms and 1, 2, or 3 heteroatoms selected from O, N and S, and wherein each C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{Cy4B} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a41}, SR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo; and \each C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl forming each R^{Cy4B} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a41}, SR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$,

$NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo;

or R^{41} and R^{42}, together with the atoms to which they are attached and the nitrogen atom linking the atoms to which R^{41} and R^{42} are attached, form a 4-7 membered heterocycloalkyl ring; which is optionally further substituted by 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy4B}, halogen, C_{1-6} haloalkyl, CN, OR^{a41}, S^{Ra41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $C(=NOR^{a41})NR^{c41}R^{d41}$, $C(=NOC(O)R^{b41})NR^{c41}R^{d41}$, $C(=NR^{e41})NR^{c41}C(O)OR^{a41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo;

R^{43} is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, or Cy^{4C}; wherein each of the C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{43} is unsubstituted or substituted by 1, 2, 3, 4 or 5 substituents each independently selected from: 0, 1, 2, 3, 4 or 5 substituents selected from the group consisting of Cy^{4C}, halogen, CN, OR^{a41}, SR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo, provided that no more than one substituent of the C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{43} is Cy^{4C};

Cy^{4C} is unsubstituted or substituted C_{6-10} aryl, unsubstituted or substituted 5-10 membered heteroaryl, unsubstituted or substituted C_{3-10} cycloalkyl, or unsubstituted or substituted 4-10 membered heterocycloalkyl; wherein the ring atoms of the 5-10 membered heteroaryl or unsubstituted or substituted 4-10 membered heterocycloalkyl forming Cy^{4B} consist of carbon atoms and 1, 2 or 3 heteroatoms selected from O, N and S; and wherein the substituted C_{6-10} aryl, substituted 5-10 membered heteroaryl substituted C_{3-10} cycloalkyl, or 4-10 membered heterocycloalkyl forming Cy^{4C} is

substituted with 1, 2, 3, 4 or 5 substituents each independently selected from R^{Cy4C}, halogen, C_{1-6} haloalkyl, CN, OR^{a41}, S^{Ra41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $C(=NOR^{a41})NR^{c41}R^{d41}$, $C(=NOC(O)R^{b41})NR^{c41}R^{d41}$, $C(=NR^{e41})NR^{c41}C(O)OR^{a41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo;

each R^{Cy4C} is independently selected from C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl, wherein the ring atoms of the 5-10 membered heteroaryl or 4-10-membered heterocycloalkyl forming R^{Cy4C} consist of carbon atoms and 1, 2, or 3 heteroatoms selected from O, N and S, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl forming R^{Cy4C} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, CN, OR^{a41}, SR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo; and wherein each C_{6-10} aryl, 5-10 membered heteroaryl, C_{3-10} cycloalkyl and 4-10 membered heterocycloalkyl forming each R^{Cy4A} is independently unsubstituted or substituted with 1, 2 or 3 substituents independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, CN, OR^{a41}, SR^{a41}, $C(O)R^{b41}$, $C(O)NR^{c41}R^{d41}$, $C(O)OR^{a41}$, $OC(O)R^{b41}$, $OC(O)NR^{c41}R^{d41}$, $NR^{c41}R^{d41}$, $NR^{c41}C(O)R^{b41}$, $NR^{c41}C(O)NR^{c41}R^{d41}$, $NR^{c41}C(O)OR^{a41}$, $C(=NR^{e41})NR^{c41}R^{d41}$, $NR^{c41}C(=NR^{e41})NR^{c41}R^{d41}$, $S(O)R^{b41}$, $S(O)NR^{c41}R^{d41}$, $S(O)_2R^{b41}$, $NR^{c41}S(O)_2R^{b41}$, $S(O)_2NR^{c41}R^{d41}$ and oxo;

R^{a41}, R^{b41}, R^{c41} and R^{d41} are each independently selected from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, C_{3-7} cycloalkyl, 5-10 membered heteroaryl, 4-10 membered heterocycloalkyl, C_{6-10} aryl-C_{1-3} alkyl, 5-10 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-10 membered heterocycloalkyl-C_{1-3} alkyl, wherein said C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, C_{3-7} cycloalkyl, 5-10 membered

heteroaryl, 4-10 membered heterocycloalkyl, C_{6-10} aryl-C_{1-3} alkyl, 5-10 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-10 membered heterocycloalkyl-C_{1-3} alkyl forming R^{a41}, R^{b41}, R^{c41} and R^{d41} are each optionally substituted with 1, 2, 3, 4 or 5 substituents independently selected from C_{1-6} alkyl, halo, CN, OR^{a42}, SR^{a42}, $C(O)R^{b42}$, $C(O)NR^{c42}R^{d42}$, $C(O)OR^{a42}$, $OC(O)R^{b42}$, $OC(O)NR^{c42}R^{d42}$, $NR^{c42}R^{d42}$, $NR^{c42}C(O)R^{b42}$, $NR^{c42}C(O)NR^{c42}R^{d42}$, $NR^{c42}C(O)OR^{a42}$, $C(=NR^{e42})NR^{c42}R^{d42}$, $NR^{c42}C(=NR^{e42})NR^{c42}R^{d42}$, $S(O)R^{b42}$, $S(O)NR^{c42}R^{d42}$, $S(O)_2R^{b42}$, $NR^{c42}S(O)_2R^{b42}$, $S(O)_2NR^{c42}R^{d42}$ and oxo;

or R^{c41} and R^{d41} attached to the same N atom, together with the N atom to which they are both attached, form a 4-, 5-, 6- or 7-membered heterocycloalkyl group or 5-membered heteroaryl group, each optionally substituted with 1, 2 or 3 substituents independently selected from C_{1-6} alkyl, halo, CN, OR^{a42}, SR^{a42}, $C(O)R^{b42}$, $C(O)NR^{c42}R^{d42}$, $C(O)OR^{a42}$, $OC(O)R^{b42}$, $OC(O)NR^{c42}R^{d42}$, $NR^{c42}R^{d42}$, $NR^{c42}C(O)R^{b42}$, $NR^{c42}C(O)NR^{c42}R^{d42}$, $NR^{c42}C(O)OR^{a42}$, $C(=NR^{e42})NR^{c42}R^{d42}$, $NR^{c42}C(=NR^{e42})NR^{c42}R^{d42}$, $S(O)R^{b42}$, $S(O)NR^{c42}R^{d42}$, $S(O)_2R^{b42}$, $NR^{c42}S(O)_2R^{b42}$, $S(O)_2NR^{c42}R^{d42}$ and oxo;

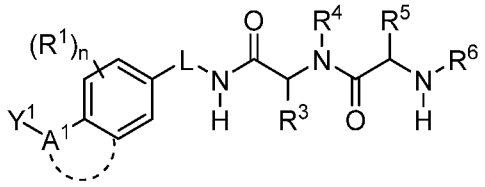
R^{a42}, R^{b42}, R^{c42} and R^{d42} are each independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, phenyl, C_{3-7} cycloalkyl, 5-6 membered heteroaryl, 4-7 membered heterocycloalkyl, phenyl-C_{1-3} alkyl, 5-6 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-7 membered heterocycloalkyl-C_{1-3} alkyl, wherein said C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, phenyl, C_{3-7} cycloalkyl, 5-6 membered heteroaryl, 4-7 membered heterocycloalkyl, phenyl-C_{1-3} alkyl, 5-6 membered heteroaryl-C_{1-3} alkyl, C_{3-7} cycloalkyl-C_{1-3} alkyl and 4-7 membered heterocycloalkyl-C_{1-3} alkyl forming R^{a42}, R^{b42}, R^{c42} and R^{d42} are each optionally substituted with 1, 2 or 3 substituents independently selected from OH, CN, amino, $NH(C_{1-6}$ alkyl), $N(C_{1-6}$ alkyl)$_2$, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy and oxo;

or R^{c42} and R^{d42} attached to the same N atom, together with the N atom to which they are both attached, form a 4-, 5-, 6- or 7-membered heterocycloalkyl group or 5-membered heteroaryl group, each of which is unsubstituted or substituted with 1, 2 or 3

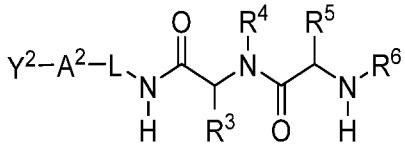
substituents independently selected from OH, CN, amino, $NH(C_{1-6}$ alkyl), $N(C_{1-6}$ alkyl)$_2$, halo, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy and oxo; and

R^{e41} and Re^{42} are each, independently, H, CN or NO_2.

In some embodiments, the small molecule is a compound Formula (**VA**) or (**VB**):



(**VA**),



(**VB**)

or a salt thereof;

wherein:

A^1 is a member selected from the group consisting of –(C=NH)–,–(C=NOR^a)–, –[C=NO(C=O)R^a]–, –[C=N[O(C=O)ZR^b]}–, a fused 5- or 6-member heterocyclyl, and a fused 5- or 6-member heteroaryl;

when A^1 is –(C=NH)–, Y^1 is selected from the group consisting of $–NH_2$, –NH(C=O)R^a, and – NH(C=O)ZR^b;

when A^1 is –(C=NOR^a)–, –[C=NO(C=O)R^a]–, or –{C=N[O(C=O)ZR^b]}–, Y^1 is $–NH_2$;

when A^1 is fused heterocyclyl or heteroaryl, Y^1 is $–NH_2$ or halo, and A^1 is substituted with m additional R^1 groups;

each R^a and R^b is independently selected from the group consisting of C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{10} aryl, and C_7-C_{12} arylalkyl; wherein R^a has m substituents selected from the group consisting of C_1-C_6 alkyl, hydroxyl, hydroxyl(C_1-C_6 alkyl), C_1-C_6 alkoxy, C_2-C_9 alkoxyalkyl, amino, C_1-C_6 alkylamino, and halo; or, alternatively, R^a and

R^b join to form an heterocyclyl ring with m substituents selected from the group consisting of C_1-C_6 alkyl, hydroxyl, C_1-C_6 alkoxy, and halo;

each Z is independently selected from the group consisting of O and S;

A^2 is a member selected from the group consisting of C_3-C_6 heteroaryl, C_6 aryl, and C_2-C_6 alkyl;

when A^2 is C_3-C_6 heteroaryl, Y^2 is selected from the group consisting of $–NH_2$, $–CH_2NH_2$, chloro, $–(C=NH)NH_2$, $–(C=NH)NH(C=O)R^a$, $–(C=NH)NH(C=O)ZR^b$, $–(C=NOR^a)NH_2$, $–[C=NO(C=O)R^a]NH_2$, and $–\{C=N[O(C=O)ZR^b]\}NH_2$; and A^2 is substituted with m additional R^1 groups;

when A^2 is C_6 aryl, Y^2 is selected from the group consisting of aminomethyl, hydroxy, and halo, and A^2 is substituted with m additional R^1 groups;

when A^2 is C_2-C_6 alkyl, Y^2 is selected from the group consisting of $–NH(C=NH)NH_2$, $–NH(C=NH)NH(C=O)R^a$, and $–NH(C=NH)NH(C=O)ZR^b$;

each R^1 is a member independently selected from the group consisting of C_1-C_6 alkyl, hydroxyl, C_1-C_6 alkoxy, amino, C_1-C_6 alkylamino, and halo;

each m and n is an independently selected integer from 0 to 3;

L is $–(O)_p–(C(R^{2a})(R^{2b}))_q–$,

each R^{2a} or R^{2b} is a member independently selected from the group consisting of hydrogen and fluoro;

p is an integer from 0 to 1;

q is an integer from 1 to 2;

R^3 is a member selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, and carboxy(C_1-C_6 alkyl); or, alternatively, R^3 and R^4 join to form an azetidine, pyrrolidine, or piperidine ring;

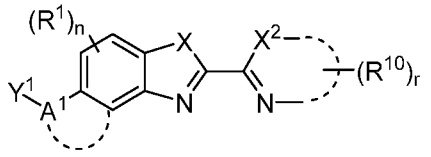
R^4 is a member selected from the group consisting of hydrogen and C_1-C_6 alkyl; or, alternatively, R^4 and R^3 join to form an azetidine, pyrrolidine, or piperidine ring;

R^5 is a member selected from the group consisting of C_3-C_7 cycloalkyl, C_4-C_8 cycloalkylalkyl, heteroaryl, and C_7-C_{12} arylalkyl or heteroarylalkyl with from 0 to 3 R^{13} substituents; or, alternatively, R^5 and R^6 join to form a heterocyclic ring with from 0 to 3 R^{13} substituents;

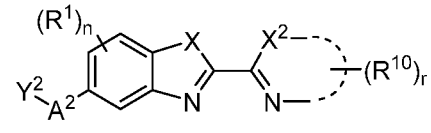
R^6 is a member selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, carboxy(C_1-C_6 alkyl), C_7-C_{12} arylalkyl or heteroarylalkyl with from 0 to 3 R^{13} substituents, amino(C_1-C_8 alkyl); and amido(C_1-C_8 alkyl); or, alternatively, R^6 and R^5 join to form a heterocyclic ring with from 0 to 3 R^{13} substituents; and

each R^{13} is a member independently selected from the group consisting of C_1-C_6 alkyl, C_6-C_{10} aryl, (C_6-C_{10} aryl)C_1-C_6 alkyl, carboxy(C_1-C_6 alkyloxy), heteroaryl, (C_6-C_{10} heteroaryl)C_1-C_6 alkyl, heterocyclyl, hydroxyl, hydroxyl(C_1-C_6 alkyl), C_1-C_6 alkoxy, C_2-C_9 alkoxyalkyl, amino, C_1-C_6 amido, C_1-C_6 alkylamino, and halo; or, alternatively, two R^{13} groups join to form a fused C_6-C_{10} aryl, C_6-C_{10} heteroaryl, or C_5-C_7 cycloalkyl ring.

In some embodiments, the small molecule is a compound of Formula (VIA) or (**VIB**):



(VIA)



(VIB)

or a salt thereof; wherein:

A^1 is a member selected from the group consisting of –(C=NH)–,–(C=NOR^a)–, –[C=NO(C=O)R^a]–, –[C=N[O(C=O)ZR^b]–, a fused 5- or 6-member heterocyclyl, and a fused 5- or 6-member heteroaryl;

when A^1 is –(C=NH)–, Y^1 is selected from the group consisting of $–NH_2$, –NH(C=O)R^a, and – NH(C=O)ZR^b;

when A^1 is –(C=NOR^a)–, –[C=NO(C=O)R^a]–, or –{C=N[O(C=O)ZR^b]}–, Y^1 is $–NH_2$;

when A^1 is fused heterocyclyl or heteroaryl, Y^1 is $–NH_2$ or halo, and A^1 is substituted with m additional R^1 groups;

each R^a and R^b is independently selected from the group consisting of C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{10} aryl, and C_7-C_{12} arylalkyl; wherein R^a has m substituents selected from the group consisting of C_1-C_6 alkyl, hydroxyl, hydroxyl(C_1-C_6 alkyl), C_1-C_6 alkoxy, C_2-C_9 alkoxyalkyl, amino, C_1-C_6 alkylamino, and halo; or, alternatively, R^a and R^b join to form an heterocyclyl ring with m substituents selected from the group consisting of C_1-C_6 alkyl, hydroxyl, C_1-C_6 alkoxy, and halo;

each Z is independently selected from the group consisting of O and S;

A^2 is a member selected from the group consisting of C_3-C_6 heteroaryl and C_2-C_6 alkyl;

when A^2 is C_3-C_6 heteroaryl, Y^2 is selected from the group consisting of $–NH_2$, $–CH_2NH_2$, chloro, –(C=NH)NH_2, –(C=NH)NH(C=O)R^a, –(C=NH)NH(C=O)ZR^b, –(C=NOR^a)NH_2, –[C=NO(C=O)R^a]NH_2, and –{C=N[O(C=O)ZR^b]}NH_2; and A^2 is substituted with m additional R^1 groups;

when A^2 is C_2-C_6 alkyl, Y^2 is selected from the group consisting of –NH(C=NH)NH_2,

–NH(C=NH)NH(C=O)R^a, and –NH(C=NH)NH(C=O)ZR^b;

each R^1 is a member independently selected from the group consisting of C_1-C_6 alkyl, hydroxyl, C_1-C_6 alkoxy, amino, C_1-C_6 alkylamino, and halo;

each m and n is an independently selected integer from 0 to 3;

X and X^2 are each a member selected from the group consisting of NR^8, CH, and CR^{10};

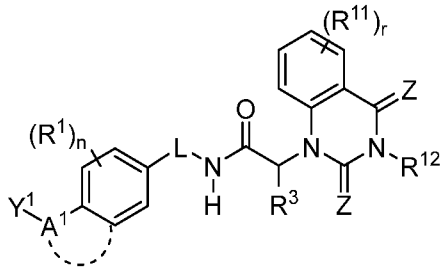
each R^8 is a member independently selected from the group consisting of hydrogen and C_1-C_6 alkyl;

each R^{10} is a member independently selected from the group consisting of C_1-C_6 alkyl, heteroaryl or C_6-C_{10} aryl with from 0 to 3 R^{13} substituents, hydroxyl, hydroxyl(C_1-C_6 alkyl), C_1-C_6 alkoxy, C_2-C_9 alkoxyalkyl, amino, C_1-C_6 alkylamino, and halo; or, alternatively, two R^{10} groups join to form a fused C_6 aryl, heteroaryl, or C_5-C_7 cycloalkyl ring with from 0 to 3 R^{13} substituents;

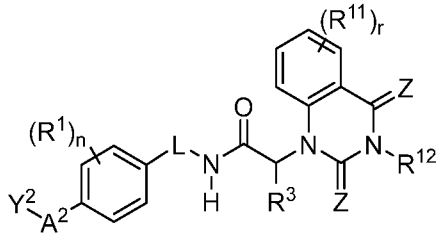
r is an integer from 0 to 4; and

each R^{13} is a member independently selected from the group consisting of C_1-C_6 alkyl, C_6-C_{10} aryl, carboxy(C_1-C_6 alkyloxy), heteroaryl, heterocyclyl, hydroxyl, hydroxyl(C_1-C_6 alkyl), C_1-C_6 alkoxy, C_2-C_9 alkoxyalkyl, amino, C_1-C_6 amido, C_1-C_6 alkylamino, and halo; or, alternatively, two R^{13} groups join to form a fused C_6-C_{10} aryl, C_6-C_{10} heteroaryl, or C_5-C_7 cycloalkyl ring.

In certain specific embodiments, the small molecule is a compound of Formula (VIIA) or (VIIB):



(VIIA),



(VIIB)

or a salt thereof;

wherein:

A^1 is a member selected from the group consisting of –(C=NH)–,–(C=NOR^a)–, –[C=NO(C=O)R^a]–, –[C=N[O(C=O)ZR^b]}–, a fused 5- or 6-member heterocyclyl, and a fused 5- or 6-member heteroaryl;

when A^1 is –(C=NH)–, Y^1 is selected from the group consisting of –NH_2, –NH(C=O)R^a, and – NH(C=O)ZR^b;

when A^1 is –(C=NOR^a)–, –[C=NO(C=O)R^a]–, or –{C=N[O(C=O)ZR^b]}–, Y^1 is –NH_2;

when A^1 is fused heterocyclyl or heteroaryl, Y^1 is –NH_2 or halo, and A^1 is substituted with m additional R^1 groups;

each R^a and R^b is independently selected from the group consisting of C_1-C_6 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{10} aryl, and C_7-C_{12} arylalkyl; wherein R^a has m substituents selected from the group consisting of C_1-C_6 alkyl, hydroxyl, hydroxyl(C_1-C_6 alkyl), C_1-C_6 alkoxy, C_2-C_9 alkoxyalkyl, amino, C_1-C_6 alkylamino, and halo; or, alternatively, R^a and R^b join to form an heterocyclyl ring with m substituents selected from the group consisting of C_1-C_6 alkyl, hydroxyl, C_1-C_6 alkoxy, and halo;

each Z is independently selected from the group consisting of O and S;

A^2 is a member selected from the group consisting of C_3-C_6 heteroaryl and C_2-C_6 alkyl;

when A^2 is C_3-C_6 heteroaryl, Y^2 is selected from the group consisting of –NH_2, – CH_2NH_2, chloro, –(C=NH)NH_2, –(C=NH)NH(C=O)R^a, –(C=NH)NH(C=O)ZR^b, –(C=NOR^a)NH_2, –[C=NO(C=O)R^a]NH_2, and –{C=N[O(C=O)ZR^b]}NH_2; and A^2 is substituted with m additional R^1 groups;

when A^2 is C_2-C_6 alkyl, Y^2 is selected from the group consisting of – NH(C=NH)NH_2,

–NH(C=NH)NH(C=O)R^a, and –NH(C=NH)NH(C=O)ZR^b;

each R^1 is a member independently selected from the group consisting of C_1-C_6 alkyl, hydroxyl, C_1-C_6 alkoxy, amino, C_1-C_6 alkylamino, and halo;

each m and n is an independently selected integer from 0 to 3;

L is $-(O)_p-(C(R^{2a})(R^{2b}))_q-$,

each R^{2a} or R^{2b} is a member independently selected from the group consisting of hydrogen and fluoro;

p is an integer from 0 to 1;

q is an integer from 1 to 2;

R^3 is a member selected from the group consisting of hydrogen, C_1-C_6 alkyl, and carboxy(C_1-C_6 alkyl);

each R^{11} is a member independently selected from the group consisting of C_1-C_6 alkyl, hydroxyl, C_1-C_6 alkoxy, amino, C_1-C_6 alkylamino, halo, and $(R^{14})(R^{14})N(CO)$-; or, alternatively, two R^{11} groups join to form a fused C_6 aryl, heteroaryl, or C_5-C_7 cycloalkyl ring with from 0 to 3 R^{13} substituents;

r is an integer from 0 to 4; and

each Z is a member independently selected from the group consisting of O and NR^8;

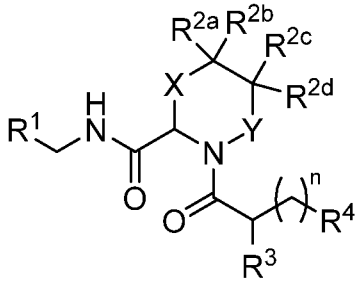
each R^8 is a member independently selected from the group consisting of hydrogen and C_1-C_6 alkyl;

each R^{12} is a member independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, and C_7-C_{14} arylalkyl with from 0 to 3 R^{13} substituents;

each R^{13} is a member independently selected from the group consisting of C_1-C_6 alkyl, hydroxyl, hydroxyl(C_1-C_6 alkyl), C_1-C_6 alkoxy, C_2-C_9 alkoxyalkyl, amino, C_1-C_6 alkylamino, and halo; or, alternatively, two R^{13} groups join to form a fused C_6 aryl, heteroaryl, or C_5-C_7 cycloalkyl ring; and

each R^{14} is a member independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, C_4-C_8 cycloalkylalkyl, C_7-C_{14} arylalkyl, and heteroaryl(C_1-C_6 alkyl); or, alternatively, two R^{13} groups join to form a fused heterocyclyl ring.

In some embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

R^1 is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, R^{2f}, R^{2g}, R^{2h}, R^{2i}, or R^{2j} are independently selected from the group consisting of hydrogen, halo, $C(=O)OR^5$, $OC(=O)R^5$, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkoxy, cyano, aminylalkyl, carboxyalkyl, NR^5R^6, $C(=O)NR^5R^6$, $N(R^5)C(=O)R^6$, $NR^5C(=O)NR^6$, $S(O)_t$, SR^5, nitro, $N(R^5)C(O)OR^6$, $C(=NR^5)NR^6R^7$, $N(R^5)C(=NR^6)NR^7R^8$, $S(O)R^5$, $S(O)NR^5R^6$, $S(O)_2R^5$, $N(R^5)S(O)_2R^6$, $S(O)_2NR^5R^6$, aryl, heteroaryl, heterocyclyl, cycloalkyl, and oxo provided that at least one occurrence of R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, R^{2f}, R^{2g}, R^{2h}, R^{2i}, or R^{2j} is not hydrogen;

R^3 is $NR^{3a}R^{3b}$;

R^{3a} and R^{3b} are each independently hydrogen, alkyl, hydroxyalkyl, haloalkyl, alkoxyalkyl, heterocyclyl, heteroaryl, heterocyclylalkyl, heteroarylalkyl, cycloalkyl, $(CH_2)_nC(=O)OR^6$, or $(CH_2)_nP(=O)(OR^6)_2$;

or R^{3a} and R^{3b}, together with the nitrogen to which they are attached, form an optionally substituted 4-7 membered heteroaryl or an optionally substituted 4-7 membered heterocyclyl;

or R^{3a} and R^4 together with the nitrogen can carbon to which they are attached, respectively, form an optionally substituted 4-7 membered heterocyclyl;

R^4 is a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl when n is 2, 3, 4, 5, or 6; or

R^4 is a substituted or unsubstituted monocyclic heteroaryl, or a substituted or unsubstituted heterocyclyl when n is 0 or 1;

R^5, R^6, R^7, and R^8 are, at each occurrence, independently hydrogen, alkyl, hydroxyalkyl, haloalkyl, alkoxyalkyl, carboxyalkyl, heterocyclyl, heteroaryl, or cycloalkyl;

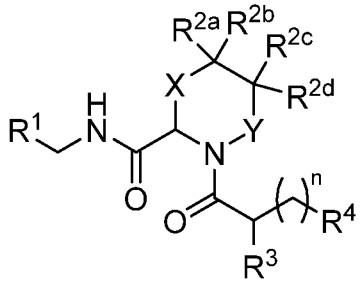
X is a direct bond, $-CR^{2e}R^{2f}-$, or $-CR^{2e}R^{2f}-CR^{2g}R^{2h}-$;

Y is a direct bond or $-CR^{2i}R^{2j}-$;

n is an integer from 0-6; and

t is 1-3.

In some embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

R^1 is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, R^{2f}, R^{2g}, R^{2h}, R^{2i}, or R^{2j} are independently selected from the group consisting of hydrogen, halo, OR^5, $C(=O)OR^5$, $OC(=O)R^5$, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkoxy, cyano, aminylalkyl, carboxyalkyl, NR^5R^6, $C(=O)NR^5R^6$, $N(R^5)C(=O)R^6$, $NR^5C(=O)NR^6$, $S(O)_t$, SR^5, nitro, $N(R^5)C(O)OR^6$, $C(=NR^5)NR^6R^7$, $N(R^5)C(=NR^6)NR^7R^8$, $S(O)R^5$, $S(O)NR^5R^6$, $S(O)_2R^5$, $N(R^5)S(O)_2R^6$, $S(O)_2NR^5R^6$, aryl, heteroaryl, heterocyclyl, cycloalkyl, and oxo provided that at least one occurrence of R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, R^{2f}, R^{2g}, R^{2h}, R^{2i}, or R^{2j} is not hydrogen;

R^3 is $NR^{3a}R^{3b}$;

R^{3a} and R^{3b} are each independently hydrogen, alkyl, hydroxyalkyl, haloalkyl, alkoxyalkyl, $-CH_2C(C=O)OH$, $-CH_2C(=O)O$alkyl, heterocyclyl, heteroaryl, heterocyclylalkyl, heteroarylalkyl, or cycloalkyl;

or R^{3a} and R^{3b}, together with the nitrogen to which they are attached, form an optionally substituted 4-7 membered heteroaryl or an optionally substituted 4-7 membered heterocyclyl;

R^4 is a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl when n is 2, 3, 4, 5, or 6; or

R^4 is a substituted or unsubstituted monocyclic heteroaryl, or a substituted or unsubstituted heterocyclyl when n is 0 or 1;

R^5, R^6, R^7, and R^8 are, at each occurrence, independently hydrogen, alkyl, hydroxyalkyl, haloalkyl, alkoxyalkyl, carboxyalkyl, heterocyclyl, heteroaryl, or cycloalkyl;

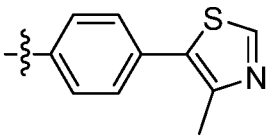
X is a direct bond, $-[C(R^{2e})R^{2f}]-$, or $-[C(R^{2e})R^{2f}]-[C(R^{2g})R^{2h}]-$;

Y is a direct bond or $-[C(R^{2i})R^{2j}]-$;

n is an integer from 0-6; and
t is 1-3,

provided that:

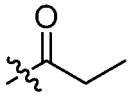
a) when one occurrence of R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, R^{2f}, R^{2g}, R^{2h}, R^{2i}, or R^{2j} is OH, R^1 does not have the following structure:



;

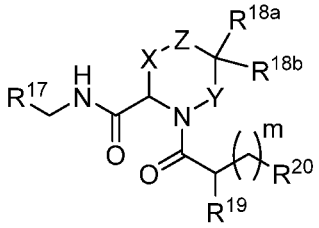
b) when one occurrence of R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, R^{2f}, R^{2g}, or R^{2h} is -OH, n is an integer from 2-6; and

c) when one occurrence of R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, R^{2f}, R^{2g}, R^{2h}, R^{2i}, or R^{2j} is an unsubstituted phenyl, neither R^{3a} nor R^{3b} has the following structure:



.

In some embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

R^{17} is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

R^{18a}, R^{18b}, R^{18c}, R^{18d}, R^{18e}, R^{18f}, R^{18g}, R^{18h}, R^{18i}, or R^{18j} are independently selected from the group consisting of hydrogen, halo, $-OR^{21}$, $C(=O)OR^{21}$, $OC(=O)R^{21}$, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkoxy, cyano, aminylalkyl, carboxyalkyl, $NR^{21}R^{22}$, $C(=O)NR^{21}R^{22}$, $N(R^{21})C(=O)R^{22}$, $NR^{21}C(=O)NR^{22}$, $S(O)_t$, SR^{21}, nitro, $N(R^{21})C(O)OR^{22}$, $C(=NR^{21})NR^{22}R^{23}$, $N(R^{21})C(=NR^{22})NR^{23}R^{24}$, $S(O)R^{21}$, $S(O)NR^{21}R^{22}$, $S(O)_2R^{21}$, $N(R^{21})S(O)_2R^{22}$, $S(O)_2NR^{21}R^{22}$, aryl, heteroaryl, heterocyclyl, cycloalkyl, and oxo provided that at least one occurrence of R^{18a}, R^{18b}, R^{18c}, R^{18d}, R^{18e}, R^{18f}, R^{18g}, R^{18h}, R^{18i}, or R^{18j} is not hydrogen;

R^{19} is $NR^{19a}R^{19b}$;

R^{19a} and R^{19b} are each independently hydrogen, alkyl, hydroxyalkyl, haloalkyl, alkoxyalkyl, heterocyclyl, heteroaryl, heterocyclylalkyl, heteroarylalkyl, cycloalkyl, $(CH_2)_nC(=O)OR^5$, or $(CH_2)_nP(=O)(OR^5)_2$;

or R^{19a} and R^{19b}, together with the nitrogen to which they are attached, form an optionally substituted 4-7 membered heteroaryl or an optionally substituted 4-7 membered heterocyclyl;

R^{20} is a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

R^{21}, R^{22}, R^{23}, and R^{24} are, at each occurrence, independently hydrogen, alkyl, hydroxyalkyl, haloalkyl, alkoxyalkyl, carboxyalkyl, heterocyclyl, heteroaryl, or cycloalkyl;

X is a direct bond, $-CR^{2e}R^{2f}-$, or $-CR^{2e}R^{2f}-CR^{2g}R^{2h}-$;

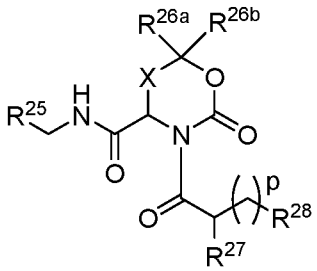
Y is a direct bond or $-CR^{2i}R^{2j}-$;

Z is O or S;

m is an integer from 0-6; and

t is 1-3.

In certain specific embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

R^{25} is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

R^{26a}, R^{26b}, R^{26c}, or R^{26d} are independently selected from the group consisting of hydrogen, halo, $-OR^{29}$, $C(=O)OR^{29}$, $OC(=O)R^{29}$, hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkoxy, cyano, aminylalkyl, carboxyalkyl, $NR^{29}R^{30}$, $C(=O)NR^{29}R^{30}$, $N(R^{29})C(=O)R^{30}$, $NR^{29}C(=O)NR^{30}$, $S(O)_t$, SR^{29}, nitro, $N(R^{29})C(O)OR^{30}$, $C(=NR^{29})NR^{30}R^{31}$, $N(R^{29})C(=NR^{30})NR^{31}R^{32}$, $S(O)R^{29}$, $S(O)NR^{29}R^{30}$, $S(O)_2R^{30}$,

$N(R^{29})S(O)_2R^{30}$, $S(O)_2NR^{29}R^{30}$, aryl, heteroaryl, heterocyclyl, cycloalkyl, and oxo provided that at least one occurrence of R^{26a}, R^{26b}, R^{26c}, or R^{26d} is not hydrogen;

R^{27} is $NR^{27a}R^{27b}$;

R^{27a} and R^{27b} are each independently hydrogen, alkyl, hydroxyalkyl, haloalkyl, alkoxyalkyl, heterocyclyl, heteroaryl, heterocyclylalkyl, heteroarylalkyl, cycloalkyl, $(CH_2)_nC(=O)OR^{29}$, or $(CH_2)_nP(=O)(OR^{29})_2$;

or R^{27a} and R^{27b}, together with the nitrogen to which they are attached, form an optionally substituted 4-7 membered heteroaryl or an optionally substituted 4-7 membered heterocyclyl;

R^{28} is a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

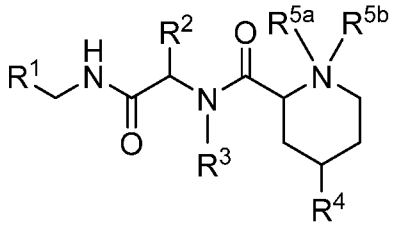
R^{29}, R^{30}, R^{31}, and R^{32} are, at each occurrence, independently hydrogen, alkyl, hydroxyalkyl, haloalkyl, alkoxyalkyl, carboxyalkyl, heterocyclyl, heteroaryl, or cycloalkyl;

X is a direct bond or $-CR^{26c}R^{26d}-$;

p is an integer from 0-6; and

t is 1-3.

In some embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

R^1 is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

R^2 is hydrogen, alkyl, alkoxy, haloalkyl, haloalkoxy, or cycloalkyl;

R^3 is hydrogen, alkyl, haloalkyl, or cycloalkyl;

or R^2 and R^3, together with the carbon and nitrogen to which they are attached, respectively, form an optionally substituted 4-7 membered heterocyclyl;

R^4 is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

R^{5a} is hydrogen, alkyl, haloalkyl, cycloalkyl, phosphonalkyl, $(CH_2)_nC(=O)OR^6$, $C(=O)R^6$, $C(=O)OR^6$, or $C(=O)NR^6R^7$;

R^{5b} is an electron pair or alkyl;

R^6 and R^7 are, at each occurrence, independently hydrogen, alkyl, haloalkyl, cycloalkyl, or arylalkyl;

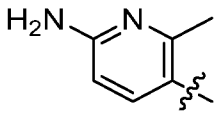
R^8 is alkyl, haloalkyl, aminylalkyl, substituted or unsubstituted arylalkyl; and

n is 1, 2, 3, 4, 5, 6, 7, or 8,

provided that

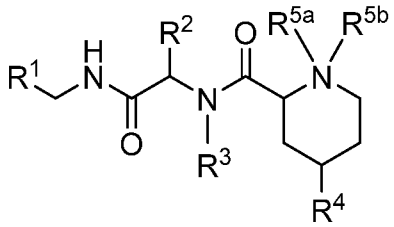
A) R^{5a} is alkyl, haloalkyl, cycloalkyl, phosphonalkyl, $(CH_2)_nC(=O)OR^6$, $C(=O)R^6$, $C(=O)OR^6$, or $C(=O)NR^6R^7$ or R^1 is substituted with one or more substituents selected from the group consisting of a substituted heteroaryl, $C(=NH)NHC(=O)OR^8$, $C(=NOC(=O)R^8)NH_2$, $C(=NOC(=O)OR^8)NH_2$, and $C(=NOH)NH_2$; and

B) when R^{5a} is alkyl or $(CH_2)_nC(=O)OR^6$, R^1 does not have the following structure:



unless R^2 and R^3, together with the carbon and nitrogen to which they are attached, respectively, form an optionally substituted 4-7 membered heterocyclyl.

In some embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

R^1 is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

R^2 is hydrogen, alkyl, alkoxy, haloalkyl, haloalkoxy, or cycloalkyl;

R^3 is hydrogen, alkyl, haloalkyl, or cycloalkyl;

or R^2 and R^3, together with the carbon and nitrogen to which they are attached, respectively, form an optionally substituted 4-7 membered heterocyclyl;

R^4 is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

R^{5a} is hydrogen, alkyl, haloalkyl, cycloalkyl, phosphonalkyl, $(CH_2)_nC(=O)OR^6$, $C(=O)R^6$, $C(=O)OR^6$, or $C(=O)NR^6R^7$;

R^{5b} is an electron pair or alkyl;

R^6 and R^7 are, at each occurrence, independently hydrogen, alkyl, haloalkyl, cycloalkyl, or arylalkyl;

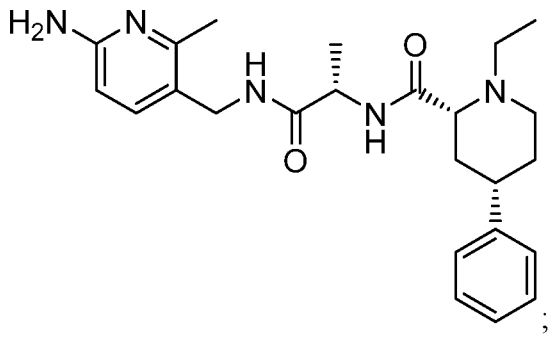
R^8 is alkyl, haloalkyl, aminylalkyl, substituted or unsubstituted arylalkyl; and

n is 1, 2, 3, 4, 5, 6, 7, or 8,

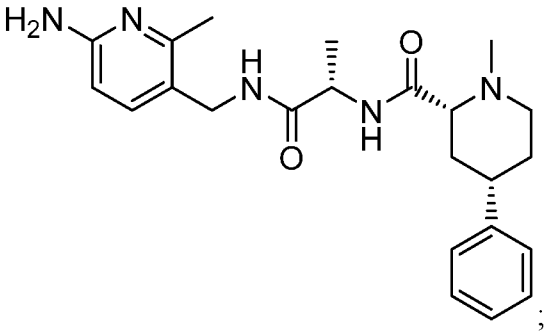
provided that

A) R^{5a} is alkyl, haloalkyl, cycloalkyl, phosphonalkyl, $(CH_2)_nC(=O)OR^6$, $C(=O)R^6$, $C(=O)OR^6$, or $C(=O)NR^6R^7$ or R^1 is substituted with one or more substituents selected from the group consisting of a substituted heteroaryl, $C(=NH)NHC(=O)OR^8$, $C(=NOC(=O)R^8)NH_2$, $C(=NOC(=O)OR^8)NH_2$, and $C(=NOH)NH_2$; and

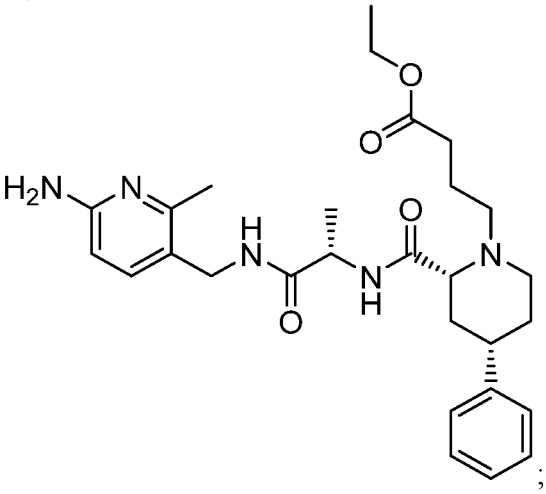
B) the compound of Structure (I) does not have one of the following structures:



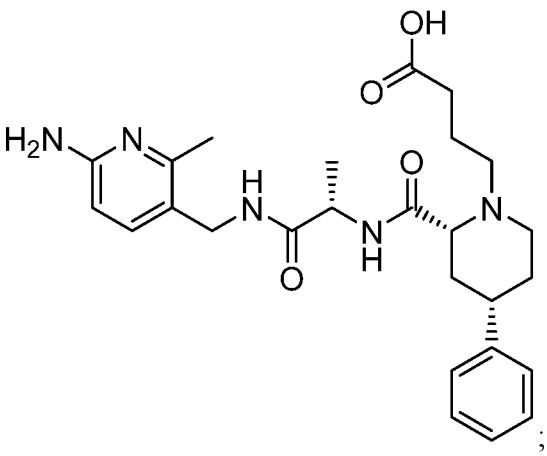
;



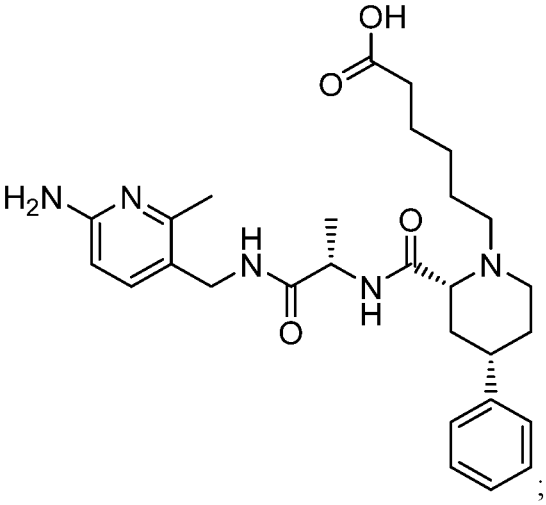
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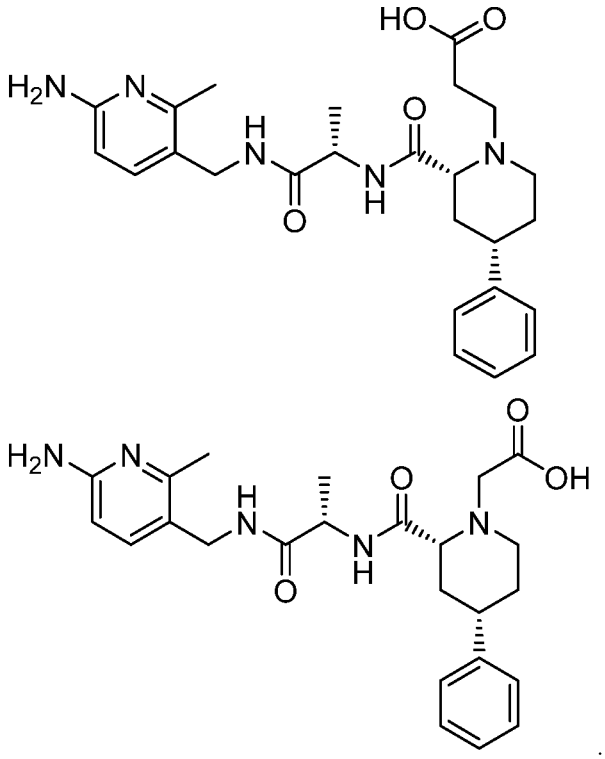
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;

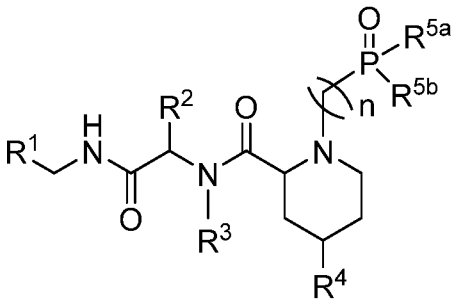


;



or

In some embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

R^1 is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

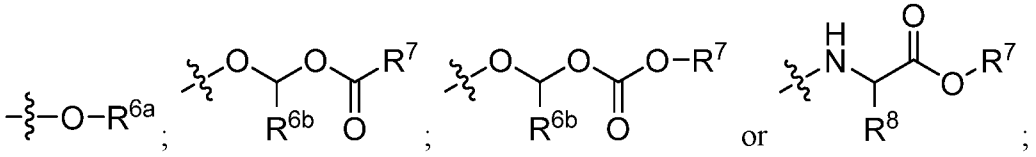
R^2 is hydrogen, alkyl, alkoxy, haloalkyl, haloalkoxy, or cycloalkyl;

R^3 is hydrogen, alkyl, haloalkyl, or cycloalkyl;

or R^2 and R^3, together with the carbon and nitrogen to which they are attached, respectively, form an optionally substituted 4-7 membered heterocyclyl;

R^4 is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted heterocyclyl;

R^{5a} and R^{5b} at each occurrence, independently have one of the following structures:



or R^{5a} and R^{5b}, together with the phosphorus atom to which they are attached form an optionally substituted 4-7 membered heterocyclyl;

R^{6a} is alkyl, haloalkyl, aryl, heteroaryl, cycloalkyl, or heterocyclyl;

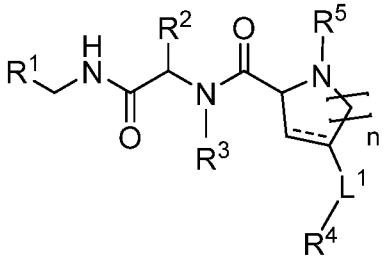
R^{6b} is, at each occurrence, independently hydrogen or alkyl;

R^7 is, at each occurrence, independently alkyl, haloalkyl, heteroaryl, cycloalkyl, heterocyclyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, or heterocyclylalkyl;

R^8 is an amino acid side chain; and

n is 1, 2, 3, 4, 5, 6, 7, or 8.

In some embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

==== represents a double or single bond;

R^1 is a substituted or unsubstituted aryl or a substituted or unsubstituted heteroaryl;

R^2 is hydrogen, alkyl, alkoxy, haloalkyl, hydroxyalkyl, haloalkoxy, or cycloalkyl;

R^3 is hydrogen, alkyl, haloalkyl, or cycloalkyl, or R^2 and R^3, together with the carbon and nitrogen to which they are attached, respectively, form an optionally substituted 4-7 membered heterocyclyl;

R^4 is a substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted heterocyclyl;

R^5 is hydrogen, alkyl, haloalkyl, cycloalkyl, phosphonalkyl, $(CH_2)_mC(=O)OR^6$, $C(=O)R^6$, $C(=O)OR^6$, $(CH_2)_mNR^6S(O)_2R^7$, or $C(=O)NR^6R^7$;

R^6 and R^7 are, at each occurrence, independently hydrogen, alkyl, haloalkyl, cycloalkyl, or arylalkyl;

L^1 is a direct bond, $-CR^{8a}R^{8b}-$, $-S(O)_t-$, NR^{8c}, or -O-;

R^{8a} and R^{8b} are each independently hydrogen, alkyl, or R^{8a} and R^{8b}, together with the carbon to which they are attached form an optionally substituted 3-6 membered cycloalkyl;

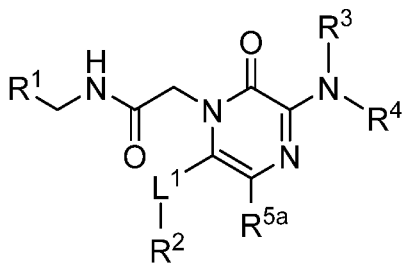
R^{8c} is hydrogen, alkyl, haloalkyl, (C=O)alkyl, (C=O)Oalkyl, (C=O)cycloalkyl, (C=O)Ocycloalkyl, (C=O)aryl, (C=O)Oaryl, (C=O)heteroaryl, (C=O)Oheteroaryl, (C=O)heterocyclyl, (C=O)O heterocyclyl, a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, a substituted or unsubstituted heterocyclyl, a substituted or unsubstituted arylalkyl, a substituted or unsubstituted heteroarylalkyl, a substituted or unsubstituted cycloalkylalkyl, or a substituted or unsubstituted heterocyclylalkyl;

n is 1 or 2;

m is 1, 2, 3, 4, 5, or 6; and

t is 0, 1, or 2.

In some embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

R^1 is a substituted or unsubstituted heteroaryl;

R^2 is a substituted or unsubstituted aryl or a substituted or unsubstituted heteroaryl;

R^3 is hydrogen or alkyl;

R^4 is alkyl, a substituted or unsubstituted arylalkyl, a heterocyclyl substituted with substituents selected from the group consisting of a substituted or unsubstituted phenyl or a substituted or unsubstituted pyridinyl, or R^3 and R^4, together with the nitrogen and carbon to which they are attached, respectively, form an optionally substituted 4-10 membered heterocyclyl;

R^{5a} is hydrogen or halo;

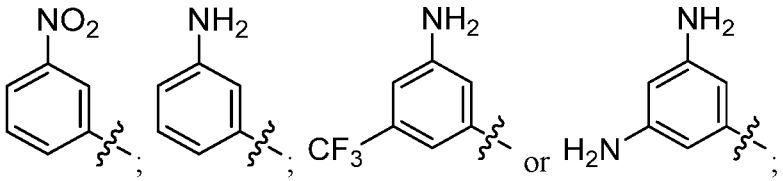
R^{5b} is hydrogen, alkyl, haloalkyl, (C=O)alkyl, (C=O)Oalkyl, (C=O)cycloalkyl, (C=O)Ocycloalkyl, (C=O)aryl, (C=O)Oaryl, (C=O)heteroaryl, (C=O)Oheteroaryl, (C=O)heterocyclyl, (C=O)Oheterocyclyl, a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, a substituted or unsubstituted cycloalkyl, a substituted or unsubstituted heterocyclyl, a substituted or unsubstituted arylalkyl, a substituted or unsubstituted heteroarylalkyl, a substituted or unsubstituted cycloalkylalkyl, or a substituted or unsubstituted heterocyclylalkyl;

L^1 is a direct bond, $-CH_2-$, $-S(O)_t-$, NR^{5b}, -O-, -C=C-, or -C≡C-; and

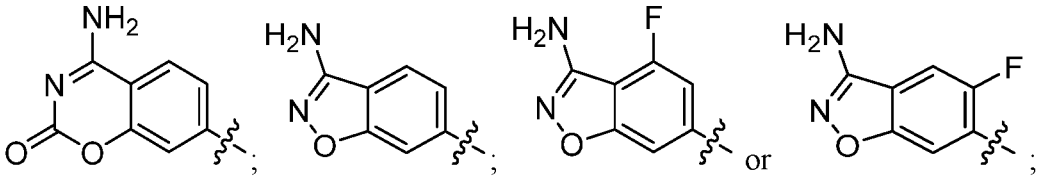
t is 0, 1, or 2,

provided that:

A) R^2 does not have one of the following structures:

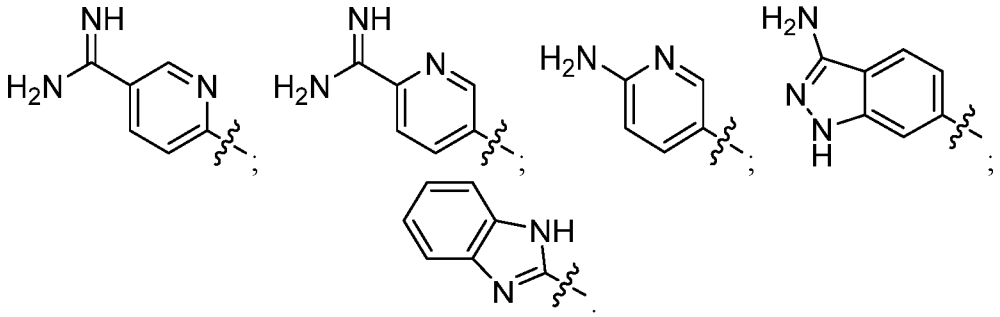


B) R^1 does not have one of the following structures:

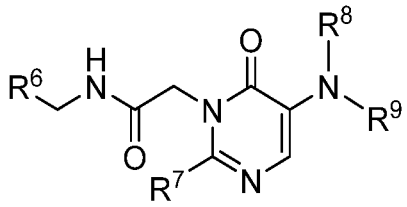


; and

C) when R^2 is unsubstituted phenyl, R^1 does not have one of the following structures:



In certain more specific embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

R^6 is a substituted or unsubstituted aryl or a substituted or unsubstituted heteroaryl;

R^7 is alkyl, $-SR^{10}$ a substituted or unsubstituted aryl, or a substituted or unsubstituted heteroaryl;

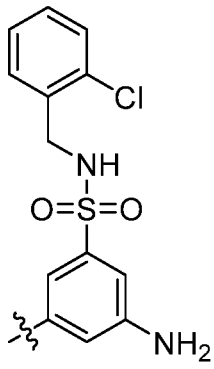
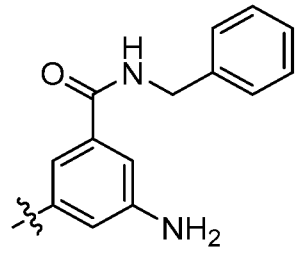
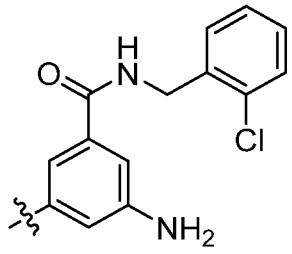
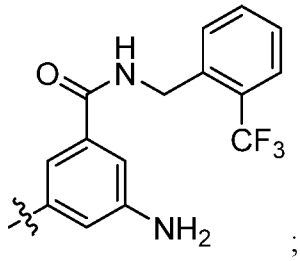
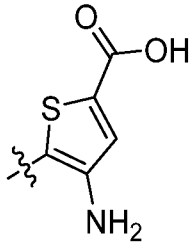
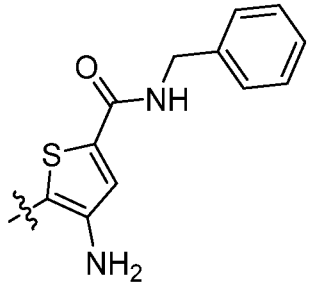
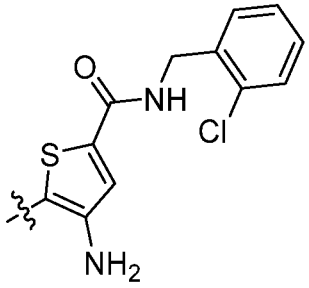
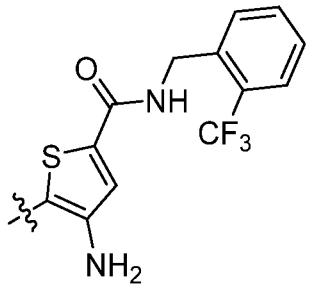
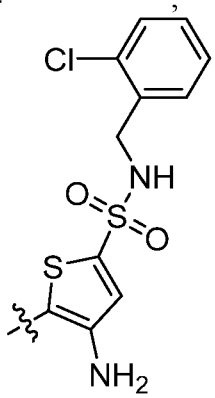
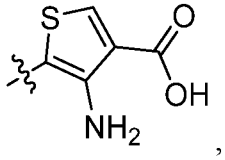
R^8 is hydrogen, alkyl, haloalkyl, or cycloalkyl;

R^9 is a substituted or unsubstituted arylalkyl, a substituted or unsubstituted heteroarylalkyl, or R^8 and R^9, together with the nitrogen to which they are attached, form an optionally substituted 4-10 membered heterocyclyl;

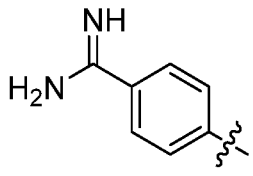
R^{10} is hydrogen, alkyl, haloalkyl, or cycloalkyl;

provided that:

A) when R^7 is unsubstituted phenyl, 3-((methylsulfonyl)amino)phenyl, 2-methylphenyl, 3-(dimethylamino)phenyl, 3-(methylamino)phenyl, 3-methylphenyl, 3-aminomethylphenyl, 3-aminophenyl, unsubstituted pyridinyl, 3-(methylamino)-2-thienyl, 3,4-diamino-2-thienyl, 3-((methylsulfonyl)amino)-2-thienyl, 3-amino-2-thienyl, 3-amino-5-5(aminocarbonyl)phenyl, or has one of the following structures:

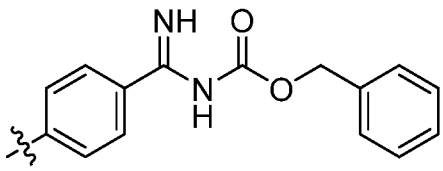











R^6 does not have the following structure:

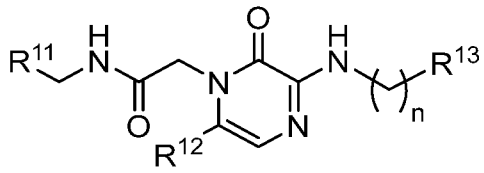


; and

B) when R^7 is unsubstituted phenyl, R^6 does not have the following structure:

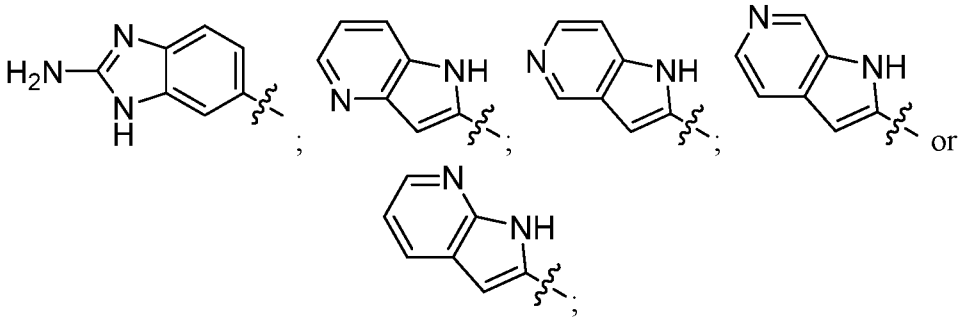


In some embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

R^{11} has one of the following structures:



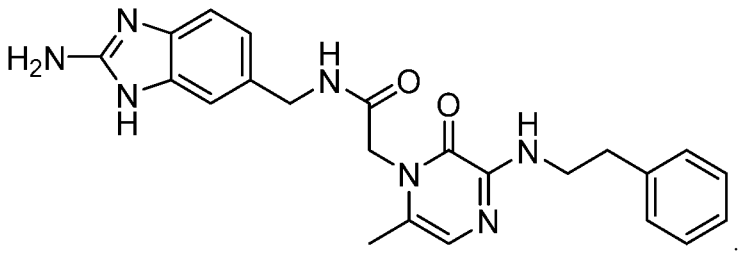
R^{12} is methyl or halo;

R^{13} is a substituted or unsubstituted aryl; and

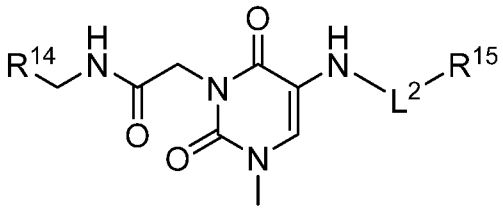
n is 1 or 2

provided that:

the compound of Structure (III) does not have the following structure:



In some more specific embodiments, the small molecule is a compound having the following Structure:



or a stereoisomer, tautomer, or pharmaceutically acceptable salt thereof, wherein:

R^{14} is a substituted or unsubstituted aryl or a substituted or unsubstituted heteroaryl;

R^{15} is a substituted or unsubstituted arylalkyl, or a substituted or unsubstituted heteroarylalkyl;

L^2 is a direct bond, -C(=O), or -$S(=O)_t$-; and

t is 0, 1, or 2.

EXPRESSION INHIBITORS OF MASP-2

In another embodiment of this aspect of the invention, the MASP-2 inhibitory agent is a MASP-2 expression inhibitor capable of inhibiting MASP-2-dependent complement activation. In the practice of this aspect of the invention, representative MASP-2 expression inhibitors include MASP-2 antisense nucleic acid molecules (such as antisense mRNA, antisense DNA or antisense oligonucleotides), MASP-2 ribozymes and MASP-2 RNAi molecules.

Anti-sense RNA and DNA molecules act to directly block the translation of MASP-2 mRNA by hybridizing to MASP-2 mRNA and preventing translation of MASP-2 protein. An antisense nucleic acid molecule may be constructed in a number of different ways provided that it is capable of interfering with the expression of MASP-2. For example, an antisense nucleic acid molecule can be constructed by inverting the coding region (or a portion thereof) of MASP-2 cDNA (SEQ ID NO:4) relative to its normal orientation for transcription to allow for the transcription of its complement.

The antisense nucleic acid molecule is usually substantially identical to at least a portion of the target gene or genes. The nucleic acid, however, need not be perfectly identical to inhibit expression. Generally, higher homology can be used to compensate for the use of a shorter antisense nucleic acid molecule. The minimal percent identity is typically greater than about 65%, but a higher percent identity may exert a more effective repression of expression of the endogenous sequence. Substantially greater percent identity of more than about 80% typically is preferred, though about 95% to absolute identity is typically most preferred.

The antisense nucleic acid molecule need not have the same intron or exon pattern as the target gene, and non-coding segments of the target gene may be equally effective in achieving antisense suppression of target gene expression as coding segments. A DNA sequence of at least about 8 or so nucleotides may be used as the antisense nucleic acid molecule, although a longer sequence is preferable. In the present invention, a

representative example of a useful inhibitory agent of MASP-2 is an antisense MASP-2 nucleic acid molecule which is at least ninety percent identical to the complement of the MASP-2 cDNA consisting of the nucleic acid sequence set forth in SEQ ID NO:4. The nucleic acid sequence set forth in SEQ ID NO:4 encodes the MASP-2 protein consisting of the amino acid sequence set forth in SEQ ID NO:5.

The targeting of antisense oligonucleotides to bind MASP-2 mRNA is another mechanism that may be used to reduce the level of MASP-2 protein synthesis. For example, the synthesis of polygalacturonase and the muscarine type 2 acetylcholine receptor is inhibited by antisense oligonucleotides directed to their respective mRNA sequences (U.S. Patent No. 5,739,119, to Cheng, and U.S. Patent No. 5,759,829, to Shewmaker). Furthermore, examples of antisense inhibition have been demonstrated with the nuclear protein cyclin, the multiple drug resistance gene (MDG1), ICAM-1, E-selectin, STK-1, striatal $GABA_A$ receptor and human EGF (see, e.g., U.S. Patent No. 5,801,154, to Baracchini; U.S. Patent No. 5,789,573, to Baker; U.S. Patent No. 5,718,709, to Considine; and U.S. Patent No. 5,610,288, to Reubenstein).

A system has been described that allows one of ordinary skill to determine which oligonucleotides are useful in the invention, which involves probing for suitable sites in the target mRNA using RNAse H cleavage as an indicator for accessibility of sequences within the transcripts. Scherr, M., et al., *Nucleic Acids Res. 26*:5079-5085, 1998; Lloyd, et al., *Nucleic Acids Res. 29*:3665-3673, 2001. A mixture of antisense oligonucleotides that are complementary to certain regions of the MASP-2 transcript is added to cell extracts expressing MASP-2, such as hepatocytes, and hybridized in order to create an RNAse H vulnerable site. This method can be combined with computer-assisted sequence selection that can predict optimal sequence selection for antisense compositions based upon their relative ability to form dimers, hairpins, or other secondary structures that would reduce or prohibit specific binding to the target mRNA in a host cell. These secondary structure analysis and target site selection considerations may be performed using the OLIGO primer analysis software (Rychlik, I., 1997) and the BLASTN 2.0.5 algorithm software (Altschul, S.F., et al., *Nucl. Acids Res. 25*:3389-3402, 1997). The antisense compounds directed towards the target sequence preferably comprise from about 8 to about 50 nucleotides in length. Antisense oligonucleotides comprising from about 9 to about 35 or so nucleotides are particularly preferred. The inventors contemplate all oligonucleotide compositions in the range of 9 to 35 nucleotides

(i.e., those of 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, or 35 or so bases in length) are highly preferred for the practice of antisense oligonucleotide-based methods of the invention. Highly preferred target regions of the MASP-2 mRNA are those that are at or near the AUG translation initiation codon, and those sequences that are substantially complementary to 5' regions of the mRNA, e.g., between the –10 and +10 regions of the *MASP-2* gene nucleotide sequence (SEQ ID NO:4). Exemplary MASP-2 expression inhibitors are provided in TABLE 4.

TABLE 4: EXEMPLARY EXPRESSION INHIBITORS OF MASP-2

SEQ ID NO:30 (nucleotides 22-680 of SEQ ID NO:4)	Nucleic acid sequence of MASP-2 cDNA (SEQ ID NO:4) encoding CUBIEGF
SEQ ID NO:31 5'CGGGCACACCATGAGGCTGCTG ACCCTCCTGGGC3	Nucleotides 12-45 of SEQ ID NO:4 including the MASP-2 translation start site (sense)
SEQ ID NO:32 5'GACATTACCTTCCGCTCCGACTC CAACGAGAAG3'	Nucleotides 361-396 of SEQ ID NO:4 encoding a region comprising the MASP-2 MBL binding site (sense)
SEQ ID NO:33 5'AGCAGCCCTGAATACCCACGGCC GTATCCCAAA3'	Nucleotides 610-642 of SEQ ID NO:4 encoding a region comprising the CUBII domain

As noted above, 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. These modifications allow one to introduce certain desirable properties that are not offered through naturally occurring oligonucleotides, such as reduced toxic properties, increased stability against nuclease degradation and enhanced cellular uptake. In illustrative embodiments, the antisense compounds of the invention differ from native DNA by the modification of the phosphodiester backbone to extend the life of the antisense oligonucleotide in which the phosphate substituents are replaced by phosphorothioates. Likewise, one or both ends of the oligonucleotide may be substituted by one or more acridine derivatives that intercalate between adjacent basepairs within a strand of nucleic acid.

Another alternative to antisense is the use of "RNA interference" (RNAi). Double-stranded RNAs (dsRNAs) can provoke gene silencing in mammals *in vivo*. The natural function of RNAi and co-suppression appears to be protection of the genome against invasion by mobile genetic elements such as retrotransposons and viruses that produce aberrant RNA or dsRNA in the host cell when they become active (see, e.g., Jensen, J., et al., *Nat. Genet. 21*:209-12, 1999). The double-stranded RNA molecule may be prepared by synthesizing two RNA strands capable of forming a double-stranded RNA molecule, each having a length from about 19 to 25 (e.g., 19-23 nucleotides). For example, a dsRNA molecule useful in the methods of the invention may comprise the RNA corresponding to a sequence and its complement listed in TABLE 4. Preferably, at least one strand of RNA has a 3' overhang from 1-5 nucleotides. The synthesized RNA strands are combined under conditions that form a double-stranded molecule. The RNA sequence may comprise at least an 8 nucleotide portion of SEQ ID NO:4 with a total length of 25 nucleotides or less. The design of siRNA sequences for a given target is within the ordinary skill of one in the art. Commercial services are available that design siRNA sequence and guarantee at least 70% knockdown of expression (Qiagen, Valencia, Calif).

The dsRNA may be administered as a pharmaceutical composition and carried out by known methods, wherein a nucleic acid is introduced into a desired target cell. Commonly used gene transfer methods include calcium phosphate, DEAE-dextran, electroporation, microinjection and viral methods. Such methods are taught in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., 1993.

Ribozymes can also be utilized to decrease the amount and/or biological activity of MASP-2, such as ribozymes that target MASP-2 mRNA. Ribozymes are catalytic RNA molecules that can cleave nucleic acid molecules having a sequence that is completely or partially homologous to the sequence of the ribozyme. It is possible to design ribozyme transgenes that encode RNA ribozymes that specifically pair with a target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the antisense constructs.

Ribozymes useful in the practice of the invention typically comprise a hybridizing region of at least about nine nucleotides, which is complementary in nucleotide sequence to at least part of the target MASP-2 mRNA, and a catalytic region that is adapted to cleave the target MASP-2 mRNA (see generally, EPA No. 0 321 201; WO88/04300; Haseloff, J., et al., *Nature 334*:585-591, 1988; Fedor, M.J., et al., *Proc. Natl. Acad. Sci. USA 87*:1668-1672, 1990; Cech, T.R., et al., *Ann. Rev. Biochem. 55*:599-629, 1986).

Ribozymes can either be targeted directly to cells in the form of RNA oligonucleotides incorporating ribozyme sequences, or introduced into the cell as an expression vector encoding the desired ribozymal RNA. Ribozymes may be used and applied in much the same way as described for antisense polynucleotides.

Anti-sense RNA and DNA, ribozymes and RNAi molecules useful in the methods of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art, such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various well known modifications of the DNA molecules may be introduced as a means of increasing stability and half-life. Useful modifications include, but are not limited to, the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

V. PHARMACEUTICAL COMPOSITIONS AND DELIVERY METHODS DOSING

In another aspect, the invention provides compositions for inhibiting the adverse effects of MASP-2-dependent complement activation in a subject suffering from a disease or condition as disclosed herein, comprising administering to the subject a composition comprising a therapeutically effective amount of a MASP-2 inhibitory agent

and a pharmaceutically acceptable carrier. The MASP-2 inhibitory agents can be administered to a subject in need thereof, at therapeutically effective doses to treat or ameliorate conditions associated with MASP-2-dependent complement activation. A therapeutically effective dose refers to the amount of the MASP-2 inhibitory agent sufficient to result in amelioration of symptoms associated with the disease or condition.

Toxicity and therapeutic efficacy of MASP-2 inhibitory agents can be determined by standard pharmaceutical procedures employing experimental animal models, such as the murine MASP-2 -/- mouse model expressing the human MASP-2 transgene described in Example 1. 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 agents 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 agent 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.

In some embodiments, therapeutic efficacy of the MASP-2 inhibitory agents for treating, inhibiting, alleviating or preventing fibrosis in a mammalian subject suffering, or at risk of developing a disease or disorder caused or exacerbated by fibrosis and/or inflammation is determined by one or more of the following: a reduction in one of more markers of inflammation and scarring (e.g., TGFβ-1, CTFF, IL-6, apoptosis, fibronectin, laminin, collagens, EMT, infiltrating macrophages) in renal tissue; a reduction in the release of soluble markers of inflammation and fibrotic renal disease into urine and plasma (e.g., by the measurement of renal excretory functions).

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 agent 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 MASP-2 protein present in a living subject and the binding affinity of the

MASP-2 inhibitory agent. 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.

Generally, the dosage of administered compositions comprising MASP-2 inhibitory agents 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 agents, such as anti-MASP-2 antibodies, can be administered in dosage ranges from about 0.010 to 10.0 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 the composition comprises a combination of anti-MASP-2 antibodies and MASP-2 inhibitory peptides.

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. 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 agent 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, the reduction of C4 cleavage and C4b deposition (measured, for example as described in Example 10), or the reduction of C3 cleavage and C3b deposition (measured, for example, as described in Example 10).

ADDITIONAL AGENTS

In certain embodiments, methods of preventing, treating, reverting and/or inhibiting fibrosis and/or inflammation include administering an MASP-2 inhibitory agent (e.g., a MASP-2 inhibitory antibody) as part of a therapeutic regimen along with one or more other drugs, biologics, or therapeutic interventions appropriate for inhibiting fibrosis and/or inflammation. In certain embodiments, the additional drug, biologic, or therapeutic intervention is appropriate for particular symptoms associated with a disease or disorder caused or exacerbated by fibrosis and/or inflammation. By way of example, MASP-2 inhibitory antibodies may be administered as part of a therapeutic regimen along with one or more immunosuppressive agents, such as methotrexate, cyclophosphamide, azathioprine, and mycophenolate mofetil. By way of further example, MASP-2 inhibitory antibodies may be administered as part of a therapeutic regimen along with one or more agents designed to increase blood flow (e.g., nifedipine, amlodipine, diltiazem, felodipine, or nicardipine). By way of further example, MASP-2 inhibitory antibodies may be administered as part of a therapeutic regimen along with one or more agents intended to decrease fibrosis, such as d-penicillamine, colchicine, PUVA, Relaxin, cyclosporine, TGF beta blockers and/or p38 MAPK blockers. By way of further example, MASP-2 inhibitory antibodies may be administered as part of a therapeutic regimen along with steroids or broncho-dilators.

The compositions and methods comprising MASP-2 inhibitory agents (e.g., MASP-2 inhibitory antibodies) may optionally comprise one or more additional

therapeutic agents, which may augment the activity of the MASP-2 inhibitory agent or that provide related therapeutic functions in an additive or synergistic fashion. For example, in the context of treating a subject suffering from a disease or disorder caused or exacerbated by fibrosis and/or inflammation one or more MASP-2 inhibitory agents may be administered in combination (including co-administration) with one or more additional antifibrotic agents and/or one or more anti-viral and/or anti-inflammatory and/or immunosuppressive agents.

MASP-2 inhibitory agents (e.g., MASP-2 inhibitory antibodies) can be used in combination with other therapeutic agents such as general antiviral drugs, or immunosuppressive drugs such as corticosteroids, immunosuppressive or cytotoxic agents, and/or antifibrotic agents.

In some embodiments of the methods described herein, MASP-2 inhibitory agents (e.g., MASP-2 inhibitory antibodies or small molecule inhibitors of MASP-2) are used as a monotherapy for the treatment of a subject suffering from coronavirus or influenza virus. In some embodiments of the methods described herein, MASP-2 inhibitory agents (e.g., MASP-2 inhibitory antibodies or small molecule inhibitors of MASP-2) are used in combination with other therapeutic agents, such as antiviral agents, therapeutic antibodies, corticosteroids and/or other agents that are shown to be efficacious for the treatment of a subject suffering from coronavirus or influenza virus. In some embodiments, a pharmaceutical composition comprises a MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibodies or small molecule inhibitors of MASP-2) and at least one additional therapeutic agent such as an antiviral agent (e.g., remdesivir), a therapeutic antibody to a target other than MASP-2, a corticosteroid, an anticoagulant, such as low molecular weight herparin (e.g., enoxaparin) and an antibiotic (e.g., azithromycin).

In such combination therapies, a MASP-2 inhibitory agent may be formulated with or administered concurrently with, prior to, or subsequent to, one or more other desired COVID-19 therapeutic agent such as an antiviral agent (e.g., remdesivir), a therapeutic antibody to a target other than MASP-2, a corticosteroid, or an anticoagulant. Each component of a combination therapy may be formulated in a variety of ways that are known in the art. For example, the MASP-2 inhibitory agent and second agent of the combination therapy may be formulated together or separately. The MASP-2 inhibitory

agent and additional agent may be suitably administered to the COVID-19 patient at one time or over a series of treatments.

Exemplary antiviral agents include, for example darunavir (which may be used with ritonavir or cobicistat to increase darunavir levels), favilavir, lopinavir, ritonavir, remdesivir, galidesivir, ebastine, danoprevir, ASC09, emtricitabine, tenofovir, umifnovir, baloxavir marboxil, azvudine and/or ISR-50. Exemplary therapeutic antibodies include, for example, vascular growth factor inhibitors (e.g., bevacizumab), PD-1 blocking antibodies (e.g., thymosin, camrelizumab), CCR5 antagonists (e.g., leronlimab), IL-6 receptor antagonists (e.g., sarilumab, tocilizumab), IL-6 targeted inhibitors (e.g., siltuximab), anti-GMCSF antibodies (e.g., gimsilumab, TJM2), GMCSF receptor alpha blocking antibodies (e.g., mavrilimumab), anti-C5 antibodies (e.g., eculizumab, ravulizumab), and/or anti-C5a antibodies (IFX-1).

In some embodiments of the methods described herein, MASP-2 inhibitory agents (e.g., MASP-2 inhibitory antibodies, e.g., OMS646, or small molecule inhibitors of MASP-2) are used in combination with an antiviral agent such as remdesivir for the treatment of a subject suffering from COVID-19.

Other agents that may be efficacious for the treatment of coronavirus and/or influenza virus include, for example, chloroquine/hydroxychloroquine, camostat mesylate, ruxolinib, peginterferon alfa-2b, novaferon, ifenprodil, recombinant ACE2, APN01, brilacidin, BXT-25, BIO-11006, fingolimod, WP1122, interferon beta-1a, nafamostat, losartan and/or alteplase.

PHARMACEUTICAL CARRIERS AND DELIVERY VEHICLES

In general, the MASP-2 inhibitory agent compositions of the present invention, 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 anti-MASP-2 antibodies and inhibitory peptides useful in the invention 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 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.

For intra-articular delivery, the MASP-2 inhibitory agent 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 oral administration of non-peptidergic agents, the MASP-2 inhibitory agent may be carried in an inert filler or diluent such as sucrose, cornstarch, or cellulose.

For topical administration, the MASP-2 inhibitory agent may be carried in 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 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 excipients, such as dispersing or wetting agents, suspending agents, diluents, buffers, penetration enhancers, emulsifiers, binders, thickeners, flavouring agents (for oral administration).

PHARMACEUTICAL CARRIERS FOR ANTIBODIES AND PEPTIDES

More specifically with respect to anti-MASP-2 antibodies and inhibitory peptides, 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 anti-MASP-2 antibodies and inhibitory peptides. 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 anti-MASP-2 antibodies and inhibitory peptides 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.

PHARMACEUTICALLY ACCEPTABLE CARRIERS FOR EXPRESSION INHIBITORS

More specifically with respect to expression inhibitors useful in the methods of the invention, compositions are provided that comprise an expression inhibitor as described above and a pharmaceutically acceptable carrier or diluent. The composition may further comprise a colloidal dispersion system.

Pharmaceutical compositions that include expression inhibitors may include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not

limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. The preparation of such compositions typically involves combining the expression inhibitor with one or more of the following: buffers, antioxidants, low molecular weight polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with non-specific serum albumin are examples of suitable diluents.

In some embodiments, the compositions may be prepared and formulated as emulsions which are typically heterogeneous systems of one liquid dispersed in another in the form of droplets (see, Idson, in *Pharmaceutical Dosage Forms*, Vol. 1, Rieger and Banker (eds.), Marcek Dekker, Inc., N.Y., 1988). Examples of naturally occurring emulsifiers used in emulsion formulations include acacia, beeswax, lanolin, lecithin and phosphatides.

In one embodiment, compositions including nucleic acids can be formulated as microemulsions. A microemulsion, as used herein refers to a system of water, oil, and amphiphile, which is a single optically isotropic and thermodynamically stable liquid solution (see Rosoff in *Pharmaceutical Dosage Forms*, Vol. 1). The method of the invention may also use liposomes for the transfer and delivery of antisense oligonucleotides to the desired site.

Pharmaceutical compositions and formulations of expression inhibitors for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, as well as aqueous, powder or oily bases and thickeners and the like may be used.

MODES OF ADMINISTRATION

The pharmaceutical compositions comprising MASP-2 inhibitory agents 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), intra-arterial, 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 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.

MASP-2 inhibitory antibodies and polypeptides 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 and peptides 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 and polypeptides 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 and polypeptides 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 and polypeptides 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 agent 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 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 agent may be achieved in the context of surgical methods for treating disease or disorder caused or exacerbated by fibrosis and/or inflammation such as for example during procedures such as surgery.

TREATMENT REGIMENS

In prophylactic applications, the pharmaceutical compositions comprising a MASP-2 inhibitory agent (e.g., a MASP-2 inhibitory antibody or MASP-2 inhibitory small molecule compound) are administered to a subject susceptible to, or otherwise at risk of developing coronavirus-induced acute respiratory distress syndrome or influenza virus-induced acute respiratory distress syndrome in an amount sufficient to inhibit MASP-2-dependent complement activation and thereby reduce, eliminate or reduce the risk of developing symptoms of the respiratory syndrome. In both prophylactic and therapeutic regimens, compositions comprising MASP-2 inhibitory agents may be administered in several dosages until a sufficient therapeutic outcome has been achieved in the subject. 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 treatment of an acute condition associated with fibrosis and/or inflammation. Alternatively, the composition may be administered at periodic intervals over an extended period of time for treatment of chronic conditions associated with fibrosis and/or inflammation.

In both prophylactic and therapeutic regimens, compositions comprising MASP-2 inhibitory agents 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 agent comprises a MASP-2 antibody, which suitably 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. For pediatric patients, dosage can be adjusted in proportion to the patient's weight. 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 treatment of a subject suffering from or at risk for developing a disease or disorder caused or exacerbated by fibrosis and/or inflammation. 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 suffering from or at risk for developing a disease or disorder caused or exacerbated by fibrosis and/or inflammation.

In both prophylactic and therapeutic regimens, compositions comprising MASP-2 inhibitory agents may be administered in several dosages until a sufficient therapeutic outcome has been achieved in the subject.

VI. Use of the MASP-2/C1-INH complex as a Biomarker for Severe COVID-19

In another aspect, the disclosure provides a biomarker for MASP-2-mediated lectin pathway activation, namely a fluid-phase MASP-2/C1-INH complex, a change in the presence and/or concentration of which are associated with the presence or risk of developing acute disease associated with COVID-19 infection, the presence or risk of developing one or more long-term sequelae associated with COVID-19 infection, and/or the clinically meaningful treatment of COVID-19 infection with a complement inhibitor. Also provided are compositions, kits and methods for interrogating the concentration of the fluid-phase MASP-2/C1-INH complex in a biological fluid, such as a biological fluid obtained from a subject infected with COVID-19. The compositions and methods are useful for, among other things, evaluating risk for developing acute disease associated with COVID-19, diagnosing COVID-19 and/or COVID-19-induced long term sequelae, monitoring progression or abatement of COVID-19-related disease, and/or monitoring response to treatment with a complement inhibitor, such as a MASP-2 inhibitory agent, or optimizing such treatment.

As described in Examples 25 to 30 herein, to determine the activation state of the lectin pathway (LP) effector enzyme human MASP-2 (set forth as SEQ ID NO:6), a feature was utilized that takes advantage of the fact that human C1 Inhibitor (C1-INH) (set forth as SEQ ID NO:86) which acts as a pseudo-substrate once MASP-2 has been activated, forms a covalent fluid-phase MASP-2/C1-INH complex. Thus, the level of MASP-2/C1-INH complex in a sample of plasma or serum provides a clear measure of recent LP activation.

As described in Examples 25 to 30, the inventors have observed that the concentrations of the MASP-2/C1-INH in the blood (e.g., serum and/or plasma) are abnormally high in patients with severe COVID-19 and also in subjects previously infected with COVID-19 and suffering from long-term sequelae. The inventors have also observed that, following recovery, the concentration of the MASP-2/C1-INH complex decreases to normal levels in most instances. As further described in Examples 29 and 30, the inventors determined that subjects suffering from acute COVID-19 had high levels of MASP-2/C1-INH prior to treatment with narsoplimab which rapidly decreased after treatment with narsoplimab. The inventors believe that monitoring a patient infected with SARS-CoV-2 for an increase in the concentration of MASP-2/C1-INH complex is useful for diagnosing a patient as having, or at risk for developing acute COVID-19, and also for diagnosing a subject as having, or at risk for developing post-acute COVID-19 (also referred to as Long-COVID-19) and optionally treating a subject identified as having such risk with a complement inhibitor, such as a MASP-2 inhibitor. Monitoring the status of the MASP-2/C1-INH complex can also be useful for determining whether a COVID-19 patient is responding to therapy with a complement inhibitor, such as a MASP-2 inhibitor, and optionally adjusting the dosage of the MASP-2 inhibitor as needed to bring the level of MASP-2/C1-INH into the normal range.

In accordance with the foregoing, in one embodiment, the present disclosure provides, among other things, compositions, kits and methods of measuring the amount of MASP-2/C1-INH complex as a biomarker for MASP-2-mediated lectin pathway activation, and whose concentration in a biological fluid is abnormally elevated in patients afflicted with acute COVID-19 disease associated with infection with SARS-Cov2 and/or those subjects previously infected with SARS-Cov2 and suffering from, or at risk for developing Long-COVID-19 sequelae.

Accordingly, in one embodiment, the present invention is directed to a monoclonal antibody (mAb C#7 or mAb C#8) that specifically binds to MASP-2 and is capable of binding to MASP-2 in complex with C1-INH (also referred to as MASP-2/C1-INH complex), and the use of this antibody in methods of detecting the presence or amount of MASP-2/C1-INH complex in a biological sample. In another embodiment, the present invention is directed to an immunoassay comprising the use of a MASP-2 specific monoclonal antibody and a C1-INH specific antibody to measure the presence or amount of MASP-2/C1-INH complex in a mammalian subject suffering from or at risk for developing an infection with a coronavirus or influenza virus to determine the activation status of the lectin pathway, optionally before and after treatment with a complement inhibitory agent, such as a MASP-2 inhibitory agent, such as a MASP-2 inhibitory antibody, (e.g., narsoplimab) wherein the MASP-2 inhibitory antibody is capable of inhibiting the lectin pathway.

In one embodiment, the presence or amount of MASP-2/C1-INH complex is useful as a biomarker for the determination of the presence or risk of developing severe COVID-19 or Long-Term COVID-19 in a subject infected with SARS-CoV-2, wherein a higher level of MASP-2/C1-INH in the subject as compared to a normal uninfected subject or pool of subjects, or threshold value, is indicates that the subject is suffering from severe COVID-19, or has a higher risk of developing severe COVID-19, or is suffering from Long-COVID-19, or has an increased risk of developing Long-COVID-19. In some embodiments, the method further comprises administering a complement inhibitory agent to a subject determined to have an increased level of MASP-2/C1-INH complex. In some embodiments, the present disclosure provides a method of determining the efficacy of a complement inhibitor, such as a MASP-2 inhibitory agent (e.g., a MASP-2 inhibitory antibody such as narsoplimab) and/or monitoring the dosing in a subject undergoing treatment with a complement inhibitory agent, in the subject. In some embodiments, the subject is suffering from a coronavirus, such as COVID-19 or an influenza virus or other lectin pathway disease or disorder (e.g., HSCT-TMA, IgAN, GvHD or other lectin pathway disease or disorder).

A. Anti-MASP-2 Monoclonal Antibodies for Use in a highly sensitive ELISA assay and bead-based assay for detecting MASP-2/C1-INH complexes in a Biological Sample

As described in Examples 25 to 30 herein, the inventors have generated anti-MASP-2 antibody mAb clone #C7 and #C8 suitable for use in the detection assays for MASP-2/C1-INH complex and methods described herein.

As described in Example 25 and 26, the variable heavy and light chain fragments of mAb clone #C7 and mAb clone #C8 have been cloned and sequenced.

The heavy chain and light chain variable regions of the anti-MASP-2 mAb clone #C7 and clone #C8 are provided below.

SEQ ID NO:87: mAb clone #C7 HC variable region

SEQ ID NO:88: mAb clone #C7 LC variable region

SEQ ID NO:97: mAb clone #C8 HC variable region

SEQ ID NO:98: mAb clone #C8 LC variable region

Accordingly, in one aspect, the present invention provides an isolated monoclonal antibody, or antigen-binding fragment thereof, that specifically binds to MASP-2 while in complex with C1-INH, wherein said antibody comprises (a) HC-CDR1, HC-CDR-2 and HC-CDR2 in the heavy chain variable region set forth as SEQ ID NO:87 and LC-CDR1, LC-CDR-2, LC-CDR-3 in the light chain variable region set forth as SEQ ID NO:88 or (b) HC-CDR1, HC-CDR-2 and HC-CDR2 in the heavy chain variable region set forth as SEQ ID NO:97 and LC-CDR1, LC-CDR-2, LC-CDR-3 in the light chain variable region set forth as SEQ ID NO:98. In one embodiment, the isolated antibody comprises a heavy chain variable region comprising HC-CDR-1 comprising SEQ ID NO:89, HC-CDR2 comprising SEQ ID NO:90 and HC-CDR3 comprising SEQ ID NO:91 and a light chain variable region comprising LC-CDR1 comprising SEQ ID NO:92, LC-CDR2 comprising SEQ ID NO:83 and LC-CDR3 comprising SEQ ID NO:94. In one embodiment the anti-MASP-2 antibody is a humanized, chimeric or fully human antibody. In one embodiment the anti-MASP-2 antibody fragment is selected from the group consisting of Fv, Fab, Fab', $F(ab)_2$ and $F(ab')_2$. In one embodiment, the anti-MASP-2 antibody is a single-chain molecule. In one embodiment, the anti-MASP-2 antibody is an IgG molecule selected from the group consisting of IgG1, IgG2 and IgG4. In one embodiment, the anti-MASP-2 antibody or antigen-binding fragment thereof is labeled with a detectable moiety, for example a detectable moiety suitable for use in an immunoassay as further described herein. In one embodiment, the anti-MASP-2 antibody

or fragment thereof is immobilized on a substrate, such as a substrate suitable for use in an immunoassay, as further described herein.

In one embodiment, the anti-MASP-2 antibody or fragment thereof (i.e., an antibody or fragment thereof that specifically binds to human MASP-2 in complex with C1-INH) comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region comprising SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region comprising SEQ ID NO:88, wherein the CDRs are numbered according to the Kabat numbering system. In one embodiment, the anti-MASP-2 antibody or fragment thereof (i.e., an antibody or fragment thereof that specifically binds to human MASP-2 in complex with C1-INH) comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region comprising SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region comprising SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

In one embodiment, the anti-MASP-2 antibody or fragment thereof comprises a binding domain comprising the following six CDRs: (a) an HC-CDR1 comprising the amino acid sequence SEQ ID NO:89; (b) an HC-CDR2 comprising the amino acid sequence SEQ ID NO:90, (c) an HC-CDR3 comprising the amino acid sequence SEQ ID NO:91; (d) a LC-CDR1 comprising the amino acid sequence SEQ ID NO:92; (**e**) a LC-CDR2 comprising the amino acid sequence SEQ ID NO:93 and (f) a LC-CDR3 comprising the amino acid sequence SEQ ID NO:94.

In one embodiment the anti-MASP-2 antibody or fragment thereof comprises a VH domain having at least 95% sequence identity (such as at least 96%, at least 97%, at least 98%, or at least 99% identity) to the amino acid sequence of SEQ ID NO:87 or SEQ ID NO:97. In one embodiment, the MASP-2-specific antibody or fragment thereof comprises a VL domain having at least 95% sequence identity (such as at least 96%, at least 97%, at least 98%, or at least 99% identity) to the amino acid sequence of SEQ ID NO:88. In one embodiment, the anti-MASP-2 antibody or fragment thereof comprises a VH comprising SEQ ID NO:87 and a VL comprising SEQ ID NO:88 or SEQ ID NO:98.

In one embodiment, the anti-MASP-2 antibody or fragment thereof comprises a binding domain comprising the following six CDRs: (a) an HC-CDR1 comprising SEQ ID NO:89, (b) an HC-CDR2 comprising SEQ ID NO:90; (c) an HC-CDR3 comprising SEQ ID NO: 91; (d) a LC-CDR1 comprising SEQ ID NO:92, (e) a LC-CDR2 comprising

SEQ ID NO:93 and (f) a LC-CDR3 comprising SEQ ID NO:94. In one embodiment the anti-MASP-2 antibody or fragment thereof comprises a VH domain having at least 95% sequence identity (such as at least 96%, at least 97%, at least 98%, or at least 99% identity) to the amino acid sequence of SEQ ID NO:87. In one embodiment, the anti-MASP-2 antibody or fragment thereof comprises a VL domain having at least 95% sequence identity (such as at least 96%, at least 97%, at least 98%, or at least 99% identity) to the amino acid sequence of SEQ ID NO:88. In one embodiment, the anti-MASP-2 antibody or fragment thereof comprises a VH comprising SEQ ID NO:87 and a VL comprising SEQ ID NO:88.

In another embodiment, the present disclosure provides a nucleic acid encoding the complementarity determining regions (CDRs) of a heavy chain variable region of an anti-MASP-2 antibody, or antigen-binding fragment thereof, that specifically binds to human MASP-2 while in complex with C1-INH, wherein the heavy chain variable region comprises an amino acid sequence set forth as SEQ ID NO:95, and wherein the CDRs are numbered according to the Kabat numbering system. In another embodiment, the present disclosure provides a nucleic acid encoding the complementarity determining regions (CDRs) of a light chain variable region of an anti-MASP-2 antibody, or antigen-binding fragment thereof that specifically binds to human MASP-2 while in complex with C1-INH wherein the light chain variable region comprises an amino acid sequence set forth as SEQ ID NO:96, and wherein the CDRs are numbered according to the Kabat numbering system.

In another embodiment, the present disclosure provides a cloning or expression vector comprising a nucleic acid encoding complementarity determining regions (CDRs) of heavy and/or light chain variable regions of an antibody, or antigen-binding fragment thereof, that specifically binds to human MASP-2, wherein (a) the heavy chain variable region comprises the amino acid sequence set forth as SEQ ID NO:87 and the light chain variable region comprises the amino acid sequence set forth as SEQ ID NO:88, or (b) the amino acid sequence set forth as SEQ ID NO:97 and the light chain variable region comprises the amino acid sequence set forth as SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

In another embodiment, the present disclosure provides a cell containing a cloning or expression vector comprising a nucleic acid encoding complementarity determining regions (CDRs) of heavy and/or light chain variable regions of an antibody, or antigen-

binding fragment thereof, that specifically binds to human MASP-2, wherein (a) the heavy chain variable region comprises the amino acid sequence set forth as SEQ ID NO:87 and the light chain variable region comprises the amino acid sequence set forth as SEQ ID NO NO:88, or (b) wherein the heavy chain variable region comprises the amino acid sequence set forth as SEQ ID NO:97 and the light chain variable region comprises the amino acid sequence set forth as SEQ ID NO NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

In another embodiment, the present disclosure provides a method for producing an anti-MASP-2 antibody comprising culturing a cell containing an expression vector which contains a nucleic acid that encodes one or both of the heavy and light chain polypeptides of the MASP-2 antibodies or antigen-binding fragments disclosed herein. The cell or culture of cells is cultured under conditions and for a time sufficient to allow expression by the cell (or culture of cells) of the antibody or antigen-binding fragment thereof encoded by the nucleic acid. The method can also include isolating the antibody or antigen binding fragment thereof from the cell (or culture of cells) or from the media in which the cell or cells were cultured.

In one embodiment, the present disclosure provides a composition comprising any of the anti-MASP-2 antibodies, or antigen-binding fragments disclosed herein.

In one embodiment, the present disclosure provides a substrate for use in an immunoassay comprising at least one or more of the anti-MASP-2 antibodies, or antigen-binding fragments disclosed herein.

In one embodiment, the present disclosure provides a kit for detecting the presence or amount of MASP-2/C1-INH complex in a test sample, such as a biological sample, said kit comprising (a) at least one container, and (b) at least one or more of any of the MASP-2 antibodies, or antigen-binding fragments disclosed herein. In some embodiments, the kit comprises an anti-MASP-2 antibody comprising a binding domain comprising (a) HC-CDR1, HC-CDR-2 and HC-CDR2 in the heavy chain variable region set forth as SEQ ID NO:87 and LC-CDR1, LC-CDR-2, LC-CDR-3 in the light chain variable region set forth as SEQ ID NO:88 or (b) HC-CDR1, HC-CDR-2 and HC-CDR2 in the heavy chain variable region set forth as SEQ ID NO:97 and LC-CDR1, LC-CDR-2, LC-CDR-3 in the light chain variable region set forth as SEQ ID NO:98. In some embodiments, the kit comprises an anti-MASP-2 antibody comprising a binding domain comprising the following six CDRs: (a) an HC-CDR1 comprising SEQ ID NO:89, (b) an

HC-CDR2 comprising SEQ ID NO:90; (c) an HC-CDR3 comprising SEQ ID NO: 91; (d) a LC-CDR1 comprising SEQ ID NO:92, (e) a LC-CDR2 comprising SEQ ID NO:93 and (f) a LC-CDR3 comprising SEQ ID NO:94. In some embodiments, the kit further comprises at least one antibody that specifically binds to the C1-INH.

Anti-MASP-2 Antibodies Labeled with a Detectable Moiety

In another aspect, the invention provides anti-MASP-2 antibodies (e.g., mAb clone #C7 or mAb clone #C8) that are labeled with a detectable moiety (i.e., a moiety that permits detection and/or quantitation). In various embodiments, the antibodies described herein are conjugated to a detectable label that may be detected directly or indirectly. In this regard, an antibody "conjugate" refers to an anti-MASP-2 antibody that is covalently linked to a detectable label. In the present invention, monoclonal antibodies, antigen-binding fragments thereof, and antibody derivatives thereof, such as a single-chain-variable-fragment antibody or an epitope tagged antibody, may all be covalently linked to a detectable label. In “direct detection”, only one detectable antibody is used, *i.e.*, a primary detectable antibody. Thus, direct detection means that the antibody that is conjugated to a detectable label may be detected, per se, without the need for the addition of a second antibody (secondary antibody).

A "detectable label" is a molecule or material that can produce a detectable (such as visually, electronically or otherwise) signal that indicates the presence and/or concentration of the label in a sample. When conjugated to an antibody, the detectable label can be used to locate and/or quantify the target to which the specific antibody is directed. Thereby, the presence and/or concentration of the target in a sample can be detected by detecting the signal produced by the detectable label. A detectable label can be detected directly or indirectly, and several different detectable labels conjugated to different specific antibodies can be used in combination to detect one or more targets.

Examples of detectable labels, which may be detected directly, include fluorescent dyes and radioactive substances and metal particles. In contrast, indirect detection requires the application of one or more additional antibodies, *i.e.*, secondary antibodies, after application of the primary antibody. Thus, the detection is performed by the detection of the binding of the secondary antibody or binding agent to the primary detectable antibody. Examples of primary detectable binding agents or antibodies requiring addition of a secondary binding agent or antibody include enzymatic detectable binding agents and hapten detectable binding agents or antibodies.

Examples of detectable labels which may be conjugated to antibodies of the present disclosure include fluorescent labels, enzyme labels, radioisotopes, chemiluminescent labels, electrochemiluminescent labels, bioluminescent labels, polymers, polymer particles, metal particles, haptens, and dyes.

Examples of fluorescent labels include 5-(and 6)-carboxyfluorescein, 5- or 6-carboxyfluorescein, 6-(fluorescein)-5-(and 6)-carboxamido hexanoic acid, fluorescein isothiocyanate, rhodamine, tetramethylrhodamine, and dyes such as Cy2, Cy3, and Cy5, optionally substituted coumarin including AMCA, PerCP, phycobiliproteins including R-phycoerythrin (RPE) and allophycoerythrin (APC), Texas Red, Princeton Red, green fluorescent protein (GFP) and analogues thereof, and conjugates of R-phycoerythrin or allophycoerythrin, inorganic fluorescent labels such as particles based on semiconductor material like coated CdSe nanocrystallites.

Examples of polymer particle labels include micro particles or latex particles of polystyrene, PMMA or silica, which can be embedded with fluorescent dyes, or polymer micelles or capsules which contain dyes, enzymes or substrates.

Examples of metal particle labels include gold particles and coated gold particles, which can be converted by silver stains. Examples of haptens include DNP, fluorescein isothiocyanate (FITC), biotin, and digoxigenin. Examples of enzymatic labels include horseradish peroxidase (HRP), alkaline phosphatase (ALP or AP), β-galactosidase (GAL), glucose-6-phosphate dehydrogenase, β-N-acetylglucosamimidase, β-glucuronidase, invertase, Xanthine Oxidase, firefly luciferase and glucose oxidase (GO). Examples of commonly used substrates for horseradishperoxidase include 3,3'-diaminobenzidine (DAB), diaminobenzidine with nickel enhancement, 3-amino-9-ethylcarbazole (AEC), Benzidine dihydrochloride (BDHC), Hanker-Yates reagent (HYR), Indophane blue (IB), tetramethylbenzidine (TMB), 4-chloro-1-naphtol (CN), .alpha.-naphtol pyronin (.alpha.-NP), o-dianisidine (OD), 5-bromo-4-chloro-3-indolylphosp- hate (BCIP), Nitro blue tetrazolium (NBT), 2-(p-iodophenyl)-3-p-nitropheny- l-5-phenyl tetrazolium chloride (INT), tetranitro blue tetrazolium (TNBT), 5-bromo-4-chloro-3-indoxyl-beta-D-galactoside/ferro-ferricyanide (BCIG/FF).

Examples of commonly used substrates for Alkaline Phosphatase include Naphthol-AS-B 1-phosphate/fast red TR (NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-AS-B1-phosphate/- fast red TR (NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-AS-B1-phosphate/new fuschin

(NABP/NF), bromochloroindolyl phosphate/nitroblue tetrazolium (BCIP/NBT), 5-Bromo-4-chloro-3-indolyl-b-- d-galactopyranoside (BCIG).

Examples of luminescent labels include luminol, isoluminol, acridinium esters, 1,2-dioxetanes and pyridopyridazines. Examples of electrochemiluminescent labels include ruthenium derivatives. Examples of radioactive labels include radioactive isotopes of iodide, cobalt, selenium, tritium, carbon, sulfur and phosphorous.

Detectable labels may be linked to the antibodies described herein (i.e., any of the anti-MASP-2 antibodies or anti-C1-INH antibodies) or to any other molecule that specifically binds to a biological marker of interest, *e.g.*, an antibody, a nucleic acid probe, or a polymer. Furthermore, one of ordinary skill in the art would appreciate that detectable labels can also be conjugated to second, and/or third, and/or fourth, and/or fifth binding agents or antibodies, etc. Moreover, the skilled artisan would appreciate that each additional binding agent or antibody used to characterize a biological marker of interest may serve as a signal amplification step. The biological marker may be detected visually using, *e.g.*, light microscopy, fluorescent microscopy, electron microscopy where the detectable substance is for example a dye, a colloidal gold particle, a luminescent reagent. Visually detectable substances bound to a biological marker may also be detected using a spectrophotometer. Where the detectable substance is a radioactive isotope detection can be visually by autoradiography, or non-visually using a scintillation counter. See, *e.g.*, Larsson, 1988, Immunocytochemistry: Theory and Practice, (CRC Press, Boca Raton, Fla.); Methods in Molecular Biology, vol. 80 1998, John D. Pound (ed.) (Humana Press, Totowa, N.J.).

In another embodiment, the anti-MASP-2 antibody (e.g., mAb clone #C7, mAb clone #C8 or anti-C1-INH antibody) is not labeled (i.e., is naked), and the presence thereof can be detected using a labeled antibody which binds to the anti-MASP-2 antibody or the anti-C1-INH antibody.

B. Compositions and Kits for measuring MASP-2/C1-INH complex in a biological sample

Compositions

In another aspect, the present disclosure provides a substrate, such as a solid support (e.g., an insoluble substrate, such as non-aqueous matrix, such as a plate or slide

made of glass, polysaccharides (e.g., agarose), polyacrylamides, polystyrene, plastic or metal, a polymer-coated bead, a tube, or a ceramic or metal chip) that comprises immobilized (or otherwise deposited) monoclonal anti-MASP-2 antibodies disclosed herein (such as anti-MASP-2 antibodies that bind to MASP-2 in complex with C1-INH, such as mAb clone #C7 or mAb clone #C8). In some embodiments, the anti-MASP-2 antibodies are immobilized (or deposited) at discrete locations (e.g., in the wells of a multiwall plate, or deposited in an array on a biochip). In some embodiments, the substrate comprising the anti-MASP-2 antibodies may be part of a kit for detecting MASP-2/C1-INH complex in a biological sample obtained from a mammalian subject.

Kits

In another aspect, the present disclosure provides kits for use in performing one or more assays disclosed herein.

In one embodiment, the present disclosure provides a kit (i.e., a packaged combination of reagents in predetermined amounts) with reagents and instructions for detecting the presence or amount of MASP-2/C1-INH complex in a test sample, such as a biological sample. Exemplary kits may contain at least one anti-MASP-2 monoclonal antibody or antigen binding fragment thereof as described herein (i.e., mAb clone #C7 or mA clone #C8) and at least one anti-C1-INH antibody. Where the anti-MASP-2 antibody or anti-C1-INH antibody is labeled with a detectable moiety, such as an enzyme, the kit will include substrates and cofactors required by the enzyme (e.g., a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such as stabilizers, buffers (e.g., a blocking buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents, which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

In addition, kits may include instructional materials disclosing means of use of an antibody of the present invention (e.g., for detection of MASP-2/C1-INH complexes, or absence thereof). For example, the kit may additionally contain means of detecting a label (e.g., enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a sheep anti-mouse-HRP or the like). The kits may

additionally include buffers and other reagents routinely used for the practice of a particular immunoassay, as is well known in the art.

Certain embodiments provide kits for detecting the presence or amount of MASP-2/C1-INH in a sample, wherein the kits contain at least one anti-MASP-2 antibody as described herein, such as an antibody or fragment comprising the CDRs from clone #C7 as set forth in TABLE 5 or an antibody or fragment thereof comprising the CDRs from clone #C8. In certain embodiments, a kit may comprise buffers, enzymes, labels, substrates, beads or other surfaces to which the antibodies of the invention are attached, and the like, and instructions for use.

Certain embodiments provide kits for detecting the presence or amount of MASP-2/C1-INH in a biological sample, wherein the kits contain at least one anti-MASP-2 antibody as described herein, such an antibody or fragment comprising the CDRs from MASP-2-specific clone mAb #C7 as set forth in TABLE 5 or an antibody or fragment thereof comprising the CDRs from clone #C8. The subject anti-MASP-2 antibodies and antigen-binding fragments thereof can be labeled with any appropriate detectable moiety as described herein. In certain embodiments, a kit may comprise buffers, enzymes, labels, substrates, beads or other surfaces to which the antibodies of the invention are attached, and the like, and instructions for use.

Items in a kit may be individually wrapped or packaged in individual receptacles, which are provided together in a larger container (e.g., a cardboard or styrofoam box).

In accordance with the foregoing, in one embodiment, the present disclosure provides a kit for measuring the presence or amount of MASP-2/C1-INH complex in a biological sample, the kit comprising at least one monoclonal antibody that specifically binds to MASP-2 in an immunoassay and optionally an anti-C1-INH specific antibody, or antigen-binding fragment thereof, that specifically binds to C1-INH. In one embodiment, the MASP-2-specific antibody or fragment thereof comprises a binding domain comprising HC-CDR-1, HC-CDR-2 and HC-CDR-3 in a heavy chain variable region comprising SEQ ID NO:87 and comprising LC-CDR-1, LC-CDR2 and LC-CDR3 in a light chain variable region comprising SEQ ID NO:88, wherein the CDRs are numbered according to the Kabat numbering system. In one embodiment, the MASP-2-specific antibody or fragment thereof comprises a binding domain comprising HC-CDR-1, HC-CDR-2 and HC-CDR-3 in a heavy chain variable region comprising SEQ ID NO:97 and comprising LC-CDR-1, LC-CDR2 and LC-CDR3 in a light chain variable region

comprising SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

In some embodiments, the kit further comprises at least one container.

In some embodiments, the kit is for carrying out an enzyme-linked immunosorbent assay (ELISA). In one embodiment, the anti-MASP-2 antibody or fragment thereof is a coating antibody. In one embodiment, the anti-MASP-2 antibody or fragment thereof is a detecting antibody. In one embodiment, the C1-INH-specific antibody or fragment thereof is a coating antibody. In one embodiment, the C1-INH-specific antibody or fragment thereof is a detecting antibody. In one embodiment, the anti-MASP-2 antibody is a coating/capture antibody and comprises a binding domain comprising (a) HC-CDR-1, HC-CDR-2 and HC-CDR-3 in a heavy chain variable region comprising SEQ ID NO:87 and comprising LC-CDR-1, LC-CDR2 and LC-CDR3 in a light chain variable region comprising SEQ ID NO:88 or (b)) HC-CDR-1, HC-CDR-2 and HC-CDR-3 in a heavy chain variable region comprising SEQ ID NO:97 and comprising LC-CDR-1, LC-CDR2 and LC-CDR3 in a light chain variable region comprising SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system and is immobilized on a substrate, such as a solid support (e.g., an insoluble substrate, such as non-aqueous matrix, such as a plate or slide made of glass, polysaccharides (e.g., agarose), polyacrylamides, polystyrene, plastic or metal, a polymer-coated bead, a tube, or a ceramic or metal chip).

In some embodiments, the kit is for carrying out a bead-based immunoflouresence assay, such as a Luminex assay and comprises (i) at least one anti-MASP-2 antibody, such as mAb #C7 or mAb #C8 immobilized on beads (such as polystyrene microspheres, or magnetic polystyrene microspheres) suitable for capturing MASP-2/C1-INH complexes from human serum or plasma. In some embodiments, the kit further comprises (ii) at least one anti-C1-INH antibodiy for use as a detection antibody to detect the captured complexes. In one embodiment, the kit further comprises (iii) an anti-C1s antibody suitable for capturing C1s/C1-INH complexes from human serum or plasma.

In various embodiments of the kits of the invention, the subject antibodies and antigen-binding fragments thereof can be labeled with any appropriate detectable moiety as described herein. In certain embodiments, the kit further comprises buffers, enzymes, labels, substrates, beads (such as polystyrene microspheres or magnetic polystyrene

microsperes) or other surfaces to which the antibodies of the invention are attached, and the like, and instructions for use.

C. Methods of Detecting MASP-2/C1-INH Complex in a Biological Sample

As described herein, the inventors have generated anti-MASP-2 antibodies that are suitable for use in an immunoassay for detecting the presence and/or amount of MASP-2/C1-INH in a biological sample, such as a biological sample obtained from a mammalian subject.

In one aspect, the anti-MASP-2 antibodies (e.g., mAb clone #C7 or mAb clone #C8) of the present invention are used in an *in vitro* immunoassay for analyzing a test sample, such as a biological sample obtained from a test subject, for the presence or amount of MASP-2/C1-INH complex. In such *in vitro* immunoassays, the anti-MASP-2 antibody, or antigen-binding fragment thereof, may be naked or may be labeled with a detectable moiety, as described herein, and may be utilized in liquid phase or bound to a substrate, as described below. For purposes of *in vitro* assays, any type of antibody such as murine, chimeric, humanized or human may be utilized, since there is no host immune response to consider.

The antibodies of the present disclosure may be employed in any known immunoassay method, such as competitive binding assays, direct and indirect sandwich assays, lateral flow assays (e.g., dipstick format), bead-based assays and immunoprecipitation assays (see e.g., Zola, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press. Inc., 1987).

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, of the MASP-2/C1-INH complex to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody (e.g., an anti-MASP-2 antibody, such as Clone #C7 or Clone #C8, which is immobilized on a solid support (e.g., substrate), and thereafter a second antibody binds to the C1-INH, thus forming an insoluble three-part complex. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay).

For example, one preferable type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme. ELISA assays, regardless of the detection system employed, generally include the immobilization of an antigen or antibody to a

substrate (e.g., a solid support), as well as the use of an appropriate detecting reagent. In an ELISA assay, the protein antigen-antibody reaction takes place on a substrate (e.g., a solid support), typically in wells on microtiter plates. Antigen and this first antibody, also called the coating or capture antibody, react and produce a stable complex, which can be visualized by addition of a second antibody, called the detection antibody, which may be directly or indirectly linked to an enzyme. Addition of a substrate for that enzyme results in a color formation, which can be measured photometrically.

In one embodiment, the anti-MASP-2 antibodies of the invention (e.g., clone #C7 or clone #C8) are used as a coating/capture antibody to detect the presence of the MASP-2/C1-INH complex in a biological sample using an enzyme-linked immunosorbent assay (ELISA) (see e.g., Gold et al. *J Clin Oncol.* 24:252-58, 2006).

In the direct competitive ELISA, a pure or semipure antigen preparation is bound to a substrate that is insoluble in the fluid or cellular extract being tested and a quantity of detectably labeled soluble antibody is added to permit detection and/or quantitation of the binary complex formed between substrate-bound antigen and labeled antibody.

In contrast, a "double-determinant" ELISA, also known as a "two-site ELISA" or "sandwich assay," requires small amounts of antigen and the assay does not require extensive purification of the antigen. Thus, the double-determinant ELISA is preferred to the direct competitive ELISA for the detection of an antigen in a clinical sample. See, for example, the use of the double-determinant ELISA for quantitation of the c-myc oncoprotein in biopsy specimens. Field et al., *Oncogene* 4: 1463 (1989); Spandidos et al., AntiCancer Res. 9: 821 (1989). In a double-determinant ELISA, a quantity of unlabeled monoclonal antibody or antibody fragment (the "capture antibody") is bound to a substrate (e.g., a solid support), the test sample is brought into contact with the capture antibody, and a quantity of detectably labeled soluble antibody (or antibody fragment) is added to permit detection and/or quantitation of the ternary complex formed between the capture antibody, antigen, and labeled antibody.

In one embodiment, the capture antibody bound to a substrate (e.g., solid support) is an anti-MASP-2 antibody or antigen-binding fragment thereof as disclosed herein that binds to MASP-2 in complex with C1-INH. In one embodiment, the capture antibody bound to a substrate (e.g., solid support) is a MASP-2 specific antibody or antigen-binding fragment thereof as disclosed herein.

Methods of performing a double-determinant ELISA are well-known by those of skill in the art. See, for example, Field et al., *Oncogene* 4: 1463 (1989); Spandidos et al., *AntiCancer Res.* 9: 821 (1989); and Moore et al., *Methods in Molecular Biology* Vol 10:273-281 (The Humana Press, Inc. 1992).

In the double-determinant ELISA, the soluble antibody or antibody fragment must bind to an epitope on the MASP-2/C1-INH complex that is distinct from the epitope recognized by the capture antibody. The double-determinant ELISA can be performed to ascertain whether the MASP-2/C1-INH complex is present in a test biological sample, such as a body fluid (e.g., blood, plasma or serum) or a biopsy sample. Alternatively, the assay can be performed to quantitate the amount of MASP-2/C1-INH complex that is present in a clinical sample of body fluid. The quantitative assay can be performed by including dilutions of MASP-2/C1-INH complex.

In vitro immunoassays can be performed in which at least one MASP-2 specific antibody or antigen-binding fragment thereof is bound to a substrate (e.g., a solid-phase carrier). For example, MASP-2 specific monoclonal antibodies or fragments thereof can be attached to a polymer, such as aminodextran, in order to link the monoclonal antibody to an insoluble substrate such as a polymer-coated bead, a plate, a tube, or a ceramic or metal chip. In one embodiment, the substrate is suitable for use in an ELISA method (e.g., a multiwell microtitre plate). In one embodiment, the substrate is a bead (e.g.,a polystyrene microsphere or magnetic polystyrene microspere) for use in a bead-based immunoflouresence assay, such as a Luminex assay as described herein. In some embodiments, the disclosure provides an immunoassay for detecting both MASP-2/C1-INH and C1s/C1-INH complexes wherein a MASP-2-specific antibody or antigen-binding fragment thereof is bound to one set of beads and a C1s-specific antibody or antigen-binding fragment thereof is bound to a second set of beads.

Other suitable *in vitro* assays will be readily apparent to those of skill in the art. The specific concentrations of detectably labeled anti-MASP-2 antibody or C1-INH specific antibody, the temperature and time of incubation, as well as other assay conditions may be varied, depending on various factors including the concentration of the MASP-2/C1-INH complex in the sample, the nature of the sample, and the like. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

<u>Assays to detect and/or measure MASP-2/C1-INH complex</u>

In accordance with the foregoing, in one aspect, the present invention provides a method of determining the presence or amount of MASP-2/C1-INH in a test sample, such as a biological sample, the method comprising (a) contacting a test sample with a MASP-2-specific monoclonal antibody or antigen-binding fragment thereof in an *in vitro* immunoassay; (b) contacting the test sample with a C1-INH specific antibody and (c) detecting the presence or absence of binding of said C1-INH antibody, wherein the presence of binding indicates the presence or amount of MASP-2/C1-INH complex in the sample.

In one embodiment, the MASP-2-specific antibody or antigen-binding fragment thereof comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region comprising SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region comprising SEQ ID NO:88. In some embodiments, the MASP-2 specific antibody or fragment thereof is a monoclonal antibody comprising the CDRs from MASP-2 specific clone #C7 , as set forth in TABLE 5. In one embodiment, the MASP-2-specific antibody or antigen-binding fragment thereof comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region comprising SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region comprising SEQ ID NO:98.

In one embodiment, the method further comprises comparing the amount of MASP-2/C1-INH detected in accordance with step (c) with a reference standard or control sample to determine the level of MASP-2/C1-INH in the test sample.

In one embodiment, the control sample is an individual or pooled sample of subjects suffering from a lectin pathway disease or disorder (e.g., COVID-19, HSCT-TMA, IgAN, GvHD or other lectin pathway disease or disorder). In one embodiment, the control sample is an individual or pooled sample of normal healthy volunteers. In one embodiment, the control sample is a baseline sample of a subject prior to treatment with a complement inhibitor (e.g., a MASP-2 inhibitory agent or other complement inhibitor). In one embodiment, the MASP-2-specific antibody or antigen-binding fragment thereof is immobilized on a substrate. In one embodiment, the immunoassay is an ELISA assay. In one embodiment, the immunoassay is a bead-based assay such as a Luminex assay.

In one embodiment, the MASP-2 specific antibody is labeled with a detectable moiety and step (b) comprises detecting the presence of said detectable moiety. In one embodiment, said MASP-2-specific antibody or antigen-binding fragment thereof is

naked (i.e., not labeled), and the presence or amount of the antibody or fragment thereof bound to MASP-2/C1-INH complex is detected using a labeled antibody which binds to the MASP-2 antibody. In one embodiment, said MASP-2-specific antibody or antigen-binding fragment thereof is immobilized on a substrate (i.e., capture/coating) and the bound MASP-2/C1-INH complex is detected with a second antibody that binds to C1-INH as described herein).

In one embodiment, the test sample is a biological sample obtained from a mammalian subject. In various embodiments, the biological sample is a fluid sample selected from the group consisting of whole blood, serum, plasma, sputum, amniotic fluid, cerebrospinal fluid, cell lysate, ascites, urine, and saliva. In one embodiment, the biological sample is selected from the group consisting of blood, serum, plasma, urine and cerebrospinal fluid. As described herein, in some embodiments, the assay methods and kits are suitable for measuring the presence and/or amount of MASP-2/C1-INH in low serum concentrations (i.e., less than 10% serum, such as from 0.1% to 9%, such as from 0.5% to 8%, such as from 1% to 5%, such as 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8% or 9% serum).

In one embodiment, the mammalian subject (e.g., human) is infected with SARS-CoV-2 and is suffering from, or at risk for developing severe COVID-19 and/or Long-COVID-19.

In one embodiment, the mammalian subject (e.g., human) has been treated with a complement inhibitor, such lectin pathway complement inhibitor, such as a MASP-2 inhibitory agent (e.g. a MASP-2 inhibitory antibody, such as narsoplimab), as further described herein.

In one embodiment, the mammalian subject (e.g., human) is suffering from a lectin pathway disease or disorder (e.g., COVID-19, HSCT-TMA, IgAN, GvHD or other lectin pathway disease or disorder.

As described herein, the methods of detecting or measuring MASP-2/C1-INH complex according to various embodiments of the present disclosure may be used to define a pharmacodynamic endpoint or therapeutic threshold, or a determination of whether to treat a subject with a complement inhibitor, such as an lectin pathway complement inhibitor, such as a MASP-2 inhibitory agent, (e.g., a MASP-2 inhibitory antibody, e.g., narsoplimab).

Although the details of an immunoassay may vary with the particular format employed, in one embodiment, the method of detecting MASP-2/C1-INH in a test sample comprises the steps of contacting the test sample with a capture antibody that specifically binds to MASP-2. The MASP-2 antibody is allowed to bind to MASP-2/C1-INH in the sample under immunologically reactive conditions, and the presence of the bound antibody is detected directly or indirectly with an anti-C1-INH antibody. The MASP-2-specific antibodies may be used, for example, as the capture antibody of an ELISA for MASP-2/C1-INH or a bead-based assay, or as a second antibody to bind to MASP-2/C1-INH captured by a capture antibody that binds to C1-INH. As is known in the art, the presence of the second antibody is typically then detected. In some embodiments, the immunoassay is performed on a solid support. In some embodiments, the immunoassay is an ELISA assay. In some embodiments, the immunoassay is a bead-based assay.

D. Methods of Diagnosis, Monitoring and Treatment of a Subject Suffering from, or at Risk for Developing Acute COVID-19, or suffering from, or at risk for developing Long-COVID-19

The inventive anti-MASP-2 antibodies, methods, reagents and kits may be used in a number of applications. For example, in certain embodiments, an assay of this invention may be used to assess the level of MASP-2/C1-INH in a subject infected with SARS-CoV-2 to determine the risk of developing acute COVID-19 (i.e., acute respiratory distress syndrome, pneumonia or some other pulmonary or other acute manifestation of COVID-19, such as thrombosis), or the likelihood of recovery from acute COVID-19, and/or the likelihood of developing, or the presence of Long-COVID-19 (i.e., COVID-19 related long term sequelae selected from the group consisting of a cardiovascular complication, a neurological complication, kidney injury, a pulmonary complication, an inflammatory condition such as Kawasaki disease, Kawasaki-like disease, multisystem inflammatory syndrome in children, multi-system organ failure, extreme fatigue, muscle weakness, low grade fever, inability to concentrate, memory lapses, changes in mood, sleep difficulties, needle pains in arms and legs, diarrhea and vomiting, loss of taste and smell, sore throat and difficulties in swallowing, new onset of diabetes and hypertension, skin rash, shortness of breath, chest pains and palpitations) and/or to assess the extent to which a complement pathway inhibitor, such as a lectin pathway complement inhibitor, such as a MASP-2 inhibitory agent (e.g., a MASP-2 inhibitory antibody such as

narsoplimab) affects the level of MASP-2/C1-INH in a biological sample obtained from the subject and thereby assess the extent of lectin pathway activation in said subject.

In some embodiments, an assay of this invention may be used to assess the extent to which a complement pathway inhibitor (e.g., a MASP-2 inhibitory agent) decreases lectin complement pathway activation *in vivo*. In some embodiments, the inventive method is performed on a biological sample obtained from a subject infected with SARS-CoV-2. In some embodiments, the level of MASP-2/C1-INH complex detected in an assay of this invention is compared with a suitable reference value. The reference value may be, e.g., a value measured from a sample obtained from a healthy patient (or a pool of healthy patients), or a value measured from a sample or pool of samples obtained from subjects suffering from severe COVID-19, or a value measured from a sample obtained from a COVID-19 patient undergoing treatment with a MASP-2 inhibitory agent (e.g., obtained prior to treatment or at a time point in a sequence of treatments), or the reference value may be from healthy serum that has been activated with an agent that activates the lectin pathway (see Example 25), or the reference value may be a predetermined threshold. In one embodiment, the control sample is an individual or pooled sample of subjects suffering from acute COVID-19. In one embodiment, the control sample is an individual or pooled sample of normal healthy volunteers. In one embodiment, the control sample is a baseline sample of a subject prior to treatment with a complement inhibitor (e.g., a MASP-2 inhibitory agent or other complement inhibitor). As described herein, the methods of detecting MASP-2/C1-INH complex according to various embodiments of the present disclosure may be used assess the extent of lectin pathway complement activation and thereby used to define a pharmacodynamic endpoint or therapeutic threshold of a complement inhibitor or a determination or whether to treat a subject with a complement inhibitor, such as an lectin pathway complement inhibitor, such as a MASP-2 inhibitory agent, (e.g., a MASP-2 inhibitory antibody, such as narsoplimab).

E. Methods of Assessing the Extent of Lectin Pathway Complement Activation in a Mammalian Subject

In one aspect, the present disclosure provides methods of assessing the extent of lectin pathway complement (APC) activation in a test sample and performing an immunoassay comprising capturing and detecting MASP-2/C1-INH complex in the test sample, wherein the level of MASP-2/C1-INH complex detected in the test sample is

indicative of the extent of lectin pathway complement activation in the test sample. In one embodiment, the test sample is a biological sample obtained from a mammalian subject and the method comprises the steps of: (a) providing a biological sample obtained from the mammalian subject; and (b) assessing the extent of lectin pathway activation in the subject by performing an immunoassay comprising at least one of capturing and detecting the level of MASP-2/C1-INH complex in the biological sample according to an inventive methods described herein. For example, in one embodiment, the immunoassay comprises capturing and detecting MASP-2/C1-INH complex in the test sample, wherein the MASP-2/C1-INH complex is either captured or detected with a MASP-2 specific monoclonal antibody. In various embodiments, the method comprises comparing the level of MASP-2/C1-INH complex detected in the test sample (e.g., biological sample) with a predetermined level or control sample, wherein the level of MASP-2/C1-INH complex detected in the test sample is indicative of the extent of lectin pathway complement activation in the test sample (e.g., biological sample). In some embodiments, the method further comprises using the result of the comparative analysis to provide diagnostic, prognostic or treatment-related information regarding the mammalian subject from which the biological sample was obtained. In some embodiments, the test sample is obtained from a subject that is currently infected with SARS-CoV-2 and the method is used to assess the risk of said subject developing acute COVID-19 disease, wherein an elevated level of MASP-2/C1-INH of at least 20%, such as at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, or at least 2-fold, or at least 3-fold or greater as compared to a normal healthy control (e.g., a subject or a pool of subjects that healthy and are not infected with SARS-CoV-2) or reference standard is indicative of an increased risk of developing acute COVID-19 disease and/or Long-COVID-19 disease or the likelihood of recovery in a subject suffering from acute COVID-19.

In some embodiments, the test sample is obtained from a subject that has been infected with SARS-CoV-2 and the method is used to assess the risk of said subject for developing Long-COVID-19 disease, wherein an elevated level of MASP-2/C1-INH of at least 2-fold or greater as compared to a normal healthy control is indicative of an increased risk of developing Long- COVID-19 disease.

In some embodiments, the present disclosure provides a method of assessing the effect on lectin pathway complement activation in vivo of an inhibitor of human

complement. Any compound which binds to or otherwise blocks the generation and/or activity of any of the human complement components may be utilized in accordance with the present disclosure. For example, an inhibitor of complement can be, e.g., a small molecule, a nucleic acid or nucleic acid analog, a peptidomimetic, or a macromolecule that is not a nucleic acid or a protein, such as an antibody, or fragment thereof. In some embodiments, the present disclosure provides a method of assessing the effect on alternative complement pathway activation in vivo of an inhibitor (e.g., an antibody or small molecule) specific to a human complement component, such as, for example an inhibitor of a complement component selected from the group consisting of C1 (C1q, C1r, C1s), C2, C3, C4, C5, C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, MASP-2, and MASP-3. In some embodiments, the present disclosure provides a method of assessing the effect of an alternative complement pathway inhibitor on alternative pathway complement activation. In some embodiments, the present disclosure provides a method of assessing the effect of an inhibitor of MASP-2 on lectin pathway complement activation.

In some embodiments, the present disclosure provides a method of assessing the effect on lectin pathway complement activation *in vivo* of a MASP-2 inhibitory agent that has been administered to a mammalian subject. In various embodiments, a MASP-2 inhibitory agent (e.g., a MASP-2 inhibitory antibody or small molecule inhibitor of MASP-2) is administered to a mammalian subject, and a biological sample is subsequently obtained. The extent of lectin pathway complement (LPC) activation in the biological sample is then assessed by performing an immunoassay comprising capturing and detecting MASP-2/C1-INH complex in the biological sample according to an inventive method described herein.

F. Methods of Monitoring the Efficacy of a MASP-2 Inhibitory Agent in a Mammalian Subject

In one embodiment, the present disclosure provides a method for monitoring the efficacy of treatment with a MASP-2 inhibitory agent in a mammalian subject, the method comprising the steps of (a) administering a dose of a MASP-2 inhibitory agent (i.e. an antibody or small molecule) to a mammalian subject at a first point in time; (b) assessing a first concentration of MASP-2/C1-INH complex in a biological sample obtained from the subject after step (a); (c) treating the subject with the MASP-2 inhibitory antibody at a second point in time; (d) assessing a second concentration of

MASP-2/C1-INH complex in a biological sample obtained from the subject after step (c); and (e) comparing the level of MASP-2/C1-INH complex assessed in step (b) with the level of MASP-2/C1-INH complex assessed in step (d) to determine the efficacy of the MASP-2 inhibitory agent (antibody or small molecule) in the mammalian subject. In one embodiment, the extent of lectin pathway activation in the subject is assessed in an immunoassay, wherein the immunoassay comprises capturing and detecting the level of MASP-2/C1-INH complex in the biological sample. Optionally the level of MASP-2/C1-INH complex detected in the biological sample is compared with a suitable reference value. The reference value may be, e.g., a value of MASP-2/C1-INH complex measured from a biological sample obtained from the subject prior to administration of the MASP-2 inhibitory antibody, an average value measured from samples obtained from a group of healthy control subjects, a value that represents a desired extent of lectin pathway activation (e.g., a level of MASP-2/C1-INH corresponding to 90% inhibition of lectin pathway activation, or 80% inhibition, or 70% inhibition, or 60% inhibition, or 50% inhibition of lectin pathway activation). For example, a first biological sample is obtained from a subject before administration of a MASP-2 inhibitory antibody and a second biological sample is obtained after administration of the MASP-2 inhibitory antibody and the level of MASP-2/C1-INH complex is measured in the samples. If the level of MASP-2/C1-INH complex in the second biological sample is less than the level of MASP-2/C1-INH complex in the first biological sample, or is lower than a control value (e.g. a threshold value corresponding to a percent inhibition of lectin pathway activation), it can be concluded that the MASP-2 inhibitory antibody inhibited lectin pathway activation to a desired extent. Alternatively, if the level of MASP-2/C1-INH complex in the second biological sample is higher than the level of MASP-2/C1-INH complex in the first biological sample, or is higher than a control value (e.g., a threshold value corresponding to a percent inhibition of lectin pathway activation), it can be concluded that the dosage of the MASP-2 inhibitory antibody (e.g., narsoplimab) should be increased, and optionally, the method further comprises administering an increased dosage of the MASP-2 inhibitory antibody (e.g., narsoplimab) to the subject. In some embodiments, if the subject is administered an increased dose of the MASP-2 inhibitory antibody, steps (b) to (e) are repeated to determine whether the increased dose of the MASP-2 inhibitory antibody is sufficient to adjust the level of MASP-2/C1-INH complex to the desired level as compared to the respective control or reference standard.

In some embodiments, the methods are used to monitor the efficacy of a MASP-2 inhibitory antibody that is administered to a human subject suffering from or at risk of developing a lectin pathway disease or disorder, such as wherein the lectin pathway disease or disorder is selected from the group consisting of acute COVID-19 disease, Long-COVID-19, or other lectin pathway diseases or disorders (e.g., HSCT-TMA, IgAN, GvHD or other lectin pathway disease or disorder).

G. **Methods of Diagnosis, Monitoring and Treatment a Subject Suffering from, or at Risk for Developing Severe COVID-19 or Long-COVID-19**

In one embodiment, the present disclosure provides a method of determining the presence or amount of MASP-2/C1-INH complex in a test sample of biological fluid obtained from a subject currently infected with SARS-CoV-2 or potentially infected with SARS-CoV-2, or suffering from severe COVID-19, or previously infected with SARS-CoV-2, the method comprising: (a) contacting a test sample of biological fluid with an antibody that binds to human MASP-2 complexed with C1-INH in an *in vitro* immunoassay; and (b) detecting the presence or absence or amount of the antibody or fragment thereof bound to the MASP-2/C1-INH complex with an antibody that binds to C1-INH, wherein detection of the presence and/or amount of MASP-2/C1-INH is indicative of MASP-2-mediated lectin pathway activation in the subject.

In another embodiment, the present disclosure provides a method of assessing the extent of MASP-2 mediated lectin pathway complement activation in a test sample of biological fluid from a subject known to be infected with SARS-CoV-2, potentially infected with SARS-CoV-2, suffering from severe COVID-19 or previously infected with SARS-CoV-2, comprising: (a) providing a test sample of biological fluid obtained from a subject known to be infected with SARS-CoV-2, potentially infected with SARS-CoV-2, suffering from severe COVID-19 or previously infected with SARS-CoV-2; (b) performing an immunoassay comprising capturing and detecting MASP-2/C1-INH complex in the test sample, and (c) comparing the presence and/or amount of the MASP-2/C1-INH detected in the test sample to a reference standard, wherein the presence or increased amount of MASP-2/CI-INH as compared to the reference sample indicates that

the subject has an increase in MASP-2-mediated complement lectin pathway which indicates that (i) the subject is currently suffering from MASP-2-mediated COVID-19 disease and is likely to benefit from treatment with a complement inhibitor such as a MASP-2 inhibitory agent (anti-MASP-2 antibody such as narsoplimab or small molecule inhibitor of MASP-2) or (ii) that the subject has an increased risk of developing COVID-19 related complications, or (iii) that the subject was previously infected with COVID-19 and is suffering from, or at risk for developing one or more long-term sequelae associated with COVID-19, or (iv) that the subject is currently suffering from acute COVID-19 and is at increased risk of a poor outcome, such as death. In some embodiments, the method further comprises administering to the subject having an increased amount of MASP-2/C1-INH complex a therapeutic agent for the treatment of COVID-19, such as a complement inhibitory agent, such as a MASP-2 inhibitory agent, such as a MASP-2 inhibitory antibody or small molecule, such as narsoplimab. In some embodiments, the COVID-19 infected subject displays symptoms of COVID-19. In some embodiments, the COVID-19 infected subject is asymptomatic. In some embodiments, the subject was previously infected with COVID-19 and is suffering from, or at risk for developing, one or more long-term sequelae associated with COVID-19. In some embodiments, the method further comprises determining the level of C1s/C1-INH complex in the test sample, wherein an increased level of C1s/C1-INH complex (i.e, at least 2-fold or greater) as compared to healthy controls is indicative of an increased likelihood of recovery from COVID-19 and a low level of C1s/C1-INH is indicative of an increased likelihood of a poor outcome.

In another aspect, the present disclosure provides a method for monitoring the efficacy of treatment with a MASP-2 inhibitory antibody in a mammalian subject suffering from one or more COVID-19-related complications, the method comprising: (a) administering a dose of a MASP-2 inhibitory antibody to a mammalian subject at a first point in time; (b) assessing a first concentration of MASP-2/C1-INH complex in a biological sample obtained from the subject after step (a); (c) treating the subject with the

MASP-2 inhibitory antibody at a second point in time; (d) assessing a second concentration of MASP-2/C1-INH complex in a biological sample obtained from the subject after step (c); and (e) comparing the level of MASP-2/C1-INH complex assessed in step (b) with the level of MASP-2/C1-INH complex assessed in step (d) to determine the efficacy of the MASP-2 inhibitory antibody in the mammalian subject.

In another aspect, the present disclosure provides a method of treating a mammalian subject suffering from, or at risk of developing a COVID-19 related disease or disorder, comprising administering a MASP-2 inhibitory antibody to the subject if the subject is determined to have: (i) a higher amount of MASP-2/C1-INH complex in one or more biological samples taken from the subject compared to a predetermined level of MASP-2/C1-INH complex or compared to the MASP-2/C1-INH complex level in one or more control samples.

VII. Exemplary Embodiments

A. MASP-2-specific mAb that binds to MASP-2 in complex with C1-INH (MASP-2/C1-INH complex)

1. A monoclonal antibody, or antigen binding fragment thereof, that specifically binds to human MASP-2 in complex with C1-INH, wherein the antibody comprises a binding domain comprising (a) HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:88, or (b) HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:98,

wherein the CDRs are numbered according to the Kabat numbering system.

2. The monoclonal antibody of paragraph 1, wherein said antibody comprises (a) a heavy chain variable region having at least 95% identify with the amino acid sequence set forth as SEQ ID NO:87 and a light chain variable region having at least 95% identify

with the amino acid sequence set forth as SEQ ID NO:88 or (b) a heavy chain variable region having at least 95% identify with the amino acid sequence set forth as SEQ ID NO:97 and a light chain variable region having at least 95% identify with the amino acid sequence set forth as SEQ ID NO:98.

3. The monoclonal antibody of paragraph 1, wherein said antibody is a humanized, chimeric or fully human antibody.

4. The monoclonal antibody or fragment thereof of any of paragraphs 1 to 3, wherein said antibody fragment selected from the group consisting of Fv, Fab, Fab', F(ab)2 and F(ab')2.

5. The monoclonal antibody of any of paragraphs 1 to 4, wherein said antibody is a single chain molecule.

6. The monoclonal antibody of any of paragraphs 1 to 4, wherein said antibody is an IgG molecule selected from the group consisting of IgG1, IgG2 and IgG4.

7. The monoclonal antibody or antigen-binding fragment thereof of any of paragraphs 1 to 6, wherein said antibody or antigen binding fragment thereof binds to human MASP-2 with a K_D of less than 10 nM.

8. The monoclonal antibody or antigen-binding fragment thereof of any of paragraphs 1 to 7, wherein, said antibody is labeled with a detectable moiety.

9. The monoclonal antibody or antigen-binding fragment thereof of any of paragraphs 1 to 8, wherein said antibody or fragment thereof is immobilized on a substrate.

10. A nucleic acid molecule encoding the amino acid sequence of an antibody, or fragment thereof, that specifically binds human MASP-2 as set forth in any of paragraphs 1-7.

11. An expression cassette comprising a nucleic acid molecule encoding an antibody, or fragment thereof, that specifically binds human MASP-2 of the invention according to paragraph 10.

12. A cell comprising at least one of the nucleic acid molecules encoding an antibody, or fragment thereof, that specifically binds human MASP-2 of the invention according to paragraph 10 or paragraph 11.

13. A composition comprising an antibody, or fragment thereof, that specifically binds human MASP-2 as set forth in any of paragraphs 1 to 9.

14. A substrate for use in an immunoassay comprising at least one antibody, or fragment thereof, that specifically binds human MASP-2 as set forth in any of paragraphs 1 to 9.

15. A kit for detecting the presence or amount of MASP-2/C1-INH complex in a test sample, said kit comprising (a) at least one container, and (b) at least one antibody, or fragment thereof, that specifically binds human MASP-2 as set forth in any of paragraphs 1 to 9.

16. The kit of paragraph 15, further comprising at least one antibody, or fragment thereof, that specifically detects C1-INH in complex with MASP-2.

17. The kit of paragraph 15 or 16, wherein the antibody that specifically binds to MASP-2 is immobilized on a substrate (e.g., a bead).

18. The kit of paragraph 16, wherein the antibody that specifically binds to C1-INH is labeled with a detectable moiety.

19. The kit of any of paragraphs 15-18, wherein the kit is for use in an immunoassay.

20. The kit of paragraph 19, wherein the immunoassay is an enzyme-linked immunosorbent assay (ELISA) or a bead-based assay.

21. The kit of paragraph 19 or 20, wherein the antibody or fragment thereof that binds to MASP-2 is a coating/capture antibody.

22. The kit of paragraph 19 or 20, wherein the antibody or fragment thereof of that binds to C1-INH is a detecting antibody.

23. The kit of any of paragraphs 15-22, wherein the kit further comprises a reference standard corresponding to the level of MASP-2/C1-INH complex in a healthy control subject or a population of healthy human subjects.

24. The kit of any of paragraphs 15-23, wherein the kit further comprises a reference standard corresponding to the level of MASP-2/C1-INH complex in a subject suffering from severe COVID-19, or a population of subjects suffering from severe COVID-19, or an amount of recombinant MASP-2/C1-INH complex corresponding to a subject suffering from severe COVID-19.

25. The kit of any of paragraphs 15-24, wherein the kit further comprises an antibody or fragment thereof that binds to C1s while in complex with C1-INH.

26. The kit of paragraph 25, wherein the antibody that binds to C1s is a capture antibody.

27. The kit of paragraph 25 or 26, wherein the antibody that specifically binds to C1s-INH is immobilized on a substrate (e.g., a bead).

B. Methods of Detecting the Amount of MASP-2/C1-INH complex in a biological sample

1. A method of measuring the amount of MASP-2/C1-INH in a biological sample comprising:

(a) providing a test biological sample from a human subject;

(b) performing an immunoassay comprising capturing and detecting MASP-2/C1-INH complex in the test sample, wherein MASP-2/C1-INH is captured with a monoclonal antibody that specifically binds to human MASP-2; and the MASP-2/C1-INH complex is detected directly or indirectly with an antibody that specifically binds to C1-INH; and

(c) comparing the level of MASP-2/C1-INH complex detected in accordance with (b) with a predetermined level or control sample wherein the level of MASP-2/C1-INH complex detected in the test sample is indicative of the extent of Lectin Pathway Complement activation.

2. The method of paragraph 1, wherein the biological sample is a fluid sample selected from the group consisting of whole blood, serum, plasma, urine and cerebrospinal fluid.

3. The method of paragraph 1 or 2, wherein the antibody that specifically binds to MASP-2 comprises a binding domain comprising (a) HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:88 or (b) HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

4. The method of paragraph 1 or 2, wherein the biological sample is a serum sample at a concentration of from 0.3 to 5%.

5. The method of any of paragraphs 1 to 4, wherein the human subject is currently infected with SARS-CoV-2, or has previously been infected with SARS-CoV-2, or wherein the subject is suffering from or at risk for developeing another lectin pathway disease or disorder (e.g., COVID-19, HSCT-TMA, IgAN, GvHD).

6. The method of paragraph 5, wherein the method further comprises determining that the subject is suffering from, or at risk for developing severe COVID-19 disease or Long-COVID-19 based on a determination that the level of MASP-2/C1-INH complex detected is higher (at least 20% higher, such as at least 30% higher, or at least 40% higher, or at least 50% higher, or at least 60% higher, or at least 70% higher or at least 80% higher or at least 90% higher, or 2-fold higher) than the pre-determined level or control reference from healthy subjects.

7. The method of any of paragraphs 1 to 6, wherein the subject is determined to have a higher than normal level of MASP-2/C1-INH complex and is identified as a candidate for treatment with a complement inhibitory agent.

8. The method of any of paragraphs 1-7 wherein the method further comprises administering a complement inhibitor to the subject identified as having a higher than normal level of MASP-2/C1-INH complex.

9. The method of paragraph 8 wherein the complement inhibitor is a MASP-2 inhibitory agent (e.g., a MASP-2 inhibitory antibody such as narsoplimab or a small molecule inhibitor of MASP-2).

10. The method of any of paragraphs 1 to 4, wherein the mammalian subject has been treated with a complement inhibitory agent, such as a lectin complement pathway

inhibitory agent, such as a MASP-2 inhibitory agent (e.g., a MASP-2 inhibitory antibody such as narsoplimab).

11. The method of paragraph 10, wherein the control sample is a sample taken from the subject prior to treatment with the MASP-2 inhibitory agent, or a sample taken at an earlier point in time during a course of treatment with the MASP-2 inhibitory agent.

12. The method of any of paragraphs 10 or 11, wherein the MASP-2 inhibitory agent is a MASP-2 inhibitory antibody.

C. Methods of Determining the Risk of a Subject infected with SARS-CoV-2 for Developing COVID-19-related ARDS or other poor outcome from acute COVID-19, or Long-term sequelae associated with COVID-19

1. A method of determining the risk of a subject that is or has been infected with SARS-CoV-2 for developing COVID-19-related ARDS or other poor outcome from acute COVID-19 or long-term sequelae associated with COVID-19 comprising:

(a) obtaining a biological sample from the subject;

(b) measuring the level of MASP-2/C1-INH complex in the sample;

(c) comparing the measured level with a predetermined level of MASP-2/C1-INH complex or a reference standard to assess the risk of developing COVID-19-related ARDS or other poor outcome from acute COVID-19, and/or long-term sequelae associated with COVID-19; and

(d) determining the risk of the subject for developing COVID-19-related ARDS or other poor outcome from acute COVID-19 and/or long-term sequelae associated with COVID-19 and reporting the results to the patient, physician or database;

(e) optionally, administering a treatment to the subject determined to be likely to develop acute disease and/or other poor outcome from acute COVID-19, and/or long-term sequelae associated with COVID-19 infection.

2. The method of paragraph 1, wherein the level of MASP-2/C1-INH complex is measured in an immunoassay.

3. The method of paragraph 3, wherein the method comprises performing an immunoassay to measure the level of MASP-2/C1-INH complex in the biological sample.

4. The method of paragraph 2 or paragraph 3, wherein the immunoassay is an ELISA assay or a bead-based assay.

5. The method of paragraph 4, wherein the immunoassay comprises the use of a capture antibody that specifically binds to MASP-2 comprises a binding domain comprising (a) HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:88, or (b) HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:98wherein the CDRs are numbered according to the Kabat numbering system.

6. The method of any of paragraphs 1-5, wherein the method further comprises assaying for or otherwise determining the level of C1s/C1-INH complex in a biological sample obtained from the subject.

D. Methods for treating, inhibiting, alleviating or preventing acute COVID-19 in a mammalian subject infected with SARS-CoV-2 and at risk for developing acute COVID-19

1. A method for treating, inhibiting, alleviating or preventing acute respiratory distress syndrome, pneumonia or some other pulmonary or other acute manifestation of COVID-19, such as thrombosis, in a mammalian subject infected with SARS-CoV-2 and at risk for developing acute COVID-19, comprising

(i) determining the level of MASP-2/C1-INH complex in a biological sample obtained from the subject, wherein an increased level of MASP-2/C1-INH complex as compared to a healthy control sample is indicative of an increased risk of developing one or more acute manifestations of COVID-19; and

(ii) administering to the subject having an increased level of MASP-2/C1-INH complex an amount of a MASP-2 inhibitory agent effective to inhibit MASP-2-dependent complement activation.

2. The method of paragraph 1, wherein the MASP-2 inhibitory agent is a MASP-2 antibody or fragment thereof.

3. The method of paragraph 2, 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:6.

4. The method of paragraph 2, wherein the MASP-2 antibody or fragment thereof specifically binds to a polypeptide comprising SEQ ID NO:6 with an affinity of at least 10 times greater than it binds to a different antigen in the complement system.

5. The method of paragraph 2, wherein the antibody or fragment thereof is selected from the group consisting of a recombinant antibody, an antibody having reduced effector function, a chimeric antibody, a humanized antibody and a human antibody.

6. The method of paragraph 1, wherein the MASP-2 inhibitory agent selectively inhibits lectin pathway complement activation without substantially inhibiting C1q-dependent complement activation.

7. The method of paragraph 1, wherein the MASP-2 inhibitory agent is a small molecule MASP-2 inhibitory compound.

8. The method of paragraph 1, wherein the MASP-2 inhibitory agent is an expression inhibitor of MASP-2.

9. The method of paragraph 2, wherein the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising CDR-H1, CDR-H2 and CDR-H3 of the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising CDR-L1, CDR-L2 and CDR-L3 of the amino acid sequence set forth as SEQ ID NO:69.

10. The method of paragraph 2, wherein the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO:67 and a light chain variable region comprising SEQ ID NO:69.

11. The method of paragraph 1, wherein step (i) comprises the use of an antibody, kit or composition according to any of paragraphs A1 to A24.

12. The method of paragraph 1, wherein step (i) comprises a method according to any of paragraphs B1-B11.

13. The method of paragraph 1, wherein step (i) comprises a method according to any of paragraphs C1-C5.

E. Methods for treating, inhibiting, alleviating or preventing Long-COVID-19 in a mammalian subject that has been infected with SARS-CoV-2 and is at risk for developing Long-COVID-19

1. A method for treating, ameliorating, preventing or reducing the risk of developing one or more COVID-19-related long-term sequelae in a mammalian subject that has been infected with SARS-CoV-2, comprising

(i) determining the level of MASP-2/C1-INH complex in a biological sample obtained from the subject, wherein an increased level of MASP-2/C1-INH complex as compared to a healthy control sample is indicative of an increased risk of developing one or more COVID-19-related long term sequelae; and

(ii) administering to the subject having an increased level of MASP-2/C1-INH complex an amount of a MASP-2 inhibitory agent effective to inhibit MASP-2-dependent complement activation.

2. The method of paragraph 1, wherein the MASP-2 inhibitory agent is a MASP-2 antibody or fragment thereof.

3. The method of paragraph 2, 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:6.

4. The method of paragraph 2, wherein the MASP-2 antibody or fragment thereof specifically binds to a polypeptide comprising SEQ ID NO:6 with an affinity of at least 10 times greater than it binds to a different antigen in the complement system.

5. The method of paragraph 2, wherein the antibody or fragment thereof is selected from the group consisting of a recombinant antibody, an antibody having reduced effector function, a chimeric antibody, a humanized antibody and a human antibody.

6. The method of paragraph 2, wherein the MASP-2 inhibitory agent selectively inhibits lectin pathway complement activation without substantially inhibiting C1q-dependent complement activation.

7. The method of paragraph 1, wherein the MASP-2 inhibitory agent is a small molecule MASP-2 inhibitory compound.

8. The method of paragraph 1, wherein the MASP-2 inhibitory agent is an expression inhibitor of MASP-2.

9. The method of paragraph 2, wherein the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising CDR-H1, CDR-H2 and CDR-H3 of the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising CDR-L1, CDR-L2 and CDR-L3 of the amino acid sequence set forth as SEQ ID NO:69.

10. The method of paragraph 2, wherein the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO:67 and a light chain variable region comprising SEQ ID NO:69.

11. The method of paragraph 1, wherein the one or more COVID-19 related long term sequelae is selected from the group consisting of a cardiovascular complication (including myocardial injury, cardiomyopathy, myocarditis, intravascular coagulation, stroke, venous and arterial complications and pulmonary thrombosis); a neurological complication (including cognitive difficulties, confusion, memory loss, also referred to as "brain fog," headache, stroke, dizziness, syncope, seizure, anorexia, insomnia, anosmia, ageusia, myoclonus, neuropathic pain, myalgias, development of neurological disease such as Alzheimer's disease, Guillian Barre Syndrome, Miller-Fisher Syndrome, Parkinson's disease) kidney injury (such as acute kidney injury (AKI); a pulmonary complication (including lung fibrosis,

dyspnea, pulmonary embolism),an inflammatory condition such as Kawasaki disease, Kawasaki-like disease, multisystem inflammatory syndrome in children, multi-system organ failure, extreme fatigue, muscle weakness, low grade fever, inability to concentrate, memory lapses, changes in mood, sleep difficulties, needle pains in arms and legs, diarrhea and vomiting, loss of taste and smell, sore throat and difficulties in swallowing, new onset of diabetes and hypertension, skin rash, shortness of breath, chest pains and palpitations.

12. The method of paragraph 1, wherein step (i) comprises the use of an antibody, kit or composition according to any of paragraphs A1 to A24.

13. The method of paragraph 1, wherein step (i) comprises a method according to any of paragraphs B1-B11.

14. The method of paragraph 1, wherein step (i) comprises a method according to any of paragraphs C1-C5.

F. Methods of monitoring the efficacy of treatment with a MASP-2 inhibitory antibody, or antigen-binding fragment thereof, in a mammalian subject in need thereof.

1. A method for monitoring the efficacy of treatment with a MASP-2 inhibitory antibody, or antigen-binding fragment thereof, in a mammalian subject in need thereof, the method comprising:

(a) administering a dose of a MASP-2 inhibitory antibody, or antigen-binding fragment thereof, to a mammalian subject at a first point in time;

(b) assessing a first level of MASP-2/C1-INH complex in a biological sample obtained from the subject after step (a);

(c) treating the subject with the MASP-2 inhibitory antibody, or antigen-binding fragment thereof, at a second point in time;

(d) assessing a second level of MASP-2/C1-INH complex in a biological sample obtained from the subject after step (c); and

(e) comparing the level of MASP-2/C1-INH complex assessed in step (b) with the level of MASP-2/C1-INH complex assessed in step (d) to determine the efficacy of the

MASP-2 inhibitory antibody or antigen-binding fragment thereof in the mammalian subject.

2. The method of paragraph 1, wherein the method further comprises adjusting the dose of the MASP-2 inhibitory antibody or antigen-binding fragment thereof.

3. The method of paragraph 2, wherein the dose of MASP-2 inhibitory antibody or antigen-binding fragment thereof administered to the subject is increased if the level of MASP-2/C1-INH complex is higher than the control or reference standard.

4. The method of paragraph 3, wherein if the subject is administered an increased dose of the MASP-2 inhibitory antibody or antigen-binding fragment thereof, steps (b) to (e) are repeated to determine whether the increased dose is sufficient to adjust the level of MASP-2/C1-INH complex to the desired level as compared to the respective control or reference standard.

5. The method of paragraph 1, wherein steps (b) and (d) comprise assessing the concentration of MASP-2/CI-INH complex in the biological samples in an immunoassay.

6. The method of paragraph 5, wherein the immunoassay is a bead-based immunofluorescence assay.

7. The method of paragraph 6, wherein the immunoassay comprises the use of a capture antibody that specifically binds to MASP-2 comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

8. The method of paragraph 6 or 7, wherein the biological sample is serum or plasma.

9. The method of paragraph 8, wherein the biological sample is from 1% to 5% serum or plasma.

10. The method of any one of paragraphs 1-9, wherein the mammalian subject is a human subject.

11. The method of paragraph 10, wherein the human subject is suffering from, or at risk of developing a lectin pathway disease or disorder selected from the group consisting of HSCT-TMA, IgAN, Lupus Nephritis and Graft-versus-Host Disease or some other lectin pathway disease or disorder.

12. The method of paragraph 1, wherein the human subject is suffering from, or at risk of developing COVID-19 or long-term sequelae associated with COVID-19.

13. The method of paragraph 1, wherein the second point in time is from 2 to 14 days after the first point in time.

14. The method of paragraph 1, wherein the second point in time is within 2 to 7 days from the first point in time.

15. The method of paragraph 1, wherein the second point in time is within 2 to 4 days from the first point in time.

VI. 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.

EXAMPLE 1

This example describes the generation of a mouse strain deficient in MASP-2 (MASP-2-/-) but sufficient of MAp19 (MAp19+/+).

Materials and Methods: The targeting vector pKO-NTKV 1901 was designed to disrupt the three exons coding for the C-terminal end of murine MASP-2, including the exon that encodes the serine protease domain, as shown in **FIGURE 3**. PKO-NTKV 1901 was used to transfect the murine ES cell line E14.1a (SV129 Ola). Neomycin-resistant and Thymidine Kinase-sensitive clones were selected. 600 ES clones were screened and, of these, four different clones were identified and verified by southern blot to contain the expected selective targeting and recombination event as shown in FIGURE 3. Chimeras were generated from these four positive clones by embryo transfer. The chimeras were then backcrossed in the genetic background C57/BL6 to create transgenic males. The transgenic males were crossed with females to generate F1s

with 50% of the offspring showing heterozygosity for the disrupted *MASP-2* gene. The heterozygous mice were intercrossed to generate homozygous MASP-2 deficient offspring, resulting in heterozygous and wild-type mice in the ration of 1:2:1, respectively.

Results and Phenotype: The resulting homozygous MASP-2-/- deficient mice were found to be viable and fertile and were verified to be MASP-2 deficient by southern blot to confirm the correct targeting event, by Northern blot to confirm the absence of MASP-2 mRNA, and by Western blot to confirm the absence of MASP-2 protein (data not shown). The presence of MAp19 mRNA and the absence of MASP-2 mRNA were further confirmed using time-resolved RT-PCR on a LightCycler machine. The MASP-2-/- mice do continue to express MAp19, MASP-1, and MASP-3 mRNA and protein as expected (data not shown). The presence and abundance of mRNA in the MASP-2-/- mice for Properdin, Factor B, Factor D, C4, C2, and C3 was assessed by LightCycler analysis and found to be identical to that of the wild-type littermate controls (data not shown). The plasma from homozygous MASP-2-/- mice is totally deficient of lectin-pathway-mediated complement activation as further described in Example 2.

Generation of a MASP-2-/- strain on a pure C57BL6 Background: The MASP-2-/- mice were back-crossed with a pure C57BL6 line for nine generations prior to use of the MASP-2-/- strain as an experimental animal model.

A transgenic mouse strain that is murine MASP-2-/-, MAp19+/+ and that expresses a human MASP-2 transgene (a murine MASP-2 knock-out and a human MASP-2 knock-in) was also generated as follows:

Materials and Methods: A minigene encoding human MASP-2 called "mini hMASP-2" (SEQ ID NO:49) as shown in FIGURE 4 was constructed which includes the promoter region of the human *MASP 2* gene, including the first 3 exons (exon 1 to exon 3) followed by the cDNA sequence that represents the coding sequence of the following 8 exons, thereby encoding the full-length MASP-2 protein driven by its endogenous promoter. The mini hMASP-2 construct was injected into fertilized eggs of MASP-2-/- in order to replace the deficient murine *MASP 2* gene by transgenically expressed human MASP-2.

EXAMPLE 2

This example demonstrates that MASP-2 is required for complement activation via the lectin pathway.

Methods and Materials:

Lectin pathway specific C4 Cleavage Assay: A C4 cleavage assay has been described by Petersen, et al., *J. Immunol. Methods 257*:107 (2001) that measures lectin pathway activation resulting from lipoteichoic acid (LTA) from *S. aureus*, which binds L-ficolin. The assay described by Petersen et al., (2001) was adapted to measure lectin pathway activation via MBL by coating the plate with LPS and mannan or zymosan prior to adding serum from MASP-2 -/- mice as described below. The assay was also modified to remove the possibility of C4 cleavage due to the classical pathway. This was achieved by using a sample dilution buffer containing 1 M NaCl, which permits high affinity binding of lectin pathway recognition components to their ligands but prevents activation of endogenous C4, thereby excluding the participation of the classical pathway by dissociating the C1 complex. Briefly described, in the modified assay serum samples (diluted in high salt (1 M NaCl) buffer) are added to ligand-coated plates, followed by the addition of a constant amount of purified C4 in a buffer with a physiological concentration of salt. Bound recognition complexes containing MASP-2 cleave the C4, resulting in C4b deposition.

Assay Methods:

1) Nunc Maxisorb microtiter plates (MaxiSorb®, Nunc, Cat. No. 442404, Fisher Scientific) were coated with 1 µg/ml mannan (M7504 Sigma) or any other ligand (e.g., such as those listed below) diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6).

The following reagents were used in the assay:

a. mannan (1 µg/well mannan (M7504 Sigma) in 100 µl coating buffer):
b. zymosan (1 µg/well zymosan (Sigma) in 100 µl coating buffer);
c. LTA (1µg/well in 100 µl coating buffer or 2 µg/well in 20 µl methanol)
d. 1 µg of the H-ficolin specific Mab 4H5 in coating buffer
e. PSA from *Aerococcus viridans* (2 µg/well in 100 µl coating buffer)
f. 100 µl/well of formalin-fixed *S. aureus* DSM20233 (OD_{550}=0.5) in coating buffer.

2) The plates were incubated overnight at 4°C.

3) After overnight incubation, the residual protein binding sites were saturated by incubated the plates with 0.1% HSA-TBS blocking buffer (0.1% (w/v) HSA in 10 mM Tris-CL, 140 mM NaCl, 1.5 mM NaN_3, pH 7.4) for 1-3 hours, then washing the plates 3X with TBS/tween/Ca^{2+} (TBS with 0.05% Tween 20 and 5 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4).

4) Serum samples to be tested were diluted in MBL-binding buffer (1 M NaCl) and the diluted samples were added to the plates and incubated overnight at 4°C. Wells receiving buffer only were used as negative controls.

5) Following incubation overnight at 4°C, the plates were washed 3X with TBS/tween/Ca^{2+}. Human C4 (100 µl/well of 1 µg/ml diluted in BBS (4 mM barbital, 145 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4)) was then added to the plates and incubated for 90 minutes at 37°C. The plates were washed again 3X with TBS/tween/Ca^{2+}.

6) C4b deposition was detected with an alkaline phosphatase-conjugated chicken anti-human C4c (diluted 1:1000 in TBS/tween/Ca^{2+}), which was added to the plates and incubated for 90 minutes at room temperature. The plates were then washed again 3X with TBS/tween/Ca^{2+}.

7) Alkaline phosphatase was detected by adding 100 µl of *p*-nitrophenyl phosphate substrate solution, incubating at room temperature for 20 minutes, and reading the OD_{405} in a microtiter plate reader.

Results: FIGURES 5A-B show the amount of C4b deposition on mannan (FIGURE 5A) and zymosan (FIGURE 5B) in serum dilutions from MASP-2+/+ (crosses), MASP-2+/- (closed circles) and MASP-2-/- (closed triangles). FIGURE 5C shows the relative C4 convertase activity on plates coated with zymosan (white bars) or mannan (shaded bars) from MASP-2-/+ mice (n=5) and MASP-2-/- mice (n=4) relative to wild-type mice (n=5) based on measuring the amount of C4b deposition normalized to wild-type serum. The error bars represent the standard deviation. As shown in FIGURES 5A-C, plasma from MASP-2-/- mice is totally deficient in lectin-pathway-mediated complement activation on mannan and on zymosan coated plates. These results clearly demonstrate that MASP-2 is an effector component of the lectin pathway.

Recombinant MASP-2 reconstitutes Lectin Pathway-Dependent C4 Activation in serum from the MASP-2-/- mice

In order to establish that the absence of MASP-2 was the direct cause of the loss of lectin pathway-dependent C4 activation in the MASP-2-/- mice, the effect of adding recombinant MASP-2 protein to serum samples was examined in the C4 cleavage assay described above. Functionally active murine MASP-2 and catalytically inactive murine MASP-2A (in which the active-site serine residue in the serine protease domain was substituted for the alanine residue) recombinant proteins were produced and purified as described below in Example 3. Pooled serum from 4 MASP-2 -/- mice was pre-incubated with increasing protein concentrations of recombinant murine MASP-2 or inactive recombinant murine MASP-2A and C4 convertase activity was assayed as described above.

Results: As shown in FIGURE 6, the addition of functionally active murine recombinant MASP-2 protein (shown as open triangles) to serum obtained from the MASP-2 -/- mice restored lectin pathway-dependent C4 activation in a protein concentration dependent manner, whereas the catalytically inactive murine MASP-2A protein (shown as stars) did not restore C4 activation. The results shown in FIGURE 6 are normalized to the C4 activation observed with pooled wild-type mouse serum (shown as a dotted line).

EXAMPLE 3

This example describes the recombinant expression and protein production of recombinant full-length human, rat and murine MASP-2, MASP-2 derived polypeptides, and catalytically inactivated mutant forms of MASP-2

Expression of Full-length human, murine and rat MASP-2:

The full length cDNA sequence of human MASP-2 (SEQ ID NO: 4) was also 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)). The full length mouse cDNA (SEQ ID NO:50) and rat MASP-2 cDNA (SEQ ID NO:53) were each subcloned into the pED expression vector. The MASP-2 expression vectors were then transfected into the adherent Chinese hamster ovary cell line DXB1 using the standard calcium phosphate transfection procedure described in Maniatis et al., 1989. Cells transfected with these constructs grew very slowly, implying that the encoded protease is cytotoxic.

In another approach, the minigene construct (SEQ ID NO:49) containing the human cDNA of MASP-2 driven by its endogenous promoter is transiently transfected into Chinese hamster ovary cells (CHO). The human MASP-2 protein is secreted into the culture media and isolated as described below.

Expression of Full-length catalytically inactive MASP-2:

Rationale: MASP-2 is activated by autocatalytic cleavage after the recognition subcomponents MBL or ficolins (either L-ficolin, H-ficolin or M-ficolin) bind to their respective carbohydrate pattern. Autocatalytic cleavage resulting in activation of MASP-2 often occurs during the isolation procedure of MASP-2 from serum, or during the purification following recombinant expression. In order to obtain a more stable protein preparation for use as an antigen, a catalytically inactive form of MASP-2, designed as MASP-2A was created by replacing the serine residue that is present in the catalytic triad of the protease domain with an alanine residue in rat (SEQ ID NO:55 Ser617 to Ala617); in mouse (SEQ ID NO:52 Ser617 to Ala617); or in human (SEQ ID NO:6 Ser618 to Ala618).

In order to generate catalytically inactive human and murine MASP-2A proteins, site-directed mutagenesis was carried out using the oligonucleotides shown in TABLE 5. The oligonucleotides in TABLE 5 were designed to anneal to the region of the human and murine cDNA encoding the enzymatically active serine and oligonucleotide contain a mismatch in order to change the serine codon into an alanine codon. For example, PCR oligonucleotides SEQ ID NOS:56-59 were used in combination with human MASP-2 cDNA (SEQ ID NO:4) to amplify the region from the start codon to the enzymatically active serine and from the serine to the stop codon to generate the complete open reading from of the mutated MASP-2A containing the Ser618 to Ala618 mutation. The PCR products were purified after agarose gel electrophoresis 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, transformed into *E. coli*.

A catalytically inactive rat MASP-2A protein was generated by kinasing and annealing SEQ ID NO:64 and SEQ ID NO:65 by combining these two oligonucleotides in equal molar amounts, heating at 100°C for 2 minutes and slowly cooling to room temperature. The resulting annealed fragment has Pst1 and Xba1 compatible ends and was inserted in place of the Pst1-Xba1 fragment of the wild-type rat MASP-2 cDNA (SEQ ID NO:53) to generate rat MASP-2A.

5 'GAGGTGACGCAGGAGGGGCATTAGTGTTT 3' (SEQ ID NO:64)

5' CTAGAAACACTAATGCCCCTCCTGCGTCACCTCTGCA 3' (SEQ ID NO:65)

The human, murine and rat MASP-2A were each further subcloned into either of the mammalian expression vectors pED or pCI-Neo and transfected into the Chinese Hamster ovary cell line DXB1 as described below.

In another approach, a catalytically inactive form of MASP-2 is constructed using the method described in Chen et al., *J. Biol. Chem.*, *276*(28):25894-25902, 2001. Briefly, the plasmid containing the full-length human MASP-2 cDNA (described in Thiel et al., *Nature 386*:506, 1997) is digested with *Xho*1 and *EcoR*1 and the MASP-2 cDNA (described herein as SEQ ID NO:4) is cloned into the corresponding restriction sites of the pFastBac1 baculovirus transfer vector (Life Technologies, NY). The MASP-2 serine protease active site at Ser618 is then altered to Ala618 by substituting the double-stranded oligonucleotides encoding the peptide region amino acid 610-625 (SEQ ID NO:13) with the native region amino acids 610 to 625 to create a MASP-2 full length polypeptide with an inactive protease domain.

Construction of Expression Plasmids Containing Polypeptide Regions Derived from Human Masp-2.

The following constructs are produced using the MASP-2 signal peptide (residues 1-15 of SEQ ID NO:5) to secrete various domains of MASP-2. A construct expressing the human MASP-2 CUBI domain (SEQ ID NO:8) is made by PCR amplifying the region encoding residues 1–121 of MASP-2 (SEQ ID NO:6) (corresponding to the N-terminal CUBI domain). A construct expressing the human MASP-2 CUBIEGF domain (SEQ ID NO:9) is made by PCR amplifying the region encoding residues 1–166 of MASP-2 (SEQ ID NO:6) (corresponding to the N-terminal CUBIEGF domain). A construct expressing the human MASP-2 CUBIEGFCUBII domain (SEQ ID NO:10) is made by PCR amplifying the region encoding residues 1-293 of MASP-2 (SEQ ID NO:6) (corresponding to the N-terminal CUBIEGFCUBII domain). The above mentioned domains are amplified by PCR using Vent$_R$ polymerase and pBS-MASP-2 as a template, according to established PCR methods. The 5' primer sequence of the sense primer (5'-CGGGATCCATGAGGCTGCTGACCCTC-3' SEQ ID NO:34) introduces a *Bam*HI restriction site (underlined) at the 5' end of the PCR products. Antisense primers for each of the MASP-2 domains, shown below in

TABLE 5, are designed to introduce a stop codon (boldface) followed by an *Eco*RI site (underlined) at the end of each PCR product. Once amplified, the DNA fragments are digested with *Bam*HI and *Eco*RI and cloned into the corresponding sites of the pFastBac1 vector. The resulting constructs are characterized by restriction mapping and confirmed by dsDNA sequencing.

TABLE 5: MASP-2 PCR PRIMERS

MASP-2 domain	**5' PCR Primer**	**3' PCR Primer**
SEQ ID NO:8 CUBI (aa 1–121 of SEQ ID NO:6)	5'CGGGATCCATGAG GCTGCTGACCCTC-3' (SEQ ID NO:34)	5'GGAATTCCTAGGCTGCA TA (SEQ ID NO:35)
SEQ ID NO:9 CUBIEGF (aa 1–166 of SEQ ID NO:6)	5'CGGGATCCATGAG GCTGCTGACCCTC-3' (SEQ ID NO:34)	5'GGAATTCCTACAGGGCG CT-3' (SEQ ID NO:36)
SEQ ID NO:10 CUBIEGFCUBII (aa 1-293 of SEQ ID NO:6)	5'CGGGATCCATGAG GCTGCTGACCCTC-3' (SEQ ID NO:34)	5'GGAATTCCTAGTAGTGG AT 3' (SEQ ID NO:37)
SEQ ID NO:4 human MASP-2	5'ATGAGGCTGCTGA CCCTCCTGGGCCTTC 3' (SEQ ID NO: 56) hMASP-2_forward	5'TTAAAATCACTAATTAT GTTCTCGATC 3' (SEQ ID NO: 59) hMASP-2_reverse
SEQ ID NO:4 human MASP-2 cDNA	5'CAGAGGTGACGCA GGAGGGGCAC 3' (SEQ ID NO: 58) hMASP-2_ala_forward	5'GTGCCCCTCCTGCGTCA CCTCTG 3' (SEQ ID NO: 57) hMASP-2_ala_reverse
SEQ ID NO:50 Murine MASP-2 cDNA	5'ATGAGGCTACTCA TCTTCCTGG3' (SEQ ID NO: 60) mMASP-2_forward	5'TTAGAAATTACTTATTAT GTTCTCAATCC3' (SEQ ID NO: 63) mMASP-2_reverse
SEQ ID NO:50 Murine MASP-2 cDNA	5'CCCCCCCTGCGTC ACCTCTGCAG3' (SEQ ID NO: 62) mMASP-2_ala_forward	5'CTGCAGAGGTGACGCAG GGGGGG 3' (SEQ ID NO: 61) mMASP-2_ala_reverse

Recombinant eukaryotic expression of MASP-2 and protein production of enzymatically inactive mouse, rat, and human MASP-2A.

The MASP-2 and MASP-2A expression constructs described above were 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 approximately 1.5 mg/liter of culture medium for each of the three species.

MASP-2A protein purification: The MASP-2A (Ser-Ala mutant described above) was purified by affinity chromatography on MBP-A-agarose columns. This strategy enabled rapid purification without the use of extraneous tags. MASP-2A (100-200 ml of medium diluted with an equal volume of loading buffer (50 mM Tris-Cl, pH 7.5, containing 150 mM NaCl and 25 mM $CaCl_2$) was loaded onto an MBP-agarose affinity column (4 ml) pre-equilibrated with 10 ml of loading buffer. Following washing with a further 10 ml of loading buffer, protein was eluted in 1 ml fractions with 50 mM Tris-Cl, pH 7.5, containing 1.25 M NaCl and 10 mM EDTA. Fractions containing the MASP-2A were identified by SDS-polyacrylamide gel electrophoresis. Where necessary, MASP-2A was purified further by ion-exchange chromatography on a MonoQ column (HR 5/5). Protein was dialyzed with 50 mM Tris-Cl pH 7.5, containing 50 mM NaCl and loaded onto the column equilibrated in the same buffer. Following washing, bound MASP-2A was eluted with a 0.05–1 M NaCl gradient over 10 ml.

Results: Yields of 0.25–0.5 mg of MASP-2A protein were obtained from 200 ml of medium. The molecular mass of 77.5 kDa determined by MALDI-MS is greater than the calculated value of the unmodified polypeptide (73.5 kDa) due to glycosylation. Attachment of glycans at each of the *N*-glycosylation sites accounts for the observed mass. MASP-2A migrates as a single band on SDS-polyacrylamide gels, demonstrating that it is not proteolytically processed during biosynthesis. The weight-average molecular mass determined by equilibrium ultracentrifugation is in agreement with the calculated value for homodimers of the glycosylated polypeptide.

PRODUCTION OF RECOMBINANT HUMAN MASP-2 POLYPEPTIDES

Another method for producing recombinant MASP-2 and MASP2A derived polypeptides is described in Thielens, N.M., et al., *J. Immunol.* 166:5068-5077, 2001.

Briefly, the *Spodoptera frugiperda* insect cells (Ready-Plaque Sf9 cells obtained from Novagen, Madison, WI) are grown and maintained in Sf900II serum-free medium (Life Technologies) supplemented with 50 IU/ml penicillin and 50 mg/ml streptomycin (Life Technologies). The *Trichoplusia ni* (High Five) insect cells (provided by Jadwiga Chroboczek, Institut de Biologie Structurale, Grenoble, France) are maintained in TC100 medium (Life Technologies) containing 10% FCS (Dominique Dutscher, Brumath, France) supplemented with 50 IU/ml penicillin and 50 mg/ml streptomycin. Recombinant baculoviruses are generated using the Bac-to-Bac system® (Life Technologies). The bacmid DNA is purified using the Qiagen midiprep purification system (Qiagen) and is used to transfect Sf9 insect cells using cellfectin in Sf900 II SFM medium (Life Technologies) as described in the manufacturer's protocol. Recombinant virus particles are collected 4 days later, titrated by virus plaque assay, and amplified as described by King and Possee, in *The Baculovirus Expression System: A Laboratory Guide*, Chapman and Hall Ltd., London, pp. 111-114, 1992.

High Five cells (1.75×10^7 cells/175-cm^2 tissue culture flask) are infected with the recombinant viruses containing MASP-2 polypeptides at a multiplicity of infection of 2 in Sf900 II SFM medium at 28°C for 96 h. The supernatants are collected by centrifugation and diisopropyl phosphorofluoridate is added to a final concentration of 1 mM.

The MASP-2 polypeptides are secreted in the culture medium. The culture supernatants are dialyzed against 50 mM NaCl, 1 mM $CaCl_2$, 50 mM triethanolamine hydrochloride, pH 8.1, and loaded at 1.5 ml/min onto a Q-Sepharose Fast Flow column (Amersham Pharmacia Biotech) (2.8 x 12 cm) equilibrated in the same buffer. Elution is conducted by applying a 1.2 liter linear gradient to 350 mM NaCl in the same buffer. Fractions containing the recombinant MASP-2 polypeptides are identified by Western blot analysis, precipitated by addition of $(NH_4)_2SO_4$ to 60% (w/v), and left overnight at 4°C. The pellets are resuspended in 145 mM NaCl, 1 mM $CaCl_2$, 50 mM triethanolamine hydrochloride, pH 7.4, and applied onto a TSK G3000 SWG column (7.5 x 600 mm) (Tosohaas, Montgomeryville, PA) equilibrated in the same buffer. The purified polypeptides are then concentrated to 0.3 mg/ml by ultrafiltration on Microsep microconcentrators (m.w. cut-off = 10,000) (Filtron, Karlstein, Germany).

EXAMPLE 4

This example describes a method of producing polyclonal antibodies against MASP-2 polypeptides.

Materials and Methods:

MASP-2 Antigens: Polyclonal anti-human MASP-2 antiserum is produced by immunizing rabbits with the following isolated MASP-2 polypeptides: human MASP-2 (SEQ ID NO:6) isolated from serum; recombinant human MASP-2 (SEQ ID NO:6), MASP-2A containing the inactive protease domain (SEQ ID NO:13), as described in Example 3; and recombinant CUBI (SEQ ID NO:8), CUBEGFI (SEQ ID NO:9), and CUBEGFCUBII (SEQ ID NO:10) expressed as described above in Example 3.

Polyclonal antibodies: Six-week old Rabbits, primed with BCG (bacillus Calmette-Guerin vaccine) are immunized by injecting 100 μg of MASP-2 polypeptide at 100 μg/ml in sterile saline solution. Injections are done every 4 weeks, with antibody titer monitored by ELISA assay as described in Example 5. Culture supernatants are collected for antibody purification by protein A affinity chromatography.

EXAMPLE 5

This example describes a method for producing murine monoclonal antibodies against rat or human MASP-2 polypeptides.

Materials and Methods:

Male A/J mice (Harlan, Houston, Tex.), 8-12 weeks old, are injected subcutaneously with 100 μg human or rat rMASP-2 or rMASP-2A polypeptides (made as described in **Example 3**) in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) in 200 μl of phosphate buffered saline (PBS) pH 7.4. At two-week intervals the mice are twice injected subcutaneously with 50 μg of human or rat rMASP-2 or rMASP-2A polypeptide in incomplete Freund's adjuvant. On the fourth week the mice are injected with 50 μg of human or rat rMASP-2 or rMASP-2A 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. $5x10^8$ of the Sp2/0 and $5x10^8$ spleen cells are fused in a medium containing 50% polyethylene glycol (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 $1.5x10^5$ spleen cells per

200 µl of the suspension in Iscove medium (Gibco, Grand Island, N.Y.), supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, 100 µg/ml of streptomycin, 0.1 mM hypoxanthine, 0.4 µM aminopterin and 16 µM thymidine. Two hundred microliters of the cell suspension are added to each well of about twenty 96-well microculture plates. After about ten days culture supernatants are withdrawn for screening for reactivity with purified factor MASP-2 in an ELISA assay.

ELISA Assay: Wells of Immulon® 2 (Dynatech Laboratories, Chantilly, Va.) microtest plates are coated by adding 50 µl of purified hMASP-2 at 50 ng/ml or rat rMASP-2 (or rMASP-2A) overnight at room temperature. The low concentration of MASP-2 for coating enables the selection of high-affinity antibodies. After the coating solution is removed by flicking the plate, 200 µl 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). Fifty microliters of culture supernatants from each fusion well is collected and mixed with 50 µl of BLOTTO and then added to the individual wells of the microtest plates. After one hour of incubation, the wells are washed with PBST. The bound murine antibodies are then detected by reaction with horseradish peroxidase (HRP) conjugated goat anti-mouse IgG (Fc specific) (Jackson ImmunoResearch Laboratories, West Grove, Pa.) and diluted at 1:2,000 in BLOTTO. 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 µl of 2M H_2SO_4 per well. The Optical Density at 450 nm of the reaction mixture is read with a BioTek® ELISA Reader (BioTek® Instruments, Winooski, Vt.).

MASP-2 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 the MASP-2 inhibitory agents 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 µl/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 h at room

temperature. Wells without MASP-2 coating serve as the background controls. Aliquots of hybridoma supernatants or purified anti-MASP-2 MoAbs, at varying concentrations in blocking solution, are added to the wells. Following a 2 h incubation at room temperature, the wells are extensively rinsed with PBS. MASP-2-bound anti-MASP-2 MoAb is detected by the addition of peroxidase-conjugated goat anti-mouse IgG (Sigma Chemical) in blocking solution, which is allowed to incubate for 1h at room temperature. The plate is rinsed again thoroughly with PBS, and 100 µl 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 µl 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 in **Example 2**. The cells in positive wells are then cloned by limiting 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.

EXAMPLE 6

This example describes the generation and production of humanized murine anti-MASP-2 antibodies and antibody fragments.

A murine anti-MASP-2 monoclonal antibody is generated in Male A/J mice as described in **Example 5**. The murine antibody is then humanized as described below to reduce its immunogenicity by replacing the murine constant regions with their human counterparts to generate a chimeric IgG and Fab fragment of the antibody, which is useful for inhibiting the adverse effects of MASP-2-dependent complement activation in human subjects in accordance with the present invention.

1. Cloning of anti-MASP-2 variable region genes from murine hybridoma cells. Total RNA is isolated from the hybridoma cells secreting anti-MASP-2 MoAb (obtained as described in Example 7) using RNAzol following the manufacturer's protocol (Biotech, Houston, Tex.). First strand cDNA is synthesized from the total RNA using oligo dT as the primer. PCR is performed using the immunoglobulin constant C region-derived 3' primers and degenerate primer sets derived from the leader

peptide or the first framework region of murine V_H or V_K genes as the 5' primers. Anchored PCR is carried out as described by Chen and Platsucas (Chen, P.F., *Scand. J. Immunol.* 35:539-549, 1992). For cloning the V_K gene, double-stranded cDNA is prepared using a NotI-MAK1 primer (5'-TGCGGCCGCTGTAGGTGCTGTCTTT-3' SEQ ID NO:38). Annealed adaptors AD1 (5'-GGAATTCACTCGTTATTCTCGGA-3' SEQ ID NO:39) and AD2 (5'-TCCGAGAATAACGAGTG-3' SEQ ID NO:40) are ligated to both 5' and 3' termini of the double-stranded cDNA. Adaptors at the 3' ends are removed by NotI digestion. The digested product is then used as the template in PCR with the AD1 oligonucleotide as the 5' primer and MAK2 (5'-CATTGAAAGCTTTGGGGTAGAAGTTGTTC-3' SEQ ID NO:41) as the 3' primer. DNA fragments of approximately 500 bp are cloned into pUC19. Several clones are selected for sequence analysis to verify that the cloned sequence encompasses the expected murine immunoglobulin constant region. The Not1-MAK1 and MAK2 oligonucleotides are derived from the V_K region and are 182 and 84 bp, respectively, downstream from the first base pair of the C kappa gene. Clones are chosen that include the complete V_K and leader peptide.

For cloning the V_H gene, double-stranded cDNA is prepared using the Not1 MAG1 primer (5'-CGCGGCCGCAGCTGCTCAGAGTGTAGA-3' SEQ ID NO:42). Annealed adaptors AD1 and AD2 are ligated to both 5' and 3' termini of the double-stranded cDNA. Adaptors at the 3' ends are removed by NotI digestion. The digested product are used as the template in PCR with the AD1 oligonucleotide and MAG2 (5'-CGGTAAGCTTCACTGGCTCAGGGAAATA-3' SEQ ID NO:43) as primers. DNA fragments of 500 to 600 bp in length are cloned into pUC19. The NotI-MAG1 and MAG2 oligonucleotides are derived from the murine Cγ.7.1 region, and are 180 and 93 bp, respectively, downstream from the first bp of the murine Cγ.7.1 gene. Clones are chosen that encompass the complete V_H and leader peptide.

2. Construction of Expression Vectors for Chimeric MASP-2 IgG and Fab. The cloned V_H and V_K genes described above are used as templates in a PCR reaction to add the Kozak consensus sequence to the 5' end and the splice donor to the 3' end of the nucleotide sequence. After the sequences are analyzed to confirm the absence of PCR errors, the V_H and V_K genes are inserted into expression vector cassettes containing human C.γ1 and C. kappa respectively, to give pSV2neoV_H-huCγ1 and pSV2neoV-huCγ. CsCl gradient-purified plasmid DNAs of the heavy- and light-chain

vectors are used to transfect COS cells by electroporation. After 48 hours, the culture supernatant is tested by ELISA to confirm the presence of approximately 200 ng/ml of chimeric IgG. The cells are harvested and total RNA is prepared. First strand cDNA is synthesized from the total RNA using oligo dT as the primer. This cDNA is used as the template in PCR to generate the Fd and kappa DNA fragments. For the Fd gene, PCR is carried out using 5'-AAGAAGCTTGCCGCCACCATGGATTGGCTGTGGAACT-3' (SEQ ID NO:44) as the 5' primer and a CH1-derived 3' primer (5'-CGGGATCCTCAAACTTTCTTGTCCACCTTGG-3' SEQ ID NO:45). The DNA sequence is confirmed to contain the complete V_H and the CH1 domain of human IgG1. After digestion with the proper enzymes, the Fd DNA fragments are inserted at the HindIII and BamHI restriction sites of the expression vector cassette pSV2dhfr-TUS to give pSV2dhfrFd. The pSV2 plasmid is commercially available and consists of DNA segments from various sources: pBR322 DNA (thin line) contains the pBR322 origin of DNA replication (pBR ori) and the lactamase ampicillin resistance gene (Amp); SV40 DNA, represented by wider hatching and marked, contains the SV40 origin of DNA replication (SV40 ori), early promoter (5' to the dhfr and neo genes), and polyadenylation signal (3' to the dhfr and neo genes). The SV40-derived polyadenylation signal (pA) is also placed at the 3' end of the Fd gene.

For the kappa gene, PCR is carried out using 5'-AAGAAAGCTTGCCGCCACCATGTTCTCACTAGCTCT-3' (SEQ ID NO:46) as the 5' primer and a C_K-derived 3' primer (5'-CGGGATCCTTCTCCCTCTAACACTCT-3' SEQ ID NO:47). DNA sequence is confirmed to contain the complete V_K and human C_K regions. After digestion with proper restriction enzymes, the kappa DNA fragments are inserted at the HindIII and BamHI restriction sites of the expression vector cassette pSV2neo-TUS to give pSV2neoK. The expression of both Fd and .kappa genes are driven by the HCMV-derived enhancer and promoter elements. Since the Fd gene does not include the cysteine amino acid residue involved in the inter-chain disulfide bond, this recombinant chimeric Fab contains non-covalently linked heavy- and light-chains. This chimeric Fab is designated as cFab.

To obtain recombinant Fab with an inter-heavy and light chain disulfide bond, the above Fd gene may be extended to include the coding sequence for additional 9 amino acids (EPKSCDKTH SEQ ID NO:48) from the hinge region of human IgG1. The

BstEII-BamHI DNA segment encoding 30 amino acids at the 3' end of the Fd gene may be replaced with DNA segments encoding the extended Fd, resulting in pSV2dhfrFd/9aa.

3. Expression and Purification of Chimeric Anti-MASP-2 IgG

To generate cell lines secreting chimeric anti-MASP-2 IgG, NSO cells are transfected with purified plasmid DNAs of pSV2neoV$_H$-huC.γ1 and pSV2neoV-huC kappa by electroporation. Transfected cells are selected in the presence of 0.7 mg/ml G418. Cells are grown in a 250 ml spinner flask using serum-containing medium.

Culture supernatant of 100 ml spinner culture is loaded on a 10-ml PROSEP-A column (Bioprocessing, Inc., Princeton, N.J.). The column is washed with 10 bed volumes of PBS. The bound antibody is eluted with 50 mM citrate buffer, pH 3.0. Equal volume of 1 M Hepes, pH 8.0 is added to the fraction containing the purified antibody to adjust the pH to 7.0. Residual salts are removed by buffer exchange with PBS by Millipore membrane ultrafiltration (M.W. cut-off: 3,000). The protein concentration of the purified antibody is determined by the BCA method (Pierce).

4. Expression and purification of chimeric anti-MASP-2 Fab

To generate cell lines secreting chimeric anti-MASP-2 Fab, CHO cells are transfected with purified plasmid DNAs of pSV2dhfrFd (or pSV2dhfrFd/9aa) and pSV2neokappa, by electroporation. Transfected cells are selected in the presence of G418 and methotrexate. Selected cell lines are amplified in increasing concentrations of methotrexate. Cells are single-cell subcloned by limiting dilution. High-producing single-cell subcloned cell lines are then grown in 100 ml spinner culture using serum-free medium.

Chimeric anti-MASP-2 Fab is purified by affinity chromatography using a mouse anti-idiotypic MoAb to the MASP-2 MoAb. An anti-idiotypic MASP-2 MoAb can be made by immunizing mice with a murine anti-MASP-2 MoAb conjugated with keyhole limpet hemocyanin (KLH) and screening for specific MoAb binding that can be competed with human MASP-2. For purification, 100 ml of supernatant from spinner cultures of CHO cells producing cFab or cFab/9aa are loaded onto the affinity column coupled with an anti-idiotype MASP-2 MoAb. The column is then washed thoroughly with PBS before the bound Fab is eluted with 50 mM diethylamine, pH 11.5. Residual salts are removed by buffer exchange as described above. The protein concentration of the purified Fab is determined by the BCA method (Pierce).

The ability of the chimeric MASP-2 IgG, cFab, and cFAb/9aa to inhibit MASP-2-dependent complement pathways may be determined by using the inhibitory assays described in Example 2 or Example 7.

EXAMPLE 7

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 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) from *S. aureus* which binds L-ficolin.

Reagents: Formalin-fixed *S. aureous* (DSM20233) is prepared as follows: bacteria is grown overnight at 37°C in tryptic soy blood medium, washed three times with PBS, then fixed for 1 h at room temperature in PBS/0.5% formalin, and washed a further three times with PBS, before being resuspended in coating buffer (15 mM Na_2Co_3, 35 mM $NaHCO_3$, 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 (OD_{550} = 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 $CaCl_2$ (wash buffer). Human serum samples are diluted in 20 mM Tris-HCl, 1 M NaCl, 10 mM $CaCl_2$, 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 anti-MASP-2 MoAbs and inhibitory peptides 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 $CaCl_2$, 1 mM $MgCl_2$, pH 7.4 is added to each well. After 1.5 h 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 ρ-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 8

The following assay demonstrates the presence of classical pathway activation in wild-type and MASP-2-/- mice.

Methods: Immune complexes were generated *in situ* by coating microtiter plates (MaxiSorb®, Nunc, cat. No. 442404, Fisher Scientific) with 0.1% human serum albumin in 10 mM Tris, 140 mM NaCl, pH 7.4 for 1 hours at room temperature followed by overnight incubation at 4°C with sheep anti whole serum antiserum (Scottish Antibody Production Unit, Carluke, Scotland) diluted 1:1000 in TBS/tween/Ca^{2+}. Serum samples were obtained from wild-type and MASP-2-/- mice and added to the coated plates. Control samples were prepared in which C1q was depleted from wild-type and MASP-2-/- serum samples. C1q-depleted mouse serum was prepared using protein-A-coupled Dynabeads® (Dynal Biotech, Oslo, Norway) coated with rabbit anti-human C1q IgG (Dako, Glostrup, Denmark), according to the supplier's instructions. The plates were incubated for 90 minutes at 37°C. Bound C3b was detected with a polyclonal anti-human-C3c Antibody (Dako A 062) diluted in TBS/tw/ Ca^{++} at 1:1000. The secondary antibody is goat anti-rabbit IgG.

Results: FIGURE 7 shows the relative C3b deposition levels on plates coated with IgG in wild-type serum, MASP-2-/- serum, C1q-depleted wild-type and C1q-depleted MASP-2-/- serum. These results demonstrate that the classical pathway is intact in the MASP-2-/- mouse strain.

EXAMPLE 9

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

Methods: To test the effect of a MASP-2 inhibitory agent on conditions of complement activation where the classical pathway is initiated by immune complexes, triplicate 50 µl samples containing 90% NHS are incubated at 37ºC in the presence of 10 µg/ml immune complex (IC) or PBS, and parallel triplicate samples (+/-IC) are also included which contain 200 nM anti-properdin monoclonal antibody during the 37ºC incubation. After a two hour incubation at 37ºC, 13 mM EDTA is added to all samples to stop further complement activation and the samples are immediately cooled to 5ºC. The samples are then stored at -70ºC 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 10

This example describes the identification of high affinity anti-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 anti-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 anti-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 anti-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:55). 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 anti-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 Anti-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 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 anti-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, anti-MASP-2 Fab2 which inhibit MASP-2 functional activity (i.e., blocking anti-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 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 min at 37ºC with diluted rat 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. Anti-MASP-2 Fab2s at selected concentrations were tested in this assay for their ability to inhibit C3 convertase formation and consequent C3b generation.

Methods:

96-well Costar Medium Binding plates were incubated overnight at 5ºC 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 at room temperature with gentle mixing. Each well was then washed three times with 200 µL of PBS. The anti-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. A 0.5% rat serum was added to the above samples at 5ºC and 100 µL was transferred to each well. 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 37ºC 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 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 hr at room temperature with gentle mixing. Each well was washed 5 x 200 µL PBS. 100 µL/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 µL with PBS. 100 µL/well of the peroxidase substrate TMB (Kirkegaard & Perry

Laboratories) was added and incubated at room temperature for 10 min. The peroxidase reaction was stopped by adding 100 µL/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. Anti-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 µg/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. Anti-MASP-2 Fab2 at selected concentrations were tested in this assay for their 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 µg/50 µL/well. Each well was washed 3X 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 at room temperature with gentle mixing. Each well was washed 3X with 200 µL of PBS. Anti-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 µg/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 x 200 µL with PBS-Tween 20 (0.05% Tween 20 in PBS), then each well was washed with 2X 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 x 200 µL PBS. 100 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 stopped by adding 100 µL/well of 1.0 M H_3PO_4 and the OD_{450} 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 anti-MASP-2 Fab2 to the 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 is used, rather than purified recombinant MASP-2. 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 anti-MASP-2 Fab2s to the immobilized 'native' MASP-2 was then studied using a standard ELISA methodology.

Methods: 96-well Costar High Binding plates were incubated overnight at 5ºC with mannan diluted in 50 mM carbonate buffer, pH 9.5 at 1 µg/50 µL/well. Each well was washed 3X 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 temperature with gentle mixing. Each well was washed 3X with 200 µL of TBS/Tween/Ca^{++} Wash Buffer (Tris-buffered saline, 0.05% Tween 20, containing 5.0 mM $CaCl_2$, pH 7.4. 10% rat serum in High Salt Binding Buffer (20 mM Tris, 1.0 M NaCl, 10 mM $CaCl_2$, 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 5ºC. Wells were washed 3X with 200 µL of TBS/Tween/Ca^{++} Wash Buffer. Wells were then washed 2X with 200 µL PBS. 100 µL /well of selected concentration of anti-MASP-2 Fab2 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) was added and incubated for one hour at room temperature with gentle mixing. Each well was washed 5 x 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 x 200 μL PBS. 100 μL/well of the peroxidase substrate TMB (Kirkegaard & Perry Laboratories) was added and incubated at room temperature for 70 min. The peroxidase reaction was stopped by adding 100 μL/well of 1.0 M H_3PO_4 and OD_{450} was measured.

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 anti-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 result of this analysis is shown below in TABLE 6.

TABLE 6: ANTI-MASP-2 FAB2 THAT BLOCK LECTIN PATHWAY COMPLEMENT ACTIVATION

Fab2 antibody #	C3 Convertase (IC_{50} (nM)	K_d	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
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 6, of the 50 anti-MASP-2 Fab2s tested, seventeen Fab2s were identified as MASP-2 blocking Fab2 that potently inhibit C3 convertase formation with IC_{50} equal to or less than 10 nM Fab2s (a 34% positive hit rate). Eight of the seventeen Fab2s identified have IC_{50}s in the subnanomolar range. Furthermore, all seventeen of the MASP-2 blocking Fab2s shown in TABLE 6 gave essentially complete inhibition of C3 convertase formation in the lectin pathway C3 convertase assay. FIGURE 8A graphically illustrates the results of the C3 convertase formation assay for Fab2 antibody #11, which is representative of the other Fab2 antibodies tested, the results of which are shown in TABLE 6. 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.

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 anti-MASP-2 Fab2s listed in this example potently inhibits C3b generation (>95 %), thus demonstrating the specificity of this assay for 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 6. FIGURE 8B graphically illustrates the results of a binding assay with the Fab2 antibody #11. Similar binding assays were also carried out for the other Fab2s, the results of which are shown in TABLE 6. In general, the apparent K_ds obtained for binding of each 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 since in each assay the Fab2 would be binding a different conformational form of MASP-2. Never-the-less, with the exception of Fab2 #88, there appears to be a reasonably close correspondence between the IC_{50} and apparent Kd for each of the other sixteen Fab2 tested in the two assays (see TABLE 6).

Several of the blocking Fab2s were evaluated for inhibition of MASP-2 mediated cleavage of C4. FIGURE 8C graphically illustrates the results of a C4 cleavage assay, showing inhibition with Fab2 #41, with an IC_{50}=0.81 nM (see TABLE 6). As shown in FIGURE 9, all of the Fab2s tested were found to inhibit C4 cleavage with IC_{50}s similar to those obtained in the C3 convertase assay (see TABLE 6).

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 anti-MASP-2 Fab2s have been identified which 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 11

This Example describes the epitope mapping for several of the blocking anti-rat MASP-2 Fab2 antibodies that were generated as described in **Example 10**.

Methods:

As shown in **FIGURE 10**, 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 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

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)).

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.

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

DOT BLOT ANALYSIS:

Serial dilutions of the five recombinant MASP-2 polypeptides described above and shown in **FIGURE 10** (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 µg/ml anti-MASP-2 Fab2s in blocking buffer (containing 5.0 mM Ca^{2+}). 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 µg/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 anti-MASP-2 Fab2s were added in TBS

containing 5.0 mM Ca^{2+}. The plates were incubated for one hour at RT. After washing three times with TBS/tween/Ca^{2+}, HRP-conjugated anti-human Fab (AbD/Serotec) diluted 1/10,000 in TBS/ Ca^{2+} was added and the plates incubated for a further one hour at RT. 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 7.** The numerical values provided in **TABLE 7** 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 7: 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
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, indicating that this Fab2 recognizes an epitope in CCP1. Fab2 #40 and #49 bound only to complete MASP-2A. In the ELISA binding assay shown in **FIGURE 11**, Fab2 #60 also bound to the CUBI-II polypeptide, albeit with a slightly lower apparent affinity.

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

EXAMPLE 12

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.

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 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.

Methods:

Expression of full-length catalytically inactive MASP-2:

The full-length cDNA sequence of human MASP-2 (SEQ ID NO: 4), encoding the human MASP-2 polypeptide with leader sequence (SEQ ID NO:5) 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)).

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 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.

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 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

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 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.

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. 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 μg/mL. The positive control was OMS100 Fab2 and was tested at 0.4 μg/mL. C3c formation was monitored in the presence and absence of the scFv/IgG clones.

Mannan was diluted to a concentration of 20 μg/mL (1 μg/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.

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 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 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 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 IC_{50} 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 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.

TABLE 8: Comparison of functional potency in IC_{50} (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	26	>1000	140
17N16mc	68	nd	nd
17N16m_d17N9	48	15	230

Presented below are the heavy-chain variable region (VH) sequences for the mother clones and daughter clones shown above in TABLE 8.

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

17D20_35VH-21N11VL heavy chain variable region (VH) (SEQ ID NO:67, encoded by SEQ ID NO:66)

QVTLKESGPVLVKPTETLTLTCTVSGFSLS**RG**KMGVSWIRQPPGKALEW**LAHIFSSDEKSYRTSL**KSRLTISKDTSKNQVVLTMTNMDPVDTAT**YYCARIRRG GIDY**WGQGTLVTVSS

d17N9 heavy chain variable region (VH) (SEQ ID NO:68)

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSTSAAWNWIRQSPSRGLEW**LGRTY YRSKWYNDYAV**SVKSRITINPDTSKNQFSLQLNSVTPEDT**AVYYCARDPFGVPF DI**WGQGTMVTVSS

Presented below are the light-chain variable region (VL) sequences for the mother clones and daughter clones shown above in TABLE 8.

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 light chain variable region (VL) (SEQ ID NO:69, encoded by SEQ ID NO:70)

QPVLTQPPSLSVSPGQTASITCS**GEKLGDKYAYW**YQQKPGQSPVLVMYQ **DKQRPSG**IPERFSGSNSGNTATLTISGTQAMDEADYYCQ**AWDSSTAVF**GGGTKL TVL

17N16m_d17N9 light chain variable region (VL) (SEQ ID NO:71)

SYELIQPPSVSVAPGQTATITCA**GDNLGKKRVHW**YQQRPGQAPVLVIYD **DSDRPSG**IPDRFSASNSGNTATLTITRGEAGDEADYYCQ**VWDIATDHV**VFGGGT KLTVLAAAGSEQKLISE

The MASP-2 antibodies OMS100 and MoAb_d3521N11VL, (comprising a heavy chain variable region set forth as SEQ ID NO:67 and a light chain variable region set forth as SEQ ID NO:69, also referred to as "OMS646" and "mAb6"), 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 OMS646 and OMS100 antibodies are highly specific for MASP-2 and do not bind to MASP-1/3. Neither antibody bound to MAp19 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.

The MASP-2 antibody OMS646 was determined to avidly bind to recombinant MASP-2 (Kd 60-250pM) with >5000 fold selectivity when compared to C1s, C1r or MASP-1 (see TABLE 9 below):

TABLE 9: Affinity and Specificity of OMS646 MASP-2 antibody-MASP-2 interaction as assessed by solid phase ELISA studies

Antigen	K_D (pM)
MASP-1	>500,000
MASP-2	62±23*
MASP-3	>500,000
Purified human C1r	>500,000
Purified human C1s	~500,000

*Mean±SD; n=12

OMS646 specifically blocks lectin-dependent activation of terminal complement components

Methods:

The effect of OMS646 on membrane attack complex (MAC) deposition was analyzed using pathway-specific conditions for the lectin pathway, the classical pathway and the alternative pathway. For this purpose, the Wieslab Comp300 complement screening kit (Wieslab, Lund, Sweden) was used following the manufacturer's instructions.

Results:

FIGURE 12A graphically illustrates the level of MAC deposition in the presence or absence of anti-MASP-2 antibody (OMS646) under lectin pathway-specific assay conditions. FIGURE 12B graphically illustrates the level of MAC deposition in the presence or absence of anti-MASP-2 antibody (OMS646) under classical pathway-specific assay conditions. FIGURE 12C graphically illustrates the level of MAC deposition in the presence or absence of anti-MASP-2 antibody (OMS646) under alternative pathway-specific assay conditions.

As shown in FIGURE 12A, OMS646 blocks lectin pathway-mediated activation of MAC deposition with an IC_{50} value of approximately 1nM. However, OMS646 had no effect on MAC deposition generated from classical pathway-mediated activation (FIGURE 12B) or from alternative pathway-mediated activation (FIGURE 12C).

Pharmacokinetics and Pharmacodynamics of OMS646 following Intravenous (IV) or Subcutaneous (SC) Administration to Mice

The pharmacokinetics (PK) and pharmacodynamics (PD) of OMS646 were evaluated in a 28 day single dose PK/PD study in mice. The study tested dose levels of 5mg/kg and 15mg/kg of OMS646 administered subcutaneously (SC), as well as a dose level of 5mg/kg OMS646 administered intravenously (IV).

With regard to the PK profile of OMS646, FIGURE 13 graphically illustrates the OMS646 concentration (mean of n=3 animals/groups) as a function of time after administration of OMS646 at the indicated dose. As shown in FIGURE 13, at 5mg/kg SC, OMS646 reached the maximal plasma concentration of 5-6 µg/mL approximately 1-2

days after dosing. The bioavailability of OMS646 at 5 mg/kg SC was approximately 60%. As further shown in FIGURE 13, at 15 mg/kg SC, OMS646 reached a maximal plasma concentration of 10-12 μg/mL approximately 1 to 2 days after dosing. For all groups, the OMS646 was cleared slowly from systemic circulation with a terminal half-life of approximately 8-10 days. The profile of OMS646 is typical for human antibodies in mice.

The PD activity of OMS646 is graphically illustrated in FIGURES 14A and 14B. FIGURES 14A and 14B show the PD response (drop in systemic lectin pathway activity) for each mouse in the 5mg/kg IV (FIGURE 14A) and 5mg/kg SC (FIGURE 14B) groups. The dashed line indicates the baseline of the assay (maximal inhibition; naïve mouse serum spiked *in vitro* with excess OMS646 prior to assay). As shown in FIGURE 14A, following IV administration of 5mg/kg of OMS646, systemic lectin pathway activity immediately dropped to near undetectable levels, and lectin pathway activity showed only a modest recovery over the 28 day observation period. As shown in FIGURE 14B, in mice dosed with 5mg/kg of OMS646 SC, time-dependent inhibition of lectin pathway activity was observed. Lectin pathway activity dropped to near-undetectable levels within 24 hours of drug administration and remained at low levels for at least 7 days. Lectin pathway activity gradually increased with time, but did not revert to pre-dose levels within the 28 day observation period. The lectin pathway activity versus time profile observed after administration of 15mg/kg SC was similar to the 5 mg/kg SC dose (data not shown), indicating saturation of the PD endpoint. The data further indicated that weekly doses of 5mg/kg of OMS646, administered either IV or SC, is sufficient to achieve continuous suppression of systemic lectin pathway activity in mice.

EXAMPLE 13

This Example describes the generation of recombinant antibodies that inhibit MASP-2 comprising a heavy chain and/or a light chain variable region comprising one or more CDRs that specifically bind to MASP-2 and at least one SGMI core peptide sequence (also referred to as an SGMI-peptide bearing MASP-2 antibody or antigen binding fragment thereof).

Background/Rationale:

The generation of specific inhibitors of MASP-2, termed SGMI-2, is described in Heja et al., *J Biol Chem* 287:20290 (2012) and Heja et al., *PNAS* 109:10498 (2012), each of which is hereby incorporated herein by reference. SGMI-2 is a 36 amino acid peptide

which was selected from a phage library of variants of the *Schistocerca gregaria* protease inhibitor 2 in which six of the eight positions of the protease binding loop were fully randomized. Subsequent *in vitro* evolution yielded mono-specific inhibitors with single digit nM K_I values (Heja et al., *J. Biol. Chem.* 287:20290, 2012). Structural studies revealed that the optimized protease binding loop forms the primary binding site that defines the specificity of the two inhibitors. The amino acid sequences of the extended secondary and internal binding regions are common to the two inhibitors and contribute to the contact interface (Heja et al., 2012. *J. Biol. Chem.* 287:20290). Mechanistically, SGMI-2 blocks the lectin pathway of complement activation without affecting the classical pathway (Heja et al., 2012. *Proc. Natl. Acad. Sci.* 109:10498).

The amino acid sequences of the SGMI-2 inhibitors are set forth below:

SGMI-2-full-length: LEVTCEPGTTFKDKCNTCRCGSDGKSAVCTKLWCNQ (SEQ ID NO:72)

SGMI-2-medium: TCEPGTTFKDKCNTCRCGSDGKSAVCTKLWCNQ (SEQ ID NO:73)

SGMI-2-short: ……..TCRCGSDGKSAVCTKLWCNQ (SEQ ID NO:74)

As described in this Example, and also described in WO2014/144542, SGMI-2 peptide-bearing MASP-2 antibodies and fragments thereof were generated by fusing the SGMI-2 peptide amino acid sequence (e.g., SEQ ID NO: 72, 73 or 74) onto the amino or carboxy termini of the heavy and/or light chains of a human MASP-2 antibody. The SGMI-2 peptide-bearing MASP-2 antibodies and fragments have enhanced inhibitory activity, as compared to the naked MASP-2 scaffold antibody that does not contain the SGMI-2 peptide sequence, when measured in a C3b or C4b deposition assay using human serum, as described in WO2014/144542, and also have enhanced inhibitory activity as compared to the naked MASP-2 scaffold antibody when measured in a mouse model *in vivo*. Methods of generating SGMI-2 peptide bearing MASP-2 antibodies are described below.

Methods:

Expression constructs were generated to encode four exemplary SGMI-2 peptide bearing MASP-2 antibodies wherein the SGMI-2 peptide was fused either to the N- or C-

terminus of the heavy or light chain of a representative MASP-2 inhibitory antibody OMS646 (generated as described in Example 12).

TABLE 10: MASP-2 antibody/SGMI-2 fusions

Antibody reference	Peptide Location on Antibody				SEQ ID NO:
	H-N	H-C	L-N	L-C	
HL-M2 (naked MASP-2 OMS646)	-	-	-	-	67 + 70
H-M2-SGMI-2-N	SGMI-2	-	-	-	75 + 70
H-M2-SGMI-2-C	-	SGMI-2	-	-	76 +70
L-M2-SGMI-2-N	-	-	SGMI-2	-	67+ 77
L-M2-SGMI-2-C	-	-	-	SGMI-2	67+ 78

Abbreviations in Table 10:
"H-N"= amino terminus of heavy chain
"H-C"=carboxyl terminus of heavy chain
"L-N"=amino terminus of light chain
"L-C"=carboxyl terminus of light chain
"M2"=MASP-2 ab scaffold (representative OMS646)

For the N-terminal fusions shown in TABLE 10, a peptide linker ('GTGGGSGSSS' SEQ ID NO: 79) was added between the SGMI-2 peptide and the variable region.

For the C-terminal fusions shown in TABLE 10, a peptide linker ('AAGGSG' SEQ ID NO: 80) was added between the constant region and the SGMI-2 peptide, and a second peptide "GSGA" (SEQ ID NO: 81) was added at the C-terminal end of the fusion polypeptide to protect C-terminal SGMI-2 peptides from degradation.

Amino acid sequences are provided below for the following representative MASP-2 antibody/SGMI-2 fusions:

H-M2ab6-SGMI-2-N (SEQ ID NO:75, encoded by SEQ ID NO:82):

LEVTCEPGTTFKDKCNTCRCGSDGKSAVCTKLWCNQ*GTGGGSGSSS*QVTLKESG
PVLVKPTETLTLTCTVSGFSLSRGKMGVSWIRQPPGKALEWLAHIFSSDEKSYRT
SLKSRLTISKDTSKNQVVLTMTNMDPVDTATYYCARIRRGGIDYWGQGTLVTVS

SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCP
APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK
AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
K

[491 aa protein, aa 1-36=SGMI-2 (underlined), aa37-46=linker (italicized); aa47-164=heavy chain variable region of MASP-2 ab#6 (underlined); aa165-491=IgG4 constant region with hinge mutation.]

H-M2ab6-SGMI-2-C (SEQ ID NO:76, encoded by SEQ ID NO:83):

QVTLKESGPVLVKPTETLTLTCTVSGFSLSRGKMGVSWIRQPPGKALEWLAHIFS
SDEKSYRTSLKSRLTISKDTSKNQVVLTMTNMDPVDTATYYCARIRRGGIDYWG
QGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESK
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS
SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQK
SLSLSLGK*AAGGSG*LEVTCEPGTTFKDKCNTCRCGSDGKSAVCTKLWCNQ*GSGA*

[491aa protein, aa1-118=heavy chain variable region of MASP-2 ab#6 (underlined); aa 119-445=IgG4 constant region with hinge mutation; aa 446-451= 1st linker (italicized); aa 452-487=SGMI-2; aa488-491=2nd linker (italicized).]

L-M2ab6-SGMI-2-N (SEQ ID NO:77, encoded by SEQ ID NO:84):

LEVTCEPGTTFKDKCNTCRCGSDGKSAVCTKLWCNQ*GTGGGSGSSS*QPVLTQPPS
LSVSPGQTASITCSGEKLGDKYAYWYQQKPGQSPVLVMYQDKQRPSGIPERFSG
SNSGNTATLTISGTQAMDEADYYCQAWDSSTAVFGGGTKLTVLGQPKAAPSVTL
FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKY
AASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

[258aa protein, aa1-36=SGMI-2 (underlined); aa37-46=linker (italicized); aa47-152=light chain variable region of MASP-2 ab#6 (underlined); aa153-258=human Ig lambda constant region]

L-M2ab6-SGMI-2-C (SEQ ID NO:78, encoded by SEQ ID NO:85):

QPVLTQPPSLSVSPGQTASITCSGEKLGDKYAYWYQQKPGQSPVLVMYQDKQRP
SGIPERFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTAVFGGGTKLTVLGQ
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT
PSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS*AAGGSG*
LEVTCEPGTTFKDKCNTCRCGSDGKSAVCTKLWCNQ*GSGA*

[258aa protein, aa1-106=light chain variable region of MASP-2 ab#6 (underlined); aa 107-212=human Ig lambda constant region; aa 213-218=1st linker; aa219-254=SGMI-2; aa255-258=2nd linker]

Functional Assays:

The four MASP-2-SGMI-2 fusion antibody constructs were transiently expressed in Expi293F cells (Invitrogen), purified by Protein A affinity chromatography, and tested in 10% normal human serum for inhibition of C3b deposition in a mannan-coated bead assay as described below.

Testing the MASP-2-SGMI-2 fusions in the mannan-coated bead assay for C3b deposition

The MASP-2-SGMI-2 fusion antibodies assessed for lectin pathway inhibition in an assay of C3b deposition on mannan-coated beads. This assay, which determines degree of activity by flow cytometry, offers greater resolution than the Wieslab® assay. The lectin pathway bead assay was carried out as follows: mannan was adsorbed to 7 µM-diameter polystyrene beads (Bangs Laboratories; Fishers, IN, USA) overnight at 4°C in carbonate-bicarbonate buffer (pH 9.6). The beads were washed in PBS and exposed to 10% human serum, or 10% serum pre-incubated with antibodies or inhibitors. The serum-bead mixture was incubated at room temperature for one hour while agitating. Following the serum incubation, the beads were washed, and C3b deposition on the beads was measured by detection with an anti-C3c rabbit polyclonal antibody (Dako North

America; Carpinteria, CA, USA) and a PE-Cy5 conjugated goat anti-rabbit secondary antibody (Southern Biotech; Birmingham, AL, USA). Following the staining procedure, the beads were analyzed using a FACSCalibur flow cytometer. The beads were gated as a uniform population using forward and side scatter, and C3b deposition was apparent as FL3-positive particles (FL-3, or "FL-3 channel" indicates the 3rd or red channel on the cytometer). The Geometric Mean Fluorescence Intensity (MFI) for the population for each experimental condition was plotted relative to the antibody/inhibitor concentration to evaluate lectin pathway inhibition.

The IC_{50} values were calculated using the GraphPad PRISM software. Specifically, IC_{50} values were obtained by applying a variable slope (four parameter), nonlinear fit to log (antibody) versus mean fluorescence intensity curves obtained from the cytometric assay.

The results are shown in TABLE 11.

TABLE 11: C3b deposition (mannan-coated bead assay) in 10% human serum

Construct	**IC_{50} (nM)**
Naked N2 ab (mAb#6)	≥ 3.63 nM
H-M2-SGMI-2-N	2.11 nM
L-M2-SGMI-2-C	1.99 nM
H-M2-SGMI-2-N	2.24 nM
L-M2-SGMI-2-N	3.71 nM

Results:

The control, non-SGMI-containing MASP-2 "naked" scaffold antibody (mAb#6), was inhibitory in this assay, with an IC50 value of ≥ 3.63 nM, which is consistent with the inhibitory results observed in Example 12. Remarkably, as shown in TABLE 11, all of the SGMI-2-MASP-2 antibody fusions that were tested improved the potency of the MASP-2 scaffold antibody in this assay, suggesting that increased valency may also be beneficial in the inhibition of C3b deposition.

Testing the MASP-2-SGMI-2 fusions in the mannan-coated bead assay for C4b deposition assay with 10% human serum

A C4b deposition assay was carried out with 10% human serum using the same assay conditions as described above for the C3b deposition assay with the following modifications. C4b detection and flow cytometric analysis was carried out by staining the deposition reaction with an anti-C4b mouse monoclonal antibody (1:500, Quidel) and staining with a secondary goat anti-mouse F(ab')2 conjugated to PE Cy5 (1:200, Southern Biotech) prior to flow cytometric analysis.

Results:

The SGMI-2-bearing MASP-2- N-terminal antibody fusions (H-M2-SGMI-2-N: IC50=0.34nM), L-M2-SGMI-2-N: IC50=0.41 nM)), both had increased potency as compared to the MASP-2 scaffold antibody (HL-M2: IC50=0.78nM).

Similarly, the single SGMI-2 bearing C-terminal MASP-2 antibody fusions (H-M2-SGMI-2-C: IC_{50}=0.45nM and L-M2-SGMI-2C: IC_{50}=0.47 nM) both had increased potency as compared to the MASP-2 scaffold antibody (HL-M2: IC_{50}=1.2 nM).

Testing the MASP-2-SGMI-2 fusions in the mannan-coated bead assay for C3b deposition with 10% mouse serum.

A mannan-coated bead assay for C3b deposition was carried out as described above with 10% mouse serum. Similar to the results observed in human serum, it was determined that the SGMI-2-bearing MASP-2 fusions had increased potency as compared to the MASP-2 scaffold antibody in mouse serum.

Summary of Results: The results in this Example demonstrate that all of the SGMI-2-MASP-2 antibody fusions that were tested improved the potency of the MASP-2 scaffold antibody.

EXAMPLE 14

This Example provides results that were generated using a Unilateral Ureteric Obstruction (UUO) model of renal fibrosis in MASP-2 -/- deficient and MASP-2 +/+ sufficient mice to evaluate the role of the lectin pathway in renal fibrosis.

Background/Rationale:

Renal fibrosis and inflammation are prominent features of late stage kidney disease. Renal tubulointerstitial fibrosis is progressive process involving sustained cell

injury, aberrant healing, activation of resident and infiltrating kidney cells, cytokine release, inflammation and phenotypic activation of kidney cells to produce extracellular matrix. Renal tubulointerstitial (TI) fibrosis is the common end point of multiple renal pathologies and represents a key target for potential therapies aimed at preventing progressive renal functional impairment in chronic kidney disease (CKD). Renal TI injury is closely linked to declining renal function in glomerular diseases (Risdon R.A. et al., *Lancet* 1: 363-366, 1968; Schainuck L.I. et al, *Hum Pathol* 1: 631-640, 1970; Nath K.A., *Am J Kid Dis* 20:1-17, 1992), and is characteristic of CKD where there is an accumulation of myofibroblasts, and the potential space between tubules and peritubular capillaries becomes occupied by matrix composed of collagens and other proteoglycans. The origin of TI myofibroblasts remains intensely controversial, but fibrosis is generally preceded by inflammation characterized initially by TI accumulation of T lymphocytes and then later by macrophages (Liu Y. et al., *Nat Rev Nephrol* 7:684-696, 2011; Duffield J.S., *J Clin Invest* 124:2299-2306, 2014).

The rodent model of UUO generates progressive renal fibrosis, a hallmark of progressive renal disease of virtually any etiology (Chevalier et al., Kidney International 75:1145-1152, 2009). It has been reported that C3 gene expression was increased in wild-type mice following UUO, and that collagen deposition was significantly reduced in C3-/- knockout mice following UUO as compared to wild-type mice, suggesting a role of complement activation in renal fibrosis (Fearn et al., *Mol Immunol* 48:1666-1733, 2011). It has also been reported that C5 deficiency led to a significant amelioration of major components of renal fibrosis in a model of tubulointerstitial injury (Boor P. et al., *J of Am Soc of Nephrology*: 18:1508-1515, 2007). However, prior to the study described herein carried out by the present inventors, the particular complement components involved in renal fibrosis were not well defined. Therefore, the following study was carried out to evaluate MASP-2 (-/-) and MASP-2 (+/+) male mice in a unilateral ureteral obstruction (UUO) model.

Methods:

A MASP-2-/- mouse was generated as described in Example 1 and backcrossed for 10 generations with C57BL/6. Male wild-type (WT) C57BL/6 mice, and homozygous MASP-2 deficient (MASP-2-/-) mice on a C57BL/6 background were kept under standardized conditions of 12/12 day/night cycle, fed on standard food pellets and given free access to food and water. Ten-week-old mice, 6 per group, were anesthetized with 2.5% isoflurane in 1.5 L/min oxygen. The right ureters of two groups of ten-week-old male C56/BL6 mice, wild-type and MASP-2-/- were surgically ligated. The right kidney was exposed through a 1cm flank incision. The right ureter was completely obstructed at two points using a 6/0 polyglactin suture. Buprenorphine analgesia was provided perioperatively every 12 hours for up to 5 doses depending on pain scoring. Local bupivacaine anesthetic was given once during the surgery.

Mice were sacrificed 7 days after the surgery and kidney tissues were collected, fixed and embedded in paraffin blocks. Blood was collected from the mice by cardiac puncture under anesthesia, and mice were culled by exsanguination after nephrectomy. Blood was allowed to clot on ice for 2 hours and serum was separated by centrifugation and kept frozen as aliquots at -80°C.

Immunohistochemistry of Kidney Tissue

To measure the degree of kidney fibrosis as indicated by collagen deposition, 5 micron paraffin embedded kidney sections were stained with picrosirius red, a collagen-specific stain, as described in Whittaker P. et al., *Basic Res Cardiol* 89:397-410, 1994. Briefly described, kidney sections were de-paraffinized, rehydrated and collagen stained for 1 hour with picrosirius red aqueous solution (0.5 gm Sirius red, Sigma, Dorset UK) in 500 mL saturated aqueous solution of picric acid. Slides were washed twice in acidified water (0.5% glacial acetic acid in distilled water) for 5 minutes each, then dehydrated and mounted.

To measure the degree of inflammation as indicated by macrophage infiltration, kidney sections were stained with macrophage-specific antibody F4/80 as follows. Formalin fixed, paraffin embedded, 5 micron kidney sections were deparaffinized and rehydrated. Antigen retrieval was performed in citrate buffer at 95°C for 20 minutes followed by quenching of endogenous peroxidase activity by incubation in 3% H_2O_2 for 10 minutes. Tissue sections were incubated in blocking buffer (10% heat inactivated normal goat serum with 1% bovine serum albumin in phosphate buffered saline (PBS))

for 1 hour at room temperature followed by avidin/biotin blocking. Tissue sections were washed in PBS three times for 5 minutes after each step. F4/80 macrophage primary antibody (Santa Cruz, Dallas, TX, USA) diluted 1:100 in blocking buffer was applied for 1 hour. A biotinylated goat anti-rat secondary antibody, diluted 1:200, was then applied for 30 minutes followed by horse radish peroxidase (HRP) conjugated enzyme for 30 minutes. Staining color was developed using diaminobenzidine (DAB) substrate (Vector Labs, Peterborough UK) for 10 minutes and slides were washed in water, dehydrated and mounted without counter staining to facilitate the computer based analysis.

Image Analysis

The percentage of kidney cortical staining was determined as described in Furness P. N. et al., *J Clin Pathol* 50:118-122, 1997. Briefly described, 24 bit color images were captured from sequential non-overlapping fields of renal cortex just beneath the renal capsule around the entire periphery of the section of kidney. After each image capture NIH Image was used to extract the red channel as an 8 bit monochrome image. Unevenness in the background illumination was subtracted using a pre-recorded image of the illuminated microscope field with no section in place. The image was subjected to a fixed threshold to identify areas of the image corresponding to the staining positivity. The percentage of black pixels was then calculated, and after all the images around the kidney had been measured in this way the average percentage was recorded, providing a value corresponding to the percentage of stained area in the kidney section.

Gene Expression Analysis

Expression of several genes relevant to renal inflammation and fibrosis in mouse kidney were measured by quantitative PCT (qPCR) as follows. Total RNA was isolated from kidney cortex using Trizol® (ThermoFisher Scientific, Paisley, UK) according to the manufacturer's instructions. Extracted RNA was treated with the Turbo DNA-free kit (ThermoFisher Scientific) to eliminate DNA contamination, and then first strand cDNA was synthesized using AMV Reverse Transcription System (Promega, Madison, WI, USA). The cDNA integrity was confirmed by a single qPCR reaction using TaqMan GAPDH Assay (Applied Biosystems, Paisley UK) followed by qPCR reaction using Custom TaqMan Array 96-well Plates (Life Technologies, Paisley, UK).

Twelve genes were studied in this analysis:

Collagen type IV alpha 1 (col4α1; assay ID: Mm01210125_m1)

Transforming growth factor beta-1 (TGFβ-1; assay ID: Mm01178820_m1);
Cadherin 1 (Cdh1; Assay ID: Mm01247357_m1);
Fibronectin 1 (Fn1; Assay ID:Mm01256744_m1);
Interleukin 6 (IL6; Assay ID Mm00446191_m1);
Interleukin 10 (IL10; Assay ID Mm00439614_m1);
Interleukin 12a (IL12a; Assay ID Mm00434165_m1);
Vimentin (Vim; Assay ID Mm01333430_m1);
Actinin alpha 1 (Actn1; Assay ID Mm01304398_m1);
Tumor necrosis factor-α (TNF-α; Assay ID Mm00443260_g1)
Complement component 3 (C3; Assay ID Mm00437838_m1);
Interferon gamma (Ifn-γ; Assay ID Mm01168134)

The following housekeeping control genes were used:
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Assay ID Mm99999915_g1);
Glucuronidase beta (Gusβ; Assay ID Mm00446953_m1);
Eukaryotic 18S rRNA (18S; Assay ID Hs99999901_s1);
Hypoxanthine guanine phosphoribosyl transferase (HPRT; Assay ID Mm00446968_m1)

Twenty μL reactions were amplified using TaqMan Fast Universal Master Mix (Applied Biosystems) for 40 cycles. Real time PCR amplification data were analyzed using Applied Biosystems 7000 SDS v1.4 software.

RESULTS:

Following unilateral ureteric obstruction (UUO), obstructed kidneys experience an influx of inflammatory cells, particularly macrophages, followed by the prompt development of fibrosis as evidenced by the accumulation of collagen alongside tubular dilatation and attenuation of the proximal tubular epithelium (see Chevalier R. L. et al., *Kidney Int* 75:1145-1152, 2009).

FIGURE 15 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with Sirius red, wherein the tissue sections were obtained from wild-type and MASP-2-/- mice following 7 days of ureteric obstruction (UUO) or from sham-operated control mice. As shown in FIGURE 15, kidney sections of wild-type mice following 7 days of ureteric obstruction showed significantly greater collagen

deposition compared to MASP-2-/- mice (p value = 0.0096). The mean values ± standard error of means for UUO operated mice in wild-type and MASP-2-/- groups were 24.79±1.908 (n=6) and 16.58±1.3 (n=6), respectively. As further shown in FIGURE 15, the tissue sections from the sham-operated control wild-type and the sham operated control MASP-2-/- mice showed very low levels of collagen staining, as expected.

FIGURE 16 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with the F4/80 macrophage-specific antibody, wherein the tissue sections were obtained from wild-type and MASP-2-/- mice following 7 days of ureteric obstruction or from sham-operated control mice. As shown in FIGURE 16, compared to wild-type mice, the tissue obtained from UUO kidneys from MASP-2-/- mice exhibited significantly less macrophage infiltration following 7 days of ureteric obstruction (% macrophage area stained in WT:2.23±0.4 vs MASP-2-/-: 0.53±0.06, p=0.0035). As further shown in FIGURE 16, the tissue sections from the sham-operated wild-type and the sham-operated MASP-2-/- mice showed no detectable macrophage staining.

Gene expression analysis of a variety of genes linked to renal inflammation and fibrosis was carried out in the kidney tissue sections obtained from wild-type and MASP-2-/- mice following 7 days of ureteric obstruction and sham-operated wild-type and MASP-2-/- mice. The data shown in FIGURES 17-20 are the Log10 of relative quantitation to a wild-type sham operated sample and bars represent the standard error of means. With regard to the results of the gene expression analysis of the fibrosis-related genes, FIGURE 17 graphically illustrates the relative mRNA expression levels of collagen type IV alpha 1 (collagen-4), as measured by qPCR in kidney tissue sections obtained from wild-type and MASP-2-/- mice following 7 days of ureteric obstruction and sham-operated control mice. FIGURE 18 graphically illustrates the relative mRNA expression levels of Transforming Growth Factor Beta-1 (TGFβ-1), as measured by qPCR in kidney tissue sections obtained from wild-type and MASP-2-/- mice following 7 days of ureteric obstruction and sham-operated control mice. As shown in FIGURES 17 and 18, the obstructed kidneys from the wild-type mice demonstrated significantly increased expression of the fibrosis-related genes Collagen-type IV (FIGURE 17) and TGFβ-1 (FIGURE 18), as compared to the sham-operated kidneys in wild-type mice, demonstrating that these fibrosis-related genes are induced after UUO injury in wild-type mice, as expected. In contrast, as further shown in FIGURES 17 and 18, the obstructed

kidneys from the MASP-2-/- subjected to the UUO injury exhibited a significant reduction in the expression of Collagen-type IV (FIGURE 17, p=0.0388) and a significant reduction in the expression of TGFβ-1 (FIGURE 18, p=0.0174), as compared to the wild-type mice subjected to the UUO injury.

With regard to the results of the gene expression analysis of the inflammation-related genes, FIGURE 19 graphically illustrates the relative mRNA expression levels of Interleukin-6 (IL-6), as measured by qPCR in kidney tissue sections obtained from wild-type and MASP-2-/- mice following 7 days of ureteric obstruction and sham-operated control mice. FIGURE 20 graphically illustrates the relative mRNA expression levels of Interferon-γ, as measured by qPCR in kidney tissue sections obtained from wild-type and MASP-2-/- mice following 7 days of ureteric obstruction and sham-operated control mice. As shown in FIGURES 19 and 20, the obstructed kidneys from the wild-type mice demonstrated significantly increased expression of the inflammation-related genes Interleukin-6 (FIGURE 19) and Interferon-γ (FIGURE 20), as compared to the sham-operated kidneys in wild-type mice, demonstrating that these inflammation-related genes are induced after UUO injury in wild-type mice. In contrast, as further shown in FIGURES 19 and 20, the obstructed kidneys from the MASP-2-/- subjected to the UUO injury exhibited a significant reduction in the expression of Interleukin-6 (FIGURE 19, p=0.0109) and Interferon-γ (FIGURE 20, p=0.0182) as compared to the wild-type mice subjected to the UUO injury.

It is noted that gene expression for Vim, Actn-1, TNFα, C3 and IL-10 were all found to be significantly up-regulated in the UUO kidneys obtained from both the wild-type and the MASP-2-/- mice, with no significant difference in the expression levels of these particular genes between the wild-type and MASP-2-/- mice (data not shown). The gene expression levels of Cdh-1 and IL-12a did not change in obstructed kidneys from animals in any group (data not shown).

Discussion:

The UUO model in rodents is recognized to induce an early, active and profound injury in the obstructed kidney with reduced renal blood flow, interstitial inflammation and rapid fibrosis within one to two weeks following obstruction and has been used extensively to understand common mechanisms and mediators of inflammation and fibrosis in the kidney (see e.g., Chevalier R.L., *Kidney Int* 75:1145-1152, 2009; Yang H. et al., *Drug Discov Today Dis Models* 7:13-19, 2010).

The results described in this Example demonstrate that there is a significant reduction in collagen deposition and macrophage infiltration in UUO operated kidneys in the MASP-2(-/-) mice versus the wild-type (+/+) control mice. The unexpected results showing a significant reduction of renal injury at both the histological and gene expression levels in the MASP-2-/- animals demonstrates that the lectin pathway of complement activation contributes significantly to the development of inflammation and fibrosis in the obstructed kidney. While not wishing to be bound by a particular theory, it is believed that the lectin pathway contributes critically to the pathophysiology of fibrotic disease by triggering and maintaining pro-inflammatory stimuli that perpetuate a vicious cycle where cellular injury drives inflammation which in turn causes further cellular injury, scarring and tissue loss. In view of these results, it is expected that that inhibition or blockade of MASP-2 with an inhibitor would have a preventive and/or therapeutic effect in the inhibition or prevention of renal fibrosis, and for the inhibition or prevention of fibrosis in general (i.e., independent of the tissue or organ).

EXAMPLE 15

This Example describes analysis of a monoclonal MASP-2 inhibitory antibody for efficacy in the Unilateral Ureteric Obstruction (UUO) model, a murine model of renal fibrosis.

Background/Rationale:

Amelioration of renal tubulointerstitial fibrosis, the common end point of multiple renal pathologies, represents a key target for therapeutic strategies aimed at preventing progressive renal diseases. Given the paucity of new and existing treatments targeting inflammatory pro-fibrotic pathways in renal disease, there is a pressing need to develop new therapies. Many patients with proteinuric renal disease exhibit tubulointerstitial inflammation and progressive fibrosis which closely parallels declining renal function. Proteinuria *per se* induces tubulointerstitial inflammation and the development of proteinuric nephropathy (Brunskill N.J. et al., *J Am Soc Nephrol* 15:504-505, 2004). Regardless of the primary renal disease, tubulointerstitial inflammation and fibrosis is invariably seen in patients with progressive renal impairment and is closely correlated with declining excretory function (Risdon R.A. et al., *Lancet* 1:363-366, 1968; Schainuck L.I., et al., *Hum Pathol* 1: 631-640, 1970). Therapies with the potential to interrupt the

key common cellular pathways leading to fibrosis hold the promise of wide applicability in renal disorders.

As described in Example 14, in the UUO model of non-proteinuric renal fibrosis it was determined that MASP-2-/- mice exhibited significantly less renal fibrosis and inflammation compared to wild-type control animals, as shown by inflammatory cell infiltrates (75% reduction), and histological markers of fibrosis such as collagen (one third reduction), thereby establishing a key role of the lectin pathway in renal fibrosis.

As described in Example 13, a monoclonal MASP-2 antibody (OMS646-SGMI-2 fusion, comprising an SGMI-2 peptide fused to the C-terminus of the heavy chain of OMS646) was generated that specifically blocks the function of the human lectin pathway has also been shown to block the lectin pathway in mice. In this example, OMS646-SGMI-2 was analyzed in the UUO mouse model of renal fibrosis in wild-type mice to determine if a specific inhibitor of MASP-2 is able to inhibit renal fibrosis.

Methods:

This study evaluated the effect of a MASP-2 inhibitory antibody (10 mg/kg OMS646-SGMI-2), compared to a human IgG4 isotype control antibody (10 mg/kg ET904), and a vehicle control in male WT C57BL/6 mice. The antibodies (10mg/kg) were administered to groups of 9 mice by intraperitoneal (ip) injection on day 7, day 4 and day 1 prior to UUO surgery and again on day 2 post-surgery. Blood samples were taken prior to antibody administration and at the end of the experiment to assess lectin pathway functional activity.

The UUO surgery, tissue collection and staining with Sirius red and macrophage-specific antibody F4/80 were carried out using the methods described in Example 14.

Hydroxyproline content of mouse kidneys was measured using a specific colorimetric assay test kit (Sigma) according to manufacturer's instructions.

To assess the pharmacodynamic effect of the MASP-2 inhibitory mAb in mice, systemic lectin pathway activity was evaluated by quantitating lectin-induced C3 activation in minimally diluted serum samples collected at the indicated time after MASP-2 mAb or control mAb i.p. administration to mice. Briefly described, 7 μM diameter polystyrene microspheres (Bangs Laboratories, Fisher IN, USA) were coated with mannan by overnight incubation with 30μg/mL mannan (Sigma) in sodium bicarbonate buffer (pH 9.6), then washed, blocked with 1% fetal bovine serum in PBS and resuspended in PBS at a final concentration of $1x10^8$ beads/mL. Complement

deposition reactions were initiated by the addition of 2.5 µL of mannan-coated beads (~250,000 beads) to 50 µL of minimally diluted mouse serum samples (90% final serum concentration), followed by incubation for 40 minutes at 4°C. Following termination of the deposition reaction by the addition of 250 µL of ice-cold flow cytometry buffer (FB: PBS containing 0.1% fetal bovine serum), beads were collected by centrifugation and washed two more times with 300 µL of ice-cold FB.

To quantify lectin-induced C3 activation, beads were incubated for 1 hour at 4°C with 50 µL of rabbit anti-human C3c antibody (Dako, Carpenteria, CA, USA) diluted in FB. Following two washes with FB to remove unbound material, the beads were incubated for 30 minutes at 4°C with 50 µL of goat anti-rabbit antibody conjugated to PE-Cy5 (Southern Biotech, Birmingham, AL, USA) diluted in FB. Following two washes with FB to remove unbound material, the beads were resuspended in FB and analyzed by a FACS Calibur cytometer. The beads were gated as a uniform population using forward and side scatter, and C3b deposition in each sample was quantitated as mean fluorescent intensity (MFI).

Results:

Assessment of Collagen Deposition:

FIGURE 21 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with Siruis red, wherein the tissue sections were obtained following 7 days of ureteric obstruction from wild-type mice treated with either a MASP-2 inhibitory antibody or an isotype control antibody. As shown in FIGURE 21, tissue sections from kidneys harvested 7 days after obstruction (UUO) obtained from wild-type mice treated with MASP-2 inhibitory antibody showed a significant reduction ($p=0.0477$) in collagen deposition as compared with the amount of collagen deposition in tissue sections from obstructed kidneys obtained from wild-type mice treated with an IgG4 isotype control.

Assessment of Hydroxy proline content:

Hydroxy proline was measured in kidney tissues as an indicator of collagen content. Hydroxy proline is a parameter which is highly indicative of the pathophysiological progression of disease induced in this model.

FIGURE 22 graphically illustrates the hydroxyl proline content from kidneys harvested 7 days after obstruction (UUO) obtained from wild-type mice treated with either a MASP-2 inhibitory antibody or an isotype control antibody. As shown in

FIGURE 22, the obstructed kidney tissues from mice treated with MASP-2 inhibitory antibody demonstrated significantly less hydroxyl proline, an indicator of collagen content, than the kidneys from mice treated with the IgG4 isotype control mAb ($p=0.0439$).

Assessment of inflammation:

Obstructed kidneys from wild-type, isotype control antibody-treated animals, and wild-type animals treated with MASP-2 inhibitory antibody demonstrated a brisk infiltrate of macrophages. Careful quantification revealed no significant difference in macrophage percentage stained area between these two groups (data not shown). However, despite equivalent numbers of infiltrating macrophages, the obstructed kidneys from the MASP-2 inhibitory antibody-injected animals exhibited significantly less fibrosis as judged by Sirius red staining, compared to obstructed kidneys from isotype control injected animals, which result is consistent with the results that obstructed kidney tissues from mice treated with MASP-2 inhibitory antibody had significantly less hydroxyl proline than the kidneys treated with the IgG4 isotype control mAb.

Discussion

The results described in this Example demonstrate that the use of a MASP-2 inhibitory antibody provides protection against renal fibrosis in the UUO model, which is consistent with the results described in Example 14 demonstrating that MASP-2-/- mice have significantly reduced renal fibrosis and inflammation in the UUO model as compared to wild-type mice. The results in this Example showing reduced fibrosis in the mice treated with the MASP-2 inhibitory antibody. The finding of reduced fibrosis in the UUO kidneys in animals with a reduction or blockade of MASP-2-dependent lectin pathway activity is highly significant novel finding. Taken together, the results presented in Example 14 and in this Example demonstrate a beneficial effect of MASP-2 inhibition on renal tubulointerstitial inflammation, tubular cell injury, profibrotic cytokine release and scarring. The relief of renal fibrosis remains a key goal for renal therapeutics. The UUO model is a severe model of accelerated renal fibrosis, and an intervention that reduces fibrosis in this model, such as the use of MASP-2 inhibitory antibodies, is likely to be used to inhibit or prevent renal fibrosis. The results from the UUO model are likely to be transferable to renal disease characterized by glomerular and/or proteinuric tubular injury.

EXAMPLE 16

This Example provides results that were generated using a protein overload proteinurea model of renal fibrosis, inflammation and tubulointerstitial injury in MASP-2-/- and wild-type mice to evaluate the role of the lectin pathway in proteinuric nephropathy.

Background/Rationale:

Proteinuria is a risk factor for the development of renal fibrosis and loss of renal excretory function, regardless of the primary renal disease (Tryggvason K. et al., *J Intern Med* 254:216-224, 2003, Williams M., *Am J. Nephrol* 25:77-94, 2005). The concept of proteinuric nephropathy describes the toxic effects of excess protein entering the proximal tubule as a result of the impaired glomerular permselectivity (Brunskill N.J., *J Am Soc Nephrol* 15:504-505, 2004, Baines R.J., *Nature Rev Nephrol* 7:177-180, 2011). This phenomenon, common to many glomerular diseases, results in a pro-inflammatory scarring environment in the kidney and is characterized by alterations in proximal tubular cell growth, apoptosis, gene transcription and inflammatory cytokine production as a consequence of dysregulated signaling pathways stimulated by proteinuric tubular fluid. Proteinuric nephropathy is generally recognized to be a key contributor to progressive renal injury common to diverse primary renal pathologies.

Chronic kidney disease affects greater than 15% of the adult population in the United States and accounts for approximately 750,000 deaths each year worldwide (Lozano R. et al., *Lancet* vol 380, Issue 9859:2095-2128, 2012). Proteinuria is an indicator of chronic kidney disease as well as a factor promoting disease progression. Many patients with proteinuric renal disease exhibit tubulointerstitial inflammation and progressive fibrosis which closely parallels declining renal function. Proteinuria *per se* induces tubulointerstitial inflammation and the development of proteinuric nephropathy (Brunskill N.J. et al., *J Am Soc Nephrol* 15:504-505, 2004). In proteinuric kidney diseases, excessive amounts of albumin and other macromolecules are filtered through the glomeruli and reabsorbed by proximal tubular epithelial cells. This causes an inflammatory vicious cycle mediated by complement activation leading to cytokine and leukocyte infiltrates that cause tubule-interstitial injury and fibrosis, thereby exacerbating proteinuria and leading to loss of renal function and eventually progression to end-stage renal failure (see, e.g., Clark et al., *Canadian Medical Association Journal* 178:173-175, 2008). Therapies that modulate this detrimental cycle of inflammation and proteinuria are expected to improve outcomes in chronic kidney disease.

In view of the beneficial effects of MASP-2 inhibition in the UUO model of tubulointerstital injury, the following experiment was carried out to determine if MASP-2 inhibition would reduce renal injury in a protein overload model. This study employed protein overload to induce proteinuric kidney disease as described in Ishola et al., *European Renal Association* 21:591-597, 2006.

Methods:

A MASP-2-/- mouse was generated as described in Example 1 and backcrossed for 10 generations with BALB/c. The current study compared the results of wild-type and MASP-2-/- BALB/c mice in a protein overload proteinuria model as follows.

One week prior to the experiment, mice were unilaterally nephrectomised before protein overload challenge in order to see an optimal response. The proteinuria inducing agent used was a low endotoxin bovine serum albumin (BSA, Sigma) given i.p. in normal saline to WT (n=7) and MASP-2 -/- mice (n=7) at the following doses: one dose each of 2mg BSA/gm, 4mg BSA/gm, 6mg BSA/gm, 8mg BSA/gm, 10mg BSA/gm and 12mg BSA/gm body weight, and 9 doses of 15mg BSA/gm body weight, for a total of 15 doses administered i.p. over a period of 15 days. The control WT (n=4) and MASP-2-/- (n=4) mice received saline only administered i.p. After administration of the last dose, animals were caged separately in metabolic cages for 24 hours to collect urine. Blood was collected by cardiac puncture under anesthesia, blood was allowed to clot on ice for 2 hours and serum was separated by centrifugation. Serum and urine samples were collected at the end of the experiment on day 15, stored and frozen for analysis.

Mice were sacrificed 24 hours after the last BSA administration on day 15 and various tissues were collected for analysis. Kidneys were harvested and processed for H&E and immunostaining. Immunohistochemistry staining was carried out as follows. Formalin fixed, paraffin-embedded 5 micron kidney tissue sections from each mouse were deparaffinized and rehydrated. Antigen retrieval was performed in citrate buffer at 95°C for 20 minutes followed by incubating tissues in 3% H_2O_2 for 10 minutes. Tissues were then incubated in blocking buffer (10% serum from the species the secondary antibody was raised in and 1% BSA in PBS) with 10% avidin solution for 1 hour at room temperature. Sections were washed in PBS three times, 5 minutes each, after each step. Primary antibody was then applied in blocking buffer with 10% biotin solution for 1 hour at a concentration of 1:100 for the antibodies F4/80 (Santa Cruz cat# sc-25830), TGFβ (Santa Cruz cat# sc-7892), IL-6 (Santa Cruz cat# sc-1265) and at 1:50 for the TNFα

antibody (Santa Cruz cat# sc-1348). A biotinylated secondary antibody was then applied for 30 minutes at a concentration of 1:200 for the F4/80, TGFβ and IL-6 sections and 1:100 for the TNFα section followed by HRP conjugate enzyme for another 30 minutes. The color was developed using diaminobenzidine (DAB) substrate kit (Vector labs) for 10 minutes and slides were washed in water, dehydrated and mounted without counter staining to facilitate computer-based image analysis. Stained tissue sections from the renal cortex were analyzed by digital image capture followed by quantification using automated image analysis software.

Apoptosis was assessed in the tissue sections by staining with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) as follows. Apoptotic cells in the kidney sections were stained using ApopTag® Peroxidase kit (Millipore) as follows. Parrafin embedded, formalin fixed kidney sections from each mouse were deparaffinized, rehydrated and then protein permeabilized using proteinase K (20 µg/mL) which was applied to each specimen for 15 minutes at room temperature. Specimens were washed in PBS between steps. Endogenous peroxidase activity was quenched by incubating tissues in 3% H_2O_2 for 10 minutes. Tissues were then incubated in equilibration buffer followed by incubation with TdT enzyme for 1 hour at 37°C. After washing in stop/wash buffer for 10 minutes, anti-digoxignenin conjugate was applied for 30 minutes at room temperature followed by washing. Color was developed in DAB substrate kit for 4 minutes followed by washing in water. Tissues were counter stained in haematoxylin and mounted in DBX. The frequency of TUNEL stained (brown colored) apoptotic cells were manually counted in serially selected 20 high power fields from the cortex using Leica DBXM light microscope.

Results:

Assessment of Proteinuria

To confirm the presence of proteinuria in the mice, the total protein in serum was analyzed at day 15 and the total excreted proteins in urine was measured in urine samples collected over a 24 hour period on day 15 of the study.

FIGURE 23 graphically illustrates the total amount of serum proteins (mg/ml) measured at day 15 in the wild-type control mice (n=2) that received saline only, the wild-type mice that received BSA (n=6) and the MASP-2-/- mice that received BSA (n=6). As shown in FIGURE 23, administration of BSA increased the serum total protein level in both wild-type and MASP-2-/- groups to more than double the concentration of

the control group that received only saline, with no significant difference between the treated groups.

FIGURE 24 graphically illustrates the total amount of excreted protein (mg) in urine collected over a 24 hour period on day 15 of the study from the wild-type control mice (n=2) that received saline only, the wild-type mice that received BSA (n=6) and the MASP-2-/- mice that received BSA (n=6). As shown in FIGURE 24, on day 15 of this study, there was an approximately six-fold increase in total excreted proteins in urine in the BSA treated groups as compared to the sham-treated control group that received saline only. The results shown in FIGURES 23 and 24 demonstrate that the proteinuria model was working as expected.

Assessment of Histological Changes in the Kidney

FIGURE 25 shows representative H&E stained renal tissue sections that were harvested on day 15 of the protein overload study from the following groups of mice: (panel A) wild-type control mice; (panel B) MASP-2-/- control mice; (panel C) wild-type mice treated with BSA; and (panel D) MASP-2-/- mice treated with BSA. As shown in FIGURE 25, there is a much higher degree of tissue preservation in the MASP-2-/- overload group (panel D) compared to the wild-type overload group (panel C) at the same level of protein overload challenge. For example, Bowman's capsules in the wild-type mice treated with BSA (overload) were observed to be greatly expanded (panel C) as compared to Bowman's capsules in the wild-type control group (panel A). In contrast, Bowman's capsules in the MASP-2-/- mice (overload) treated with the same level of BSA (panel D) retained morphology similar to the MASP-2-/- control mice (panel B) and wild-type control mice (panel A). As further shown in FIGURE 25, large protein cast structures have accumulated in proximal and distal tubules of the wild-type kidney sections (panel C), which are larger and more abundant as compared to MASP-2-/- mice (panel D).

It is also noted that analysis of renal sections from this study by transmitting electron microscope showed that the mice treated with BSA had overall damage to the ciliary borders of distal and proximal tubular cells, with cellular content and nuclei bursting into the tubule lumen. In contrast, the tissue was preserved in the MASP-2-/- mice treated with BSA.

Assessment of Macrophage Infiltration in the Kidney

To measure the degree of inflammation, as indicated by macrophage infiltration, the tissue sections of the harvested kidneys were also stained with macrophage-specific antibody F4/80 using methods as described in Boor et al., *J of Am Soc of Nephrology* 18:1508-1515, 2007.

FIGURE 26 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with macrophage-specific antibody F4/80, showing the macrophage mean stained area (%), wherein the tissue sections were obtained at day 15 of the protein overload study from wild-type control mice (n=2), wild-type mice treated with BSA (n=6), and MASP-2-/- mice treated with BSA (n=5). As shown in FIGURE 26, kidney tissue sections stained with F4/80 anti-macrophage antibody showed that while both groups treated with BSA showed a significant increase in the kidney macrophage infiltration (measured as %F4/80 antibody-stained area) compared to the wild-type sham control, a significant reduction in macrophage infiltration was observed in tissue sections from BSA-treated MASP-2-/- mice as compared with macrophage infiltration in tissue sections from BSA-treated wild-type mice (p value=0.0345).

FIGURE 27A graphically illustrates the analysis for the presence of a macrophage-proteinuria correlation in each wild-type mouse (n=6) treated with BSA by plotting the total excreted proteins measured in urine from a 24 hour sample versus the macrophage infiltration (mean stained area %). As shown in FIGURE 27A, most of the samples from the wild-type kidneys showed a positive correlation between the level of proteinuria present and the degree of macrophage infiltration.

FIGURE 27B graphically illustrates the analysis for the presence of a macrophage-proteinuria correlation in each MASP-2-/- mouse (n=5) treated with BSA by plotting the total excreted proteins in urine in a 24 hour sample versus the macrophage infiltration (mean stained area %). As shown in FIGURE 27B, the positive correlation observed in wild-type mice between the level of proteinuria and the degree of macrophage infiltration (shown in FIGURE 27A) was not observed in MASP-2-/- mice. While not wishing to be bound by any particular theory, these results may indicate the presence of a mechanism of inflammation clearance at high levels of proteinuria in MASP-2-/- mice.

Assessment of Cytokine Infiltration

Interleukin 6 (IL-6), Transforming Growth Factor Beta (TGFβ) and Tumor Necrosis Factor Alpha (TNFα) are pro-inflammatory cytokines known to be up-regulated

in proximal tubules of wild-type mice in a model of proteinuria (Abbate M. et al., *Journal of the American Society of Nephrology*: JASN, 17: 2974-2984, 2006; David S. et al., Nephrology, Didalysis, Transplantation, Official Publication of the European Dialysis and Transplant Association- *European Renal Association* 12: 51-56, 1997). The tissue sections of kidneys were stained with cytokine-specific antibodies as described above.

FIGURE 28 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-TGFβ antibody (measured as % TGFβ antibody-stained area) in wild-type mice treated with BSA (n=4) and MASP-2-/- mice treated with BSA (n=5). As shown in FIGURE 28, a significant increase in the staining of TGFβ was observed in the wild-type BSA treated (overload) group as compared to the MASP-2-/- BSA treated (overload) group (p=0.026).

FIGURE 29 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-TNFα antibody (measured as % TNFα antibody-stained area) in wild-type mice treated with BSA (n=4) and MASP-2-/- mice treated with BSA (n=5). As shown in FIGURE 29, a significant increase in the staining of TNFα was observed in the wild-type BSA treated (overload) group as compared to the MASP-2-/- BSA treated (overload) group (p=0.0303).

FIGURE 30 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-IL-6 antibody (measured as % IL-6 antibody-stained area) in wild-type control mice, MASP-2-/- control mice, wild-type mice treated with BSA (n=7) and MASP-2-/- mice treated with BSA (n=7). As shown in FIGURE 30, a highly significant increase in the staining of IL-6 was observed in the wild-type BSA treated group as compared to the MASP-2-/- BSA treated group (p=0.0016).

Assessment of Apoptosis

Apoptosis was assessed in the tissue sections by staining with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and the frequency of TUNEL stained apoptotic cells were counted in serially selected 20 high power fields (HPFs) from the cortex.

FIGURE 31 graphically illustrates the frequency of TUNEL apoptotic cells counted in serially selected 20 high power fields (HPFs) from tissue sections from the renal cortex in wild-type control mice (n=1), MASP-2-/- control mice (n=1), wild-type mice treated with BSA (n=6) and MASP-2-/- mice treated with BSA (n=7). As shown in FIGURE 31, a significantly higher rate of apoptosis in the cortex was observed in kidneys

obtained from wild-type mice treated with BSA as compared to kidneys obtained from the MASP-2-/- mice treated with BSA (p=0.0001).

Overall Summary of Results and Conclusions:

The results in this Example demonstrate that MASP-2-/- mice have reduced renal injury in a protein overload model. Therefore, MASP-2 inhibitory agents, such as MASP-2 inhibitory antibodies would be expected to inhibit or prevent the detrimental cycle of inflammation and proteinuria and improve outcomes in chronic kidney disease.

EXAMPLE 17

This Example describes analysis of a monoclonal MASP-2 inhibitory antibody for efficacy in reducing and/or preventing renal inflammation and tubulointerstitial injury in a mouse protein overload proteinurea model in wild-type mice.

Background/Rationale:

As described in Example 16, in a protein overload model of proteinuria it was determined that MASP-2-/- mice exhibited significantly better outcomes (e.g., less tubulointerstitial injury and less renal inflammation) than wild-type mice, implicating a pathogenic role for the lectin pathway in proteinuric kidney disease.

As described in Example 13, a monoclonal MASP-2 inhibitory antibody (OMS646-SGMI-2) was generated that specifically blocks the function of the human lectin pathway and has also been shown to block the lectin pathway in mice. In this example, the MASP-2 inhibitory antibody OMS646-SGMI-2 was analyzed in a mouse protein overload proteinurea model for efficacy in reducing and/or preventing renal inflammation and tubulointerstitial injury in wild-type mice.

Methods:

This study evaluated the effect of MASP-2 inhibitory antibody (10 mg/kg OMS646-SGMI-2), compared to a human IgG4 isotype control antibody, ET904 (10 mg/kg), and a saline control.

Similar to the study described in Example 16, this study employed protein overload to induce proteinuric kidney disease (Ishola et al., *European Renal Association* 21:591-597, 2006). Proteinuria was induced in unilaterally nephrectomized Balb/c mice by daily i.p. injections with escalating doses (2 g/kg to 15 g/kg) of low endotoxin bovine serum albumin (BSA) for a total of 15 days, as described in Example 16.

Antibody treatments were administered by biweekly i.p. injection starting 7 days before proteinuria induction and continued throughout the study. This dosing scheme was selected based on previous PK/PD and pharmacoclogy studies demonstrating sustained lectin pathway suppression (data not shown). Mice were sacrificed on day 15 and kidneys were harvested and processed for H&E and immunostaining. Stained tissue sections from the renal cortex were analyzed by digital image capture followed by quantification using automated image analysis software.

Immunohistochemistry staining and apoptosis assessment were carried out as described in Example 16.

Results:

Assessment of Proteinuria

To confirm the presence of proteinuria in the mice, the total excreted proteins in urine was measured in urine samples collected over a 24 hour period at day 15 (the end of the experiment). It was determined that the urine samples showed a mean of almost a six-fold increase in total protein levels in the groups that were treated with BSA as compared to the control groups not treated with BSA (data not shown), confirming the presence of proteinuria in the mice treated with BSA. No significant difference was observed in the protein levels between the BSA-treated groups.

Assessment of Histological Changes

FIGURE 32 shows representative H&E stained tissue sections from the following groups of mice at day 15 after treatment with BSA: (panel A) wild-type control mice treated with saline; (panel B) isotype antibody treated control mice; and (panel C) wild-type mice treated with MASP-2 inhibitory antibody.

As shown in FIGURE 32, there is a much higher degree of tissue preservation in the MASP-2 inhibitory antibody-treated group (panel C) as compared to the wild-type group treated with saline (panel A) or isotype control (panel B) at the same level of protein overload challenge.

Assessment of Apoptosis

Apoptosis was assessed in the tissue sections by staining with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and the frequency of TUNEL stained apoptotic cells were counted in serially selected 20 high power fields (HPFs) from the cortex. FIGURE 33 graphically illustrates the frequency of TUNEL apoptotic cells counted in serially selected 20 high power fields (HPFs) from tissue

sections from the renal cortex in wild-type mice treated with saline control and BSA (n=8), wild-type mice treated with the isotype control antibody and BSA (n=8) and wild-type mice treated with the MASP-2 inhibitory antibody and BSA (n=7). As shown in FIGURE 33, a highly significantly decrease in the rate of apoptosis in the cortex was observed in kidneys obtained from the MASP-2 inhibitory antibody treated group as compared to the saline and isotype control treated group (p=0.0002 for saline control v MASP-2 inhibitory antibody; p=0.0052 for isotype control v. MASP-2 inhibitory antibody).

Assessment of Cytokine Infiltration

Interleukin 6 (IL-6), Transforming Growth Factor Beta (TGFβ) and Tumor Necrosis Factor Alpha (TNFα), which are pro-inflammatory cytokines known to be up-regulated in proximal tubules of wild-type mice in a model of proteinuria, were assessed in the kidney tissue sections obtained in this study.

FIGURE 34 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-TGFβ antibody (measured as % TGFβ antibody-stained area) in wild-type mice treated with BSA and saline (n=8), wild-type mice treated with BSA and isotype control antibody (n=7) and wild-type mice treated with BSA and MASP-2 inhibitory antibody (n=8). As shown in FIGURE 34, quantification of the TGFβ stained areas showed a significant reduction in the levels of TGFβ in the MASP-2 inhibitory antibody-treated mice as compared to the saline and isotype control antibody-treated control groups (p values= 0.0324 and 0.0349, respectively).

FIGURE 35 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-TNFα antibody (measured as % TNFα antibody-stained area) in wild-type mice treated with BSA and saline (n=8), BSA and isotype control antibody (n=7) and wild-type mice treated with BSA and MASP-2 inhibitory antibody (n=8). As shown in FIGURE 35, analysis of stained sections showed a significant reduction in the level of TNFα in the MASP-2 inhibitory antibody-treated group as compared to the saline control group (p=0.011) as well as the isotype control group (p=0.0285).

FIGURE 36 graphically illustrates the results of computer-based image analysis of stained tissue sections with anti-IL-6 antibody (measured as % IL-6 antibody-stained area) in in wild-type mice treated with BSA and saline (n=8), BSA and isotype control antibody (n=7) and wild-type mice treated with BSA and MASP-2 inhibitory antibody

(n=8). As shown in FIGURE 36, analysis of stained sections showed a significant reduction in the level of IL-6 in the MASP-2 inhibitory antibody-treated group as compared to the saline control group (p=0.0269) as well as to the isotype control group (p=0.0445).

Overall Summary of Results and Conclusions:

The results in this Example demonstrate that the use of a MASP-2 inhibitory antibody provides protection against renal injury in a protein overload model, which is consistent with the results described in Example 16 demonstrating that MASP-2-/- mice have reduced renal injury in the proteinuria model.

EXAMPLE 18

This Example provides results generated using an Adriamycin-induced nephrology model of renal fibrosis, inflammation and tubulointerstitial injury in MASP-2-/- and wild-type mice to evaluate the role of the lectin pathway in Adriamycin-induced nephropathy.

Background/Rationale:

Adriamycin is an anthracycline antitumor antibiotic used in the treatment of a wide range of cancers, including hematological malignancies, soft tissue sarcomas and many types of carcinomas. Adriamycin-induced nephropathy is well established rodent model of chronic kidney disease that has enabled a better understanding of the progression of chronic proteinuria (Lee and Harris, *Nephrology*, 16:30-38, 2011). The type of structural and functional injury in Adriamycin-induced nephropathy is very similar to that of chronic proteinuric renal disease in humans (Pippin et al., *American Journal of Renal Physiology* 296:F213-29, 2009).

Adriamycin-induced nephropathy is characterized by an injury to the podocytes followed by glomerulosclerosis, tubulointerstitial inflammation and fibrosis. It has been shown in many studies that Adriamycin-induced nephropathy is modulated by both immune and non-immune derived mechanisms (Lee and Harris, *Nephrology*, 16:30-38, 2011). Adriamycin-induced nephropathy has several strengths as a model of kidney disease. First, it is a highly reproducible and predicable model of renal injury. This is because it is characterized by the induction of renal injury within a few days of drug administration, which allows for ease of experimental design as the timing of injury is consistent. It is also a model in which the degree of tissue injury is severe while

associated with acceptable mortality (<5%) and morbidity (weight loss). Therefore, due to the severity and timing of renal injury in Adriamycin-induced nephropathy, it is a model suitable for testing interventions that protect against renal injury.

As described in Examples 16 and 17, in a protein overload model of proteinuria it was determined that MASP-2-/- mice and mice treated with a MASP-2 inhibitory antibody exhibited significantly better outcomes (e.g., less tubulointerstitial injury, and less renal inflammation) than wild-type mice, implicating a pathogenic role for the lectin pathway in proteinuric kidney disease.

In this example, MASP-2-/- mice were analyzed in comparison with wild-type mice in the Adriamycin-induced nephrology model (AN) to determine if MASP-2 deficiency reduces and/or prevents renal inflammation and tubulointerstitial injury induced by Adriamycin.

Methods:

1. Dosage and Time point optimization

An initial experiment was carried out to determine the dose of Adriamycin and time point at which BALB/c mice develop a level of renal inflammation suitable for testing therapeutic intervention.

Three groups of wild-type BALB/c mice (n=8) were injected with a single dose of Adriamycin (10.5 mg/kg) administered IV. Mice were culled at three time points: one week, two weeks and four weeks after Adriamycin administration. Control mice were injected with saline only.

Results: All mice in the three groups showed signs of glomerulosclerosis and proteinuria, as determined by H&E staining, with incrementally increasing degree of tissue inflammation as measured by macrophage infiltration in the kidney (data not shown). The degree of tissue injury was mild in the one week group, moderate in the two week group and severe in the four week group (data not shown). The two week time point was selected for the rest of the study.

2. Analysis of Adriamycin-induced nephrology in wild-type and MASP-2-/- mice

In order to elucidate the role of the lectin pathway of complement in the Adriamycin-induced nephrology, a group of MASP-2-/- mice (BALB/c) were compared to wild-type mice (BALB/c) at the same dose of Adriamycin. The MASP-2-/- mice were backcrossed with BALB/c mice for 10 generations.

Wild-type (n=8) and MASP-2-/- (n=8) were injected IV with Adriamycin (10.5 mg/kg) and three mice of each strain were give saline only as a control. All mice were culled two weeks after the treatment and tissues were collected. The degree of histopatholigical injury was assessed by H&E staining.

Results:

FIGURE 37 shows representative H&E stained tissue sections from the following groups of mice at day 14 after treatment with Adriamycin or saline only (control): (panels A-1, A-2, A-3) wild-type control mice treated with only saline; (panels B-1, B-2, B-3) wild-type mice treated with Adriamycin; and (panels C-1, C-2, C-3) MASP-2-/- mice treated with Adriamycin. Each photo (e.g., panel A-1, A-2, A-3) represents a different mouse.

As shown in FIGURE 37, there is a much higher degree of tissue preservation in the MASP-2-/- group treated with Adriamycin as compared to the wild-type group treated with the same dose of Adriamycin.

FIGURE 38 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with macrophage-specific antibody F4/80 showing the macrophage mean stained area (%) from the following groups of mice at day 14 after treatment with Adriamycin or saline only (wild-type control): wild-type control mice treated with only saline; wild-type mice treated with Adriamycin; MASP-2-/- mice treated with saline only, and MASP-2 -/- mice treated with Adriamycin. As shown in FIGURE 38, MASP-2-/- mice treated with Adriamycin have reduced macrophage infiltration (**p=0.007) compared to wild-type mice treated with Adriamycin.

FIGURE 39 graphically illustrates the results of computer-based image analysis of kidney tissue sections stained with Sirius Red, showing the collagen deposition stained area (%) from the following groups of mice at day 14 after treatment with Adriamycin or saline only (wild-type control): wild-type control mice treated with only saline; wild-type mice treated with Adriamycin; MASP-2-/- mice treated with saline only, and MASP-2 -/- mice treated with Adriamycin. As shown in FIGURE 39, MASP-2-/- mice treated with Adriamycin have reduced collagen deposition (**p=0.005) compared to wild-type mice treated with Adriamycin.

Overall Summary and Conclusions:

The amelioration of renal tubulointerstitial inflammation is a key target for the treatment of kidney disease. The results presented herein indicate that the lectin pathway

of complement activation contributes significantly to the development of renal tubulointerstitial inflammation. As further demonstrated herein, a MASP-2 inhibitory agent, such as a MASP-2 inhibitory antibody, may be used as a novel therapeutic approach in the treatment of proteinuric nephropathy, Adriamycin nephropathy and amelioration of renal tubulointerstitial inflammation.

EXAMPLE 19

This Example describes the initial results of an ongoing Phase 2 clinical trial to evaluate the safety and clinical efficacy of a fully human monoclonal MASP-2 inhibitory antibody in adults with steroid-dependent immunoglobulin A nephropathy (IgAN) and in adults with steroid-dependent membranous nephropathy (MN).

Background:

Chronic kidney diseases affect more than 20 million people in the United States (Drawz P. et al., *Ann Intern Med* 162(11); ITC1-16, 2015). Glomerulonephropathies (GNs), including IgAN and MN are kidney diseases in which the glomeruli are damaged and frequently lead to end-stage renal disease and dialysis. Several types of primary GNs exist, the most common being IgAN. Many of these patients have persistent renal inflammation and progressive deterioration. Often these patients are treated with corticosteroids or immunosuppressive agents, which have many serious long-term adverse consequences. Many patients continue to deteriorate even on these treatments. No treatments are approved for the treatment of IgAN or MN.

IgA Nephropathy

Immunoglobulin A nephropathy (IgAN) is an autoimmune kidney disease resulting in intrarenal inflammation and kidney injury. IgAN is the most common primary glomerular disease globally. With an annual incidence of approximately 2.5 per 100,000, it is estimated that 1 in 1400 persons in the U.S. will develop IgAN. As many as 40% of patients with IgAN will develop end-stage renal disease (ESRD). Patients typically present with microscopic hematuria with mild to moderate proteinuria and variable levels of renal insufficiency (Wyatt R.J., et al., *N Engl J Med* 368(25):2402-14, 2013). Clinical markers such as impaired kidney function, sustained hypertension, and heavy proteinuria (over 1 g per day) are associated with poor prognosis (Goto M et al., *Nephrol Dial Transplant* 24(10):3068-74, 2009; Berthoux F. et al., *J Am Soc Nephrol* 22(4):752-61, 2011). Proteinuria is the strongest prognostic factor independent of other

risk factors in multiple large observational studies and prospective trials (Coppo R. et al., *J Nephrol* 18(5):503-12, 2005; Reich H. N., et al., *J Am Soc Nephrol* 18(12):3177-83, 2007). It is estimated that 15-20% of patients reach ESRD within 10 years of disease onset if left untreated (D'Amico G., *Am J Kidney Dis* 36(2):227-37, 2000).

The diagnostic hallmark of IgAN is the predominance of IgA deposits, alone or with IgG, IgM, or both, in the glomerular mesangium. In IgAN, renal biopsies reveal glomerular deposition of mannan-binding lectin (MBL), a key recognition molecule for activation of MASP-2, the effector enzyme of the complement system's lectin pathway. Glomerular MBL deposits, usually co-localized with IgA and indicating complement activation, and high levels of urinary MBL are associated with an unfavorable prognosis in IgAN, with these patients demonstrating more severe histological changes and mesangial proliferation than patients without MBL deposition or high levels of urinary MBL (Matsuda M. et al., *Nephron* 80(4):408-13, 1998; Liu LL et al., Clin Exp Immunol 169(2):148-155, 2012; Roos A. et al., *J Am Soc Nephrol* 17(6):1724-34, 2006; Liu LL et al., *Clin Exp Immunol* 174(1):152-60, 2013). Remission rates also are substantially lower for patients with MBL deposition (Liu LL et al., *Clin Exp Immunol* 174(1):152-60, 2013).

Current therapy for IgAN includes blood pressure control and, frequently, corticosteroids and /or other immunosuppressive agents, such as cyclophosphamide, azathioprine, or mycofenolate mofetil, for severe disease (e.g., crescentic IgAN). The Kidney Disease Improving Global Outcomes (KDIGO) Guidelines for Glomerulonephritis (*Int. Soc of Nephrol* 2(2):139-274, 2012) recommend that corticosteroids should be administered to patients with proteinuria of greater than or equal to 1 g/day, with a usual treatment duration of 6 months. However, even with aggressive immunosuppressive treatment, which is associated with serious long-term sequelae, some patients have progressive deterioration of renal function. There is no approved treatment for IgAN, and even with the use of angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) to control blood pressure, increased proteinuria persists in some patients. None of these treatments have been shown to stop or even slow the progression of IgAN in patients who are at risk for rapid progression of the disease.

Membranous Nephropathy

The annual incidence of membranous nephropathy (MN) is approximately 10-12 per 1,000,000. Patients with MN can have a variable clinical course, but approximately 25% will develop end-stage renal disease.

Membranous nephropathy is an immune-mediated glomerular disease and one of the most common causes of the nephrotic syndrome in adults. The disease is characterized by the formation of immune deposits, primarily IgG4, on the outer aspect of the glomerular basement membrane, which contain podocyte antigens and antibodies specific to those antigens, resulting in complement activation. Initial manifestations of MN are related to the nephrotic syndrome: proteinuria, hypoalbuminemia, hyperlipidemia, and edema.

Although MN may spontaneously remit without treatment, as many as one third of patients demonstrate progressive loss of kidney function and progress to ESRD at a median of 5 years after diagnosis. Often, corticosteroids are used to treat MN and there is a need to develop alternative therapies. Additionally, patients determined to be at moderate risk for progression, based on severity of proteinuria, are treated with prednisone in conjunction with cyclophosphamide or a calcinuerin inhibitor, and these two treatments together are often associated with severe systemic adverse effects.

Methods:

Two Phase 1 clinicial trials carried out in healthy volunteers have demonstrated that both intravenous and subcutaneous dosing of a MASP-2 inhibitory antibody, OMS646, resulted in sustained lectin pathway inhibition.

This Example describes interim results from an ongoing Phase 2, uncontrolled, multicenter study of a MASP-2 inhibitory antibody, OMS646, in subjects with IgAN and MN. Inclusion criteria require that all patients in this study, regardless of renal disease subtype, have been maintained on a stable dose of corticosteroids for at least 12 weeks prior to study enrollment (i.e., the patients are steroid-dependent). The study is a single-arm pilot study with 12 weeks of treatment and a 6-week follow-up period.

Approximately four subjects are planned to be enrolled per disease. The study is designed to evaluate whether OMS646 may improve renal function (e.g., improve proteinuria) and decrease corticosteroid needs in subjects with IgAN and MN. To date, 2 patients with IgA nephropathy and 2 patients with membranous nephropathy have completed treatment in the study.

At study entry each subject must have high levels of protein in the urine despite ongoing treatment with a stable corticosteroid dose. These criteria select for patients who are unlikely to spontaneously improve during the study period.

The subjects were age ≥ 18 at screening and were only included in the study if they had a diagnosis of one of the following: IgAN diagnosed on kidney biopsy or primary MN diagnosed on kidney biopsy. The enrolled patients also had to meet all of the following inclusion criteria:

(1) have average urine albumin/creatinine ratio > 0.6 from three samples collected consecutively and daily prior to each of 2 visits during the screening period;

(2) have been on ≥ 10 mg of prednisone or equivalent dose for at least 12 weeks prior to screening visit 1;

(3) if on immunosuppressive treatment (e.g., cyclophosphamide, mycophenolate mofetil), have been on a stable dose for at least 2 months prior to Screening Visit 1 with no expected change in the dose for the study duration;

(4) have an estimated glomerular filtration rate (eGFR) ≥ 30 mL/min/1.73m^2 calculated by the MDRD equation[1];

(5) are on a physician-directed, stable, optimized treatment with angiotensin converting enzyme inhibitors (ACEI) and/or angiotensin receptor blockers (ARB) and have a systolic blood pressure of <150 mmHg and a diastolic blood pressure of <90mmHg at rest;

(6) have not used belimumab, eculizumab or rituzimab within 6 months of screening visit 1; and

(7) do not have a history of renal transplant.

[1]MDRD Equation: eGFR (mL/min/1.73m^2) = 175 x $(SCr)^{-1.154}$ x $(Age)^{-0.203}$ x (0.742 if female) x (1.212 if African American). Note: SCr=Serum Creatinine measurement should be mg/dL.

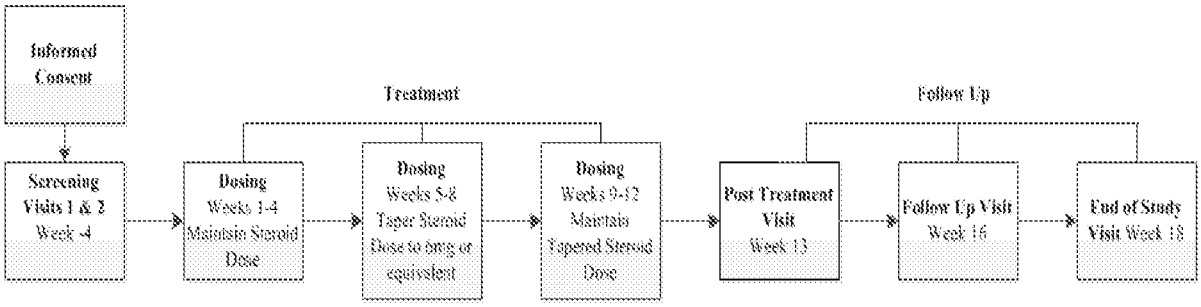
The monoclonal antibody used in this study, OMS646, is a fully human IgG4 monoclonal antibody that binds to and inhibits human MASP-2. MASP-2 is the effector enzyme of the lectin pathway. As demonstrated in Example 12, OMS646 avidly binds to recombinant MASP-2 (apparent equilibrium dissociation constant in the range of 100 pM) and exhibits greater than 5,000-fold selectivity over the homologous proteins C1s, C1r, and MASP-1. In functional assays, OMS646 inhibits the human lectin pathway with

nanomolar potency (concentration leading to 50% inhibition [IC_{50}] of approximately 3 nM) but has no significant effect on the classical pathway. OMS646 administered either by intravenous (IV) or subcutaneous (SC) injection to mice, non-human primates, and humans resulted in high plasma concentrations that were associated with suppression of lectin pathway activation in an *ex vivo* assay.

In this study, the OMS646 drug substance was provided at a concentration of 100 mg/mL, which was further diluted for IV administration. The appropriate calculated volume of OMS646 100 mg/mL injection solution was withdrawn from the vial using a syringe for dose preparation. The infusion bag was administered within four hours of preparation.

The study consists of screening (28 days), treatment (12 weeks) and follow-up (6 weeks) periods, as shown in the Study Design Schematic below.

Study Design Schematic



Within the screening period and before the first OMS646 dose, consented subjects provided three urine samples (collected once daily) on each of two three-consecutive-day periods to establish baseline values of the urine albumin-to-creatinine ratio. Following the screening period, eligible subjects received OMS646 4 mg/kg IV once weekly for 12 weeks (treatment period). There was a 6-week follow-up period after the last dose of OMS646.

During the initial 4 weeks of treatment with OMS646, subjects were maintained on their stable pre-study dose of corticosteroids. At the end of the initial 4-weeks of the 12-week treatment period, subjects underwent corticosteroid taper (i.e., the corticosteroid dose was reduced), if tolerated, over 4 weeks, followed by 4 weeks during which the resultant corticosteroid dose was maintained. The target was a taper to $\leq$ 6 mg

prednisone (or equivalent dose) daily. Over this period, the taper was discontinued in subjects who had deterioration of renal function, as determined by the investigator. Subjects were treated with OMS646 through the corticosteroid taper and through the full 12 weeks of treatment. The patients were then followed for an additional 6 weeks after their last treatment. The taper of corticosteroids and OMS646 treatment permitted assessment of whether OMS646 allowed for a decrease in the dose of corticosteroid required to maintain stable renal function.

The key efficacy measures in this study are the change in urine albumin-to-creatinine ratio (uACR) and 24-hour protein levels from baseline to 12 weeks. Measurement of urinary protein or albumin is routinely used to assess kidney involvement and persistent high levels of urinary protein correlates with renal disease progression. The uACR is used clinically to assess proteinuria.

Efficacy Analyses

The analysis value for uACR is defined as the average of all the values obtained for a time point. The planned number of uACRs is three at each scheduled time point. The baseline value of the uACR is defined as the average of the analysis values at the two screening visits.

Results:

FIGURE 40 graphically illustrates the uACRin two IgAN patients during the course of a twelve week study with weekly treatment with 4 mg/kg MASP-2 inhibitory antibody (OMS646). As shown in FIGURE 40, the change from baseline is statistically significant at time point "a" (p=0.003); time point "b" (p=0.007) and a time point "c" (p=0.033) by the untransformed analysis. TABLE 12 provides the 24-hour urine-protein data for the two IgAN patients treated with OMS646.

TABLE 12: 24-hour Urine Protein (mg/day) in OMS646-treated IgAN Patients

Time of Sample	Patient #1 (mg/24 hours)	Patient #2 (mg/24 hours)	Mean
Baseline	3876	2437	3156
Day 85	1783	455	1119
p= 0.017			

As shown in FIGURE 40 and TABLE 12, the patients with IgAN demonstrated a clinically and statistically significant improvement in kidney function over the course of the study. There were statistically significant decreases in both uACR (see FIGURE 40) and 24-hour urine protein concentration (see TABLE 12). As shown in the uACR data in FIGURE 40, the mean baseline uACR was 1264 mg/g and reached 525 mg/g at the end of treatment (p=0.011) decreasing to 128 mg/g at the end of the follow-up period. As further shown in FIGURE 40, the treatment effect was maintained throughout the follow-up period. Measures of 24-hour urine protein excretion tracked uACRs, with a mean reduction from 3156 mg/24 hours to 1119 mg/24 hours (p=0.017). Treatment effects across the two patients were highly consistent. Both patients experienced reductions of approximately 2000 mg/day and both achieved a partial remission (defined as greater than 50 percent reduction in 24-hour urine protein excretion and/or resultant protein exretion less than 1000 mg/day; complete remission defined as protein excretion less than 300 mg/day). The magnitude of the 24-hour proteinuria reductions in both IgA nephropathy patients is associated with a significant improvement in renal survival. Both IgA nephropathy patients were also able to taper their steroids substantially, each reducing the daily dose to $\leq$ 5 mg (60 mg to 0 mg; 30 mg to 5 mg).

The two MN patients also demonstrated reductions in uACR during treatment with OMS646. One MN patient had a decrease in uACR from 1003 mg/g to 69 mg/g and maintained this low level throughout the follow-up period. The other MN patient had a decrease in uACR from 1323 mg/g to 673 mg/g, with a variable course after treatment. The first MN patient showed a marked reduction in 24-hour urine protein level (10,771 mg/24 hours at baseline to 325 mg/24 hours on Day 85), achieving partial and nearly complete remission, while the other remained essentially unchanged (4272 mg/24 hours at baseline to 4502 mg/24 on Day 85). Steroids were tapered in the two MN patients from 30 mg to 15 mg and from 10 mg to 5 mg.

In summary, consistent improvements in renal function were observed in IgAN and MN subjects treated with the MASP-2 inhibitory antibody OMS646. The effects of OMS646 treatment in the patients with IgAN are robust and consistent, suggesting a strong efficacy signal. These effects are supported by the results in MN patients. The time course and magnitude of the uACR changes during treatment were consistent between all four patients with IgAN and MN. No significant safety concerns have been

observed. Patients in this study represent a difficult-to-treat group and a therapeutic effect in these patients is believed to be predictive of efficacy with a MASP-2 inhibitory antibody, such as OMS646, in IgAN and MN patients, such as patients suffering from steroid-dependent IgAN and MN (i.e., patients undergoing treatment with a stable corticosteroid dose prior to treatment with a MASP-2 inhibitory antibody), including those at risk for rapid progression to end-stage renal disease.

In accordance with the foregoing, in one embodiment, the invention provides a method of treating a human subject suffering from IgAN or MN comprising administering to the subject a composition comprising an amount of a MASP-2 inhibitory antibody effective to inhibit MASP-2-dependent complement activation. In one embodiment, the method comprises administering to the human subject suffering from IgAN or MN an amount of a MASP-2 inhibitory antibody sufficient to improve renal function (e.g., improve proteinuria). In one embodiment, the subject is suffering from steroid-dependent IgAN. In one embodiment, the subject is suffering from steroid-dependent MN. In one embodiment, the MASP-2 inhibitory antibody is administered to the subject suffering from steroid-dependent IgAN or steroid-dependent MN in an amount sufficient to improve renal function and/or decrease corticosteroid dosage in said subject.

In one embodiment, the method further comprises identifying a human subject suffering from steroid-dependent IgAN prior to the step of administering to the subject a composition comprising an amount of a MASP-2 inhibitory antibody effective to inhibit MASP-2-dependent complement activation.

In one embodiment, the method further comprises identifying a human subject suffering from steroid-dependent MN prior to the step of administering to the subject a composition comprising an amount of a MASP-2 inhibitory antibody effective to inhibit MASP-2-dependent complement activation.

In accordance with any of the disclosed embodiments herein, the MASP-2 inhibitory antibody exhibits at least one or more of the following characteristics: said antibody binds human MASP-2 with a K_D of 10 nM or less, said antibody binds an epitope in the CCP1 domain of MASP-2, said antibody inhibits C3b deposition in an *in vitro* assay in 1% human serum at an IC_{50} of 10 nM or less, said antibody inhibits C3b deposition in 90% human serum with an IC_{50} of 30 nM or less, wherein the antibody is an antibody fragment selected from the group consisting of Fv, Fab, Fab', $F(ab)_2$ and

$F(ab')_2$, wherein the antibody is a single-chain molecule, wherein said antibody is an IgG2 molecule, wherein said antibody is an IgG1 molecule, wherein said antibody is an IgG4 molecule, wherein the IgG4 molecule comprises a S228P mutation. In one embodiment, the antibody binds to MASP-2 and selectively inhibits the lectin pathway and does not substantially inhibit the classical pathway (i.e., inhibits the lectin pathway while leaving the classical complement pathway intact).

In one embodiment, the MASP-2 inhibitory antibody is administered in an amount effective to improve at least one or more clinical parameters associated renal function, such as an improvement in proteinuria (e.g., a decrease in uACR and/or a decrease in 24-hour urine protein concentration, such as greater than 20 percent reduction in 24-hour urine protein excretion, or such as greater than 30 percent reduction in 24-hour urine protein excretion, or such as greater than 40 percent reduction in 24-hour urine protein excretion, or such as greater than 50 percent reduction in 24-hour urine protein excretion).

In some embodiments, the method comprises administering a MASP-2 inhibitory antibody to a subject suffering from IgAN (such as steroid-dependent IgAN), via a catheter (e.g., intravenously) for a first time period (e.g., at least one day to a week or two weeks or three weeks or four weeks or longer) followed by administering a MASP-2 inhibitory antibody to the subject subcutaneously for a second time period (e.g., a chronic phase of at least two weeks or longer).

In some embodiments, the method comprises administering a MASP-2 inhibitory agent to a subject suffering from MN (such as steroid-dependent MN), via a catheter (e.g., intravenously) for a first time period (e.g., at least one day to a week or two weeks or three weeks or four weeks or longer) followed by administering a MASP-2 inhibitory antibody to the subject subcutaneously for a second time period (e.g., a chronic phase of at least two weeks or longer).

In some embodiments, the method comprises administering a MASP-2 inhibitory antibody to a subject suffering from IgAN (such as steroid-dependent IgAN) or MN (such as steroid-dependent MN) either intravenously, intramuscularly, or subcutaneously. Treatment may be chronic and administered daily to monthly, but preferably at least every two weeks, or at least once a week, such as twice a week or three times a week.

In one embodiment, the method comprises treating a subject suffering from IgAN (such as steroid-dependent IgAN) or MN (such as steroid-dependent MN) comprising

administering to the subject a composition comprising an amount of a MASP-2 inhibitory antibody, or antigen binding fragment thereof, comprising a heavy chain variable region comprising CDR-H1, CDR-H2 and CDR-H3 of the amino acid sequence set forth as SEQ ID NO:67 and a light-chain variable region comprising CDR-L1, CDR-L2 and CDR-L3 of the amino acid sequence set forth as SEQ ID NO:69. In some embodiments, the composition comprises a MASP-2 inhibitory antibody comprising (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:67; and ii) a heavy-chain CDR-H2 comprising the amino acid sequence from 50-65 of SEQ ID NO:67; and iii) a heavy-chain CDR-H3 comprising the amino acid sequence from 95-107 of SEQ ID NO:67 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:69; and ii) a light-chain CDR-L2 comprising the amino acid sequence from 50-56 of SEQ ID NO:69; and iii) a light-chain CDR-L3 comprising the amino acid sequence from 89-97 of SEQ ID NO:69, or (II) a variant thereof comprising a heavy-chain variable region with at least 90% identity to SEQ ID NO:67 (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity to SEQ ID NO:67) and a light-chain variable region with at least 90% identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity to SEQ ID NO:69.

In some embodiments, the method comprises administering to the subject a composition comprising an amount of a MASP-2 inhibitory antibody, or antigen binding fragment thereof, comprising a heavy-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:67 and a light-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:69.

In some embodiments, the method comprises administering to the subject a composition comprising a MASP-2 inhibitory antibody, or antigen binding fragment thereof, that specifically recognizes at least part of an epitope on human MASP-2 recognized by reference antibody OMS646 comprising a heavy-chain variable region as set forth in SEQ ID NO:67 and a light-chain variable region as set forth in SEQ ID NO:69.

In some embodiments, the method comprises administering to a subject suffering from, or at risk for developing IgAN (such as steroid-dependent IgAN) or MN (such as steroid-dependent MN), a composition comprising a MASP-2 inhibitory antibody, or

antigen binding fragment thereof comprising a heavy-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:67 and a light-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:69 in a dosage from 1 mg/kg to 10 mg/kg (i.e., 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg or 10 mg/kg) at least once weekly (such as at least twice weekly or at least three times weekly) for a period of at least 3 weeks, or for at least 4 weeks, or for at least 5 weeks, or for at least 6 weeks, or for at least 7 weeks, or for at least 8 weeks, or for at least 9 weeks, or for at least 10 weeks, or for at least 11 weeks, or for at least 12 weeks.

EXAMPLE 20

This Example describes a study using a MASP-2 inhibitory antibody, OMS646, in the treatment of a subject suffering from, or at risk for developing,coronavirus-induced acute respiratory distress syndrome.

Background/Rationale:

Acute respiratory distress syndrome is a severe complication of coronavirus infection. SARS-CoV emerged in 2002 and 2003 from coronavirus circulating in animal markets in China, leading to a global outbreak of respiratory disease, with over 8,000 human cases and 10% mortality (Rota P.A. et al., Science 300:1394-1999, 2003). In 2012, a new related coronavirus was identified in the Middle East, designated as the Middle East respiratory syndrome coronavirus (MERS-CoV), causing severe respiratory disease with greater than 35% mortality (Zaki A.M. et al., N Engl J Med 367:1814-1820, 2012). Coronavirus disease 2019 (COVID-19) is an infectious disease that emerged in 2019 and is caused by severe acute respiratory syndrome coronavirus 2 (SARS coronavirus 2 or SARS-CoV-2), a virus that is closely related to the SARS virus (World Health Organization, 2/11/2020, Novel Coronavirus Situation Report 22). COVID-19, SARS-CoV and MERS-CoV all cause a range of disease from asymptomatic cases to severe acute respiratory distress syndrome (coronavirus-induced ARDS) and respiratory failure. Those affected by COVID-19 may develop a fever, dry cough, fatigue and shortness of breath. Findings on computed tomography can show pulmonary ground glass opacities and bilateral patchy shadowing. Cases can progress to respiratory dysfunction, including pneumonia, severe acute respiratory distress syndrome, which can

lead to multi-organ failure and septic shock, and death in the most vulnerable (see e.g., Hui D.S. et al., Int J Infect Dis 91:264-266, Jan 14, 2020 and Guan et al. OI:10.1056/NEJMoa2002032). There is no vaccine or specific antiviral treatment, with management involving treatment of symptoms and supportive care. Thus, there is an urgent need to develop therapeutically effective agents to treat, inhibit and/or prevent coronavirus-induced acute respiratory distress syndrome.

It has been observed that complement activation contributes to the pathogenesis of coronavirus-induced severe acute respiratory syndrome. It was found that SARS-CoV-infected mice deficient in complement component 3 (C3-/- mice) exhibited significantly less weight loss and less respiratory dysfunction in comparison to SARS-CoV-infected C57BL/6J control mice, despite equivalent viral loads in the lung (Gralinski L.E. et al., mBio 9:e01753-18, 2018). It was further observed that there were significantly fewer neutrophils and inflammatory monocytes in the lungs of SARS-CoV-infected C3-/- mice than in the infected control mice as well as reduced lung pathology and lower cytokine and chemokine levels (e.g., IL-5, IL-6) in the lungs of the SARS-CoV-infected C3-/- mice as compared to the infected control mice (Gralinski L.E. et al., mBio 9:e01753-18, 2018).

Studies have also shown that many survivors of SARS-CoV infection develop pulmonary fibrosis, with a higher prevalence in older patients (Hui D.S. et al., Chest 128:2247-2261, 2005). There are limited options for treating pulomonary fibrosis, such as coronavirus-induced fibrosis. Traditionally, corticosteroids are used to treat ARDS and pulmonary fibrosis, however, during a viral infection, this treatment dampens the immune response and can result in worsened disease (Gross T.J. et al., *N Engl J Med* 345:517-525, 2001).

As noted previously, no effective treatment for COVID-19 is known, and the disease is spreading rapidly. Although mortality assessments are still early, the World Health Organization reported a mortality rate of 3.4% in early March 2020 (worldwideweb.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---3-march-2020). Effective treatment is needed for patients with severe COVID-19 infection.

As described herein, the lectin pathway is one of the three activation pathways of complement. The other pathways are the classical pathway and alternative pathway. All

activation pathways result in the creation of the anaphylatoxins C3a and C5a and in the creation of C5b-9 or membrane attack complex (MAC) on target cells.

The lectin pathway is part of the innate immune system and is activated by microorganisms or injured cells. Microorganisms display carbohydrate-based pathogen-associated molecular patterns (PAMPs) and injured host cells display damage-associated molecular patterns (DAMPs). DAMPs are not displayed on healthy cells but become exposed with cell injury.

Circulating lectins, such as mannose-binding lectin (MBL), ficolins, and collectins recognize and bind to PAMPs and DAMPs. Lectin binding to the PAMPs or DAMPs localizes the complement activation to the vicinity of the cell membrane. These lectins carry mannan-binding lectin-associated serine protease 2 (MASP-2), that, cleaves complement factors 2 and 4 to create the C3 convertase, which itself, then cleaves C3 to form the C5 convertase. In addition to the lectin pathway activation, the alternative pathway can also be activated and amplifies complement activation. All of this leads to insertion of the MAC into the membrane of the injured cell, further injuring the cell with more DAMP exposure. The circulating lectins carrying MASP-2 recognize and bind to the DAMPs, causing further lectin pathway activation and additional cell injury. In this manner, the lectin pathway could magnify and worsen cell injury caused by initial complement activation.

As described herein, OMS646 (also known as OMS721 or narsoplimab) is an investigational human IgG4 monoclonal antibody directed against MASP-2. As further described herein, by blocking MASP-2, activation of the lectin pathway is inhibited. This may break the cycle of complement-mediated cellular injury described above. To date, OMS646 has been administered to approximately 230 healthy volunteers, patients with thrombotic microangiopathies (TMA), and patients with glomerulonephropathies (e.g., immunoglobulin A [IgA] nephropathy.

The lectin pathway may play a key role in initiating and perpetuating complement activation in coronavirus-induced ARDS, and inhibition of the lectin pathway via a

MASP-2 inhibitory agent such as the MASP-2 inhibitory antibody OMS646 may address complement-mediated pulmonary injury related to coronavirus infection. As described herein, the inventors discovered that inhibition of mannan-binding lectin-associated serine protease-2 (MASP-2), the key regulator of the lectin pathway of the complement system, significantly reduces inflammation and fibrosis in various animal models of fibrotic disease. For example, the results presented in Examples 14 and 15 herein demonstrate a beneficial effect of MASP-2 inhibition on renal tubulointerstitial inflammation, tubular cell injury, profibrotic cytokine release and scarring. As described in Example 17, in an analysis of a monoclonal MASP-2 inhibitory antibody for efficacy in reducing and/or preventing renal inflammation and tubulointerstitial injury in a mouse protein-overload proteinuria model in wild-type mice, it was determined that there was a significant reduction in the level of IL-6 in the MASP-2 inhibitory antibody-treated group as compared to the saline control group ($p=0.0269$) as well as to the isotype control group ($p=0.0445$), as shown in FIGURE 36.

Methods:

The following study is carried out to analyze the use of OMS646 in the treatment of one or more patients suffering from coronavirus (e.g., COVID-19-virus) infection in order to measure the efficacy of OMS646 for treating, inhibiting, alleviating or preventing acute respiratory distress syndrome in said patient(s).

The methods involve identifying a subject infected with coronavirus, such as COVID-19, MERS-CoV or SARS, which may be determined by carrying out a diagnostic test, such as a molecular test (e.g., rRT-PCR) or a serology test, or by reference to a database containing such information. Exemplary tests for COVID-19, MERS-CoV and SARS are found on the Centers For Disease Control website (world-wide-web.cdc.gov/coronavirus/mers/lab/lab-testing.html#molecular).

The subject may be suffering from COVID-19-induced ARDS, or at risk for developing ARDS, such as a subject suffering from pneumonia. Pneumonia is the most common risk factor for the development of ARDS (Sweeney R.M. and McAuley, D.F.,

Lancet vol 388:2416-30, 2016).

COVID-19-induced ARDS is defined as a clinical syndrome that develops after infection with COVID-19 and fulfills one or more of the following criteria for ARDS (Sweeney R.M. and McAuley, D.F., *Lancet* vol 388:2416-30, 2016), based on the Berlin Definition (*JAMA* 307:2526, 2012):

- Oxygenation (mm Hg): mild (PaO_2/FiO_2 200-300); moderate (PaO_2/Fi O_2 100-199); severe (PaO_2/FiO_2 <100)
- Positive end-expiratory pressure (PEEP) (cm H_2O): minimum PEEP of 5 required
- Infiltrates on chest radiograph: bilateral infiltrates involving two or more quadrants on a frontal chest radiograph or CT
- Heart failure: left ventricular failure insufficient to solely account for clinical state
- Severity: based on oxygenation criteria

Treatment administration

Subjects suffering from COVID-19 and experiencing one or more respiratory symptoms, such as those criteria listed above, are dosed with 4mg/kg of OMS646 via intravenous infusion. Treatment is administered twice weekly. The dose frequency is guided by patient response to therapy. If the patient demonstrates clinical improvement that is maintained for 4 weeks, the dose may be decreased to 4mg/kg once weekly. If the patient maintains the treatment response while receiving 4mg/kg once weekly for 4 weeks, treatment may be discontinued.

A positive response to treatment is determined when an improvement is observed in respiratory function, for example, in one or more respiratory symptoms, such as in one or more criteria for ARDS.

In accordance with the foregoing, in one aspect, the present invention provides a method for treating, inhibiting, alleviating or preventing acute respiratory distress syndrome or other manifestation of the disease in a mammalian subject infected with coronavirus, comprising administering to the subject an amount of a MASP-2 inhibitory agent effective to inhibit MASP-2-dependent complement activation (i.e., inhibit lectin pathway activation). In some embodiments, the subject is suffering from one or more respiratory symptoms and the method comprises administering to the subject an amount of a MASP-2 inhibitory agent effective to improve at least one respiratory symptom (i.e., improve respiratory function).

In one embodiment, the method comprises administering the composition to a subject infected with COVID-19. In one embodiment, the method comprises administering the composition to a subject infected with SARS-CoV. In one embodiment, the method comprises administering the composition to a subject infected with MERS-CoV. In one embodiment, the subject is identified as having coronavirus (i.e., COVID-19, SARS-CoV or MERS-CoV) prior to administration of the MASP-2 inhibitory agent.

In one embodiment, the MASP-2 inhibitory agent is a small molecule that inhibits MASP-2-dependent complement activation.

In one embodiment, the MASP-2 inhibitory agent is an expression inhibitor of MASP-2.

In one embodiment, the MASP-2 inhibitory antibody is a monoclonal antibody, or fragment thereof that specifically binds to human MASP-2. In one embodiment, the MASP-2 inhibitory antibody or fragment thereof is selected from the group consisting of a recombinant antibody, an antibody having reduced effector function, a chimeric antibody, a humanized antibody, and a human antibody. In one embodiment, the MASP-2 inhibitory antibody does not substantially inhibit the classical pathway. In one embodiment, the MASP-2 inhibitory antibody inhibits C3b deposition in 90% human serum with an IC_{50} of 30 nM or less.

In one embodiment, the MASP-2 inhibitory antibody or antigen-binding fragment thereof, comprises a heavy chain variable region comprising CDR-H1, CDR-H2 and CDR-H3 of the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising CDR-L1, CDR-L2 and CDR-L3 of the amino acid sequence set forth as SEQ ID NO:69. In one embodiment, the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising the amino acid sequence set forth as SEQ ID NO:69.

In some embodiments, the method comprises administering to a subject infected with coronavirus a composition comprising a MASP-2 inhibitory antibody, or antigen binding fragment thereof comprising a heavy-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:67 and a light-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:69 in a dosage from 1 mg/kg to 10 mg/kg (i.e., 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9

mg/kg or 10 mg/kg) at least once weekly (such as at least twice weekly or at least three times weekly) for a period of at least 2 weeks (such as for at least 3 weeks, or for at least 4 weeks, or for at least 5 weeks, or for at least 6 weeks, or for at least 7 weeks, or for at least 8 weeks, or at least 9 weeks, or at least 10 weeks, or at least 11 weeks, or at least 12 weeks).

In one embodiment, the dosage of MASP-2 inhibitory antibody is about 4 mg/kg (i.e., from 3.6 mg/kg to 4.4 mg/kg).

In one embodiment, dosage of the MASP-2 inhibitory antibody is a fixed dose from about 300 mg to about 450 mg (i.e., from about 300 mg to about 400 mg, or from about 350 mg to about 400 mg), such as about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg or about 450 mg). In one embodiment, the dosage of the MASP-2 inhibitory antibody is a fixed dose of about 370 mg (±10%).

In one embodiment, the method comprises administering a fixed dosage of MASP-2 inhibitory antibody at about 370 mg (±10%) to a subject infected with coronavirus twice weekly intravenously for a treatment period of at least 8 weeks.

In one embodiment, the MASP-2 inhibitory agent is delivered to the subject systemically. In one embodiment, the MASP-2 inhibitory agent is administered orally, subcutaneously, intraperitoneally, intra-muscularly, intra-arterially, intravenously, or as an inhalant.

EXAMPLE 21

OMS646 (narsoplimab) Treatment in COVID-19 Patients

This Example describes the use of narsoplimab (OMS646) in the treatment of COVID-19 infected patients using the methods described in Example 20. The results described in this Example confirm the efficacy of narsoplimab in COVID-19 patients described in Example 20.

Background/Rationale:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; COVID-19) was identified as a clinical syndrome in Hubei province China in December 2019 and spread rapidly (Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet, 395: 1054-62, 2020). By late February 2020, a fast-growing number of COVID-19 cases were diagnosed in the northern Italian region of Lombardy (Remuzzi A, Remuzzi G. COVID-19 and Italy: what next? *The Lancet*). A primary cause of death in COVID-19 is severe respiratory dysfunction. Lung tissue in patients who have died of COVID-19 shows high concentration of SARS-CoV RNA (Wichmann D. et al., *Ann Intern Med,* 2020) and the same intense inflammatory changes seen in previously reported coronaviruses SARS-CoV (SARS) and MERS-CoV (MERS), and anti-inflammatory strategies are being evaluated for COVID-19 treatment (Xu Z. et al., *Lancet Respir Med,* 8(4):420-2, 2020; Horby P. et al., *medRxiv* 2020: 2020.06.22.20137273; Gritti G. et al., *medRxiv* 2020: 2020.04.01.20048561). Thrombosis has also been reported in SARS and COVID-19 infection (Wichmann D. et al., *Ann Intern Med* 2020; Magro C. et al., *Transl Res* 2020; Ding Y. et al., *J Pathol* 200(3):28209, 2003). Like SARS and MERS, COVID-19 can cause life-threatening acute respiratory distress syndrome (ARDS) (Guan W.J, Ni Z.Y, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020 [Epub ahead of print]).

A central pathological component of COVID-19 and of the exudative phase of ARDS is endothelial injury and activation (Varga Z. et al., *Lancet* 2020; Ackermann M. et al., *N Engl J Med* 2020; Green S. J. et al., *Microbes Infect* 22(4-5):149-50, 2020; Teuwen L.A. et al., *Nat Rev Immunol* 20(7):389-91, 2020; Goshua G. et al., *Lancet Haematol* 2020; Thompson B.T. et al., *N Engl J Med* 377(19):1904-5, 2017). The underlying cause of increased capillary permeability and pulmonary edema in ARDS, endothelial injury can also cause microvascular angiopathy and thrombosis. Endothelial injury can also cause microvascular angiopathy and thrombosis. Endothelial activation further enhances the local inflammatory environment. Importantly, as demonstrated in

human *in vitro* and animal studies, endothelial injury specifically activates the lectin pathway of complement on the endothelial cell surface (Collard CD, Väkevä A, Morrissey MA, et al. Complement Activation after Oxidative Stress: Role of the Lectin Complement Pathway. *Am J Pathol* 2000;156(5):1549–1556).

As described in Example 20, OMS646 (also known as narsoplimab), a high affinity monoclonal antibody that binds to MASP-2 and blocks lectin pathway activation, was expected to be effective for the treatment of COVID-19 patients. Consistent with the description in Example 20, MASP-2 has been directly linked to the lung injury in coronavirus infection in an animal model. See Gao et al., *medRxiv* 3/30/2020. MASP-2 also acts directly on the coagulation cascade and the contact system, cleaving prothrombin to thrombin and forming fibrin clots. Narsoplimab not only inhibits lectin pathway activation but also blocks microvascular injury associated thrombus formation as well as MASP-2-mediated activation of kallikrein and factor XII.

No disease-specific therapies have been shown effective for the treatment of COVID-19. In view of the heavy disease burden in Italy, we treated patients with severe COVID-19 infection and ARDS with narsoplimab under a compassionate use program at Papa Giovanni XXIII Hospital in Bergamo. This represents the first time that a lectin pathway inhibitor has been used to treat patients with COVID-19. Here we report this initial clinical experience.

METHODS

Study Oversight

The investigation described in this Example was conducted at the Azienda Socio-Sanitaria Territoriale Papa Giovanni XXIII in Bergamo, Italy and approved by the institutional Ethics Committee and the Agenzia Italiana del Farmaco. Laboratory values including blood counts, LDH, C Reactive protein (CRP) were collected as per standard clinical practice. All patients treated with narsoplimab (OMS646) provided informed

consent. This study was carried out using the methods described in Example 20, as further described below.

Histopathology

Standard Haematoxylin and Eosin staining (H&E) and immunohistochemistry were performed on formalin fixed-paraffin embedded samples obtained from pathological autopsies of patients with COVID-19. H&E stained sections were reviewed by two pathologists. In order to confirm diagnosis and immunohistochemical analysis of the human endothelial cell marker (CD34) was performed with Bond Ready-to-Use Antibody CD34 (Clone QBEnd/10, Leica Biosystems, Germany), a ready to use product that has been specifically optimized for use with Bond Polymer Refine Detection. The assay was performed on an automated stainer platform (Leica Bond-3, Leica, Germany) using a heat-based antigen retrieval technique as recommended by the manufacturer (Bond Epitope Retrieval solution 2 for 20 minutes). Cytoplasmatic staining of endothelium in the capillaries of pulmonary alveoli indicated positive results.

Circulating Endothelial Cells (CEC) identification and count

CEC were tested by flow cytometry analysis performed on peripheral blood samples collected with EDTA. After an erythrocyte-lysis step, samples were labeled with the following monoclonal antibodies: anti-CD45 V500 (clone 2D1, Becton Dickinson, San Jose`, CA), anti-CD34 PerCP-CY5.5 (clone 8G12, Becton Dickinson, San Jose`, CA), anti-CD146 PE (clone P1H12BD, Pharmingen, CA), for 20 min at room temperature. At least 1×10^6 events/sample with total leucocyte morphology were acquired by flow cytometry (FACSLyric, BD Biosciences). To reduce operator-induced variability, all the samples in this study were always analyzed by the same laboratory technician. CEC/ml numbers were calculated by a dual-platform counting method using the lymphocyte subset as reference population as previously reported (Almici C. et al., *Bone Marrow Transplant* 52:1637-42, 2017).

Serum levels of cytokines

Levels of interleukin-8 (IL-8), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor (TNF), and interleukin-12p70 (IL-12p70) were analyzed in a single sample of serum by flow cytometry (BD CBA Human Inflammatory Cytokines Kit, Becton Dickinson, San Jose, CA).

Patients

All narsoplimab-treated patients were admitted to the hospital between March 11 and March 23, 2020. Over this 13-day span, the total daily number of COVID-19 patients hospitalized on the wards ranged from 405 to 542. During this same time period, an average of 140 Helmet-continuous passive airway pressure (CPAP) devises were utilized on a daily basis, and a median of 82 patients (range 66-91) were managed each day in the ICU. Of these ICU patients, 61 met the Berlin criteria for ARDS (PaO2/FiO2 ratio <100 is severe ARDS; 100 – 200 is moderate; >200 and ≤300 is mild) (Ferguson N.D et al., *Intensive Care Med* 38(10): 1573-82, 2012; Fagiuoli S. et al., *N Engl J Med* 382(21)e71, 2020) on March 11, 2020 and 80 on March 23, 2020.

All patients treated in this study had laboratory-confirmed COVID-19 infection diagnosed by quantitative reverse-transcriptase-polymerase-chain-reaction assay. SARS-CoV-2 genome from nasal and respiratory samples was detected by different molecular methods including GeneFinder™ Covid-19 Plus RealAmp Kit (ELIThech Group, 92800 Puteaux, France) and Allplex™ 2019-nCoV Assay (Seegene Inc, Arrow Diagnostics S.r.l., Italy). After purification of viral RNA from clinical samples, detection of RdRp, E and N viral genes was obtained by real-time polymerase chain reaction according to World Health Organization protocol (Corman V.M. et al., Euro Surveill 25, 2020). To be eligible for treatment with narsoplimab, COVID-19-confirmed patients were required to be adults (>18 years of age), to have ARDS according to the Berlin criteria (Ferguson ND, et al. *Intensive Care* 38(10):1573–1582, 2012; see also Sweeney R.M. and McAuley, D.F., *Lancet* vol 388:2416-30, 2016; *JAMA* 307:2526, 2012) and to require non-invasive

mechanical ventilation by continuous positive airway pressure (CPAP) according to the institutional guidelines for respiratory support. While all enrolled patients empirically received azithromycin 500 mg once daily, patients with active systemic bacterial or fungal infections requiring antimicrobial therapy were not eligible for narsoplimab treatment.

Narsoplimab Treatment, Supportive Therapy and Outcome Assessment

As described in Example 20, narsoplimab (OMS646) is a fully human monoclonal antibody comprised of immunoglobulin gamma 4 (IgG4) heavy-chain and lambda light-chain constant regions. It binds to and inhibits MASP-2 with sub-nanomolar affinity. In accordance with the methods described in Example 20, narsoplimab was administered to six patients infected with COVID-19 at a dosage of 4 mg/kg intravenously twice weekly for 2 to 4 weeks, with a maximum of 6 to 8 doses (for two weeks, three weeks or four weeks). At study initiation, dosing duration was set at 2 weeks but was increased empirically when the first patient treated with narsoplimab experienced a clinical and laboratory-marker recurrence after cessation of treatment at 2 weeks, subsequently resolving with an additional week of dosing. All patients received standard supportive care per the hospital's guidelines at the time of the study, including prophylactic enoxaparin (Clexane, Sanofi Aventis) 4,000 IU/0 4 mL, azithromycin (Zitromax, Pfizer SpA, Italy) 500 mg once daily, hydroxychloroquine (Plaquenil, Sanofi Aventis) 200 mg twice daily, darunavir and cobicistat (Rezolsta, Janssen-Cilag S.p.A., Italy) 800/150 mg once daily. Beginning March 27, per updated institutional guidelines, all COVID-19 patients in the hospital received methylprednisolone 1 mg/kg. Accordingly, a total of five of the six narsoplimab-treated patients also received systemic corticosteroids (methylprednisolone 1 mg/kg) following initiation of narsoplimab treatment. All respiratory support was provided according to institutional treatment algorithms. The clinical characteristics of these six patients are summarized below in Table 13.

In addition to CEC counts and cytokine levels, clinical and laboratory measures, including blood counts, LDH and C-reactive protein (CRP) levels, were collected on all

narsoplimab-treated patients per standard clinical practice. Routine blood examinations were collected prior to each narsoplimab dose and then twice weekly. Respiratory function was evaluated daily. Chest computed tomograph (CT) scan was performed on all patients at hospital admission to document the typical interstitial pneumonia and to document pulmonary embolism if clinically indicated. Chest radiography was performed as per clinical requirement during the course of treatment.

Statistical analysis

Demographic and clinical patient data are presented as frequency with percentage for categorical variables and median with range for continuous ones. Difference in CEC values between normal and COVID-19 patients was assessed with Mann-Whitney U-test. Repeated measures analysis was performed to test differences in CEC and cytokine levels during narsoplimab treatment at appropriate timepoints; non-parametric Friedman test was used, and pairwise-comparisons were performed using paired Wilcoxon signed-rank test. Decreasing trend of LDH and CRP levels during treatment were evaluated with non-parametric Spearman test between the observations and time. Significance at 5% was fixed. Analysis was performed using R software (version 3·6·2).

Table 13 summarizes the clinical characteristics of the six narsoplimab-treated patients.

TABLE 13: Demographics of COVID-19 Patients Treated with narsoplimab

Clinical Characteristics	**All patients (N=6)**
Age – years, median (range)	56.5 years (47-63)
Sex – number (%) ….Female ….Male	 1 (17%) 5 (83%)
Weight- kilograms, median (range) BMI- kilograms/m^2, median (range)	86 (82-100) 28 (26.8-32)
Time from disease onset to hospital admission – days, median	8.5 (3-12)

(range)	
Fever[##] on admission to the hospital – number (%)	6 (100%)
Other Symptoms – number (%)	
……Cough	1 (17%)
……Anorexia	2 (33%)
……Fatigue	4 (67%)
……Shortness of breath	5 (83%)
……Nausea or Vomiting	1 (17%)
……Diarrhea	2 (33%)
……Headache	1 (17%)
Coexisting disorder – number (%)	
……Diabetes	1 (17%)[#]
……Hypertension	1 (17%)
……Dyslipidemia	2 (33%)
……Obesity (BMI) ≥30 kg/m^2	2 (33%)
……Overweight≥25 kg/m^2	4 (66%)
ARDS severity at enrollment – number (%)	
……Mild	3 (50%)
……Moderate	2 (33%)
……Severe	1 (17%)
Time from hospitalization to start of treatment – days, median (range)	2 days (1-4)
Time from CPAP placement to start of treatment – number (%)	
…….0-24 hours	4 (67%)
……24-48 hours	2 (33%)
Radiologic findings	
Abnormality on chest radiology – number (%)	
……Bilateral interstitial abnormalities	6 (100%)
Laboratory findings	
PaO_2:FiO_2 ratio – median (range)	175 (57.5-288)
Circulating endothelial cell count -median (range)	334 (0-9315)
White cell count- – per mm^3, median (range)	8335 (6420-10,120)

……>10,000 per mm^3 – number (%)	2 (33%)
……<4000 per mm^3 – number (%)	0 (0)
Lymphocyte count- per mm^3 median (range)	875 (410-1290)
Platelet count x 10^3 per mm^3 median (range)	282 (199-390)
Hemoglobin – g/dL, median (range)	13.4 (13.2-14.1)
Distribution of other findings (laboratory reference ranges)	
C-reactive protein (0.0-1.0 mg/dL)	14 (9.5-31.3)
Lactate dehydrogenase (120/246 U/L)	518.5 (238-841)
Aspartate aminotransferase (13-40 U/L)	78.5 (51-141)
Alanine aminotransferase (7-40 U/L)	73 (37-183)
Creatinine (0.3-1.3 mg/dL)	0.85 (0.38-1.33)
D-dimer* (<500 ng/mL)	1250.5 (943-1454)
Haptoglobin (36-195 mg/dL)	368.5 (270-561)
Complement C3** (79-152 mg/dL)	101 (60-126)
Complement C4** (16-38 mg/dL)	21 (2-37)
Concomitant Treatments	
Anti-retroviral Therapy – number (%)	
……Darunavir + Cobicistat	6 (100%)
Systemic steroid therapy – number (%)	5 (83%)
……After the 1st dose of narsoplimab	2 (33%)
……After the 2nd dose of narsoplimab	1 (17%)
……After the 3rd dose of narsoplimab	1 (17%)
……After the 4th dose of narsoplimab	1 (17%)

ARDS: Acute Respiratory Distress Syndrome; ICU: Intensive Care Unit; CPAP: Continuous Positive Airway Pressure.

*: data available only for 4 patients

**: data available only for 5 patients

#several patients were initially categorized as having diabetes, but were later recategorized as being overweight but not having diabetes.

defined as body temperature >37.5°C

RESULTS:

Thrombosis and Endothelial Cell Damage in COVID-19 Patients

From March 13 to March 16, soon after the dramatic beginning of the COVID-19 outbreak in Bergamo area, the Pathology department of the hospital started to perform autopsies in an initial group of 20 deceased patients. Prior to their deaths, all of these patients, as did the patients treated with narsoplimab in the current study, required advanced respiratory support with CPAP or invasive mechanical ventilation. In keeping with the clinical picture of frequently lethal pulmonary thromboembolism, the lungs and the liver of many patients were found extensively affected by thrombotic events, as described below.

At the histopathological level an arterial involvement by thrombotic process was evident in septal blood vessels of the lung in COVID-19 patients, including also areas unaffected by destructive inflammatory process. Immunohistochemical staining for CD34 (endothelial marker) demonstrated severe endothelial damage with cell shrinkage, degenerated hydropic cytoplasm and adhesion of lymphocytes on endothelial surface as shown in FIGURES 41A-D.

FIGURES 41A-D show representative images of the immunohistochemistry analysis of tissue sections taken from COVID-19 patients, showing vascular damage in these patients.

FIGURE 41A shows a representative image of the immunohistochemistry analysis of tissue sections of septal blood vessels from the lung of a COVID-19 patient. As shown in FIGURE 41A, there is arterial involvement by thrombotic process in septal blood vessels of the lung; note initial organization of the thrombus in arterial lumen (H&E, 400x).

FIGURE 41B shows a representative image of the immunohistochemistry analysis of tissue sections of septal blood vessels from the lung of a COVID-19 patient. As shown in FIGURE 41B, similar pathologic features as shown in FIGURE 41A are extensively notable in most septal vessels in lung area unaffected by destructive inflammatory process (H&E, 400x).

FIGURE 41C shows a representative image of the immunohistochemistry analysis of tissue sections of medium diameter lung septal blood vessels from a COVID-19 patient. As shown in FIGURE 41C, medium diameter lung septal blood vessel (circled) with complete

lumen thrombosis; immunohistochemical brown staining for CD34 (endothelial marker) demonstrated severe endothelial damage with cell shrinkage, degenerated hydropic cytoplasm (see arrow on the right) and adhesion of lymphocytes on endothelial surface (see arrow on the left).

FIGURE 41D shows a representative image of the immunohistochemistry analysis of tissue sections of liver parenchyma from a COVID-19 patient. As shown in FIGURE 41D, vascular alteration was also observed in liver parenchyma with large vessel partial lumen thrombosis (H&E, 400x).

Circulating Endothelial Cells (CEC) identification and count

Circulating endothelial cells (CEC) have been used as a biomarker for endothelial cell dysfunction (see Farinacci M et al., *Res Pract Thromb Haemost* 3:49-58, 2019), and it has been shown that CEC counts are elevated in patients with sepsis-related ARDS compared to those with sepsis without ARDS (Moussa M et al., *Intensive Care Med* 41(2):231-8, 2015). Results have also been published in the setting of acute Graft versus Host Disease (GvHD) where an immune-mediated attack of vascular endothelial cells leads to their detachment from the vessel wall and mobilization into the blood stream (see, e.g, Almici et al., *Bone Marrow Transplant* 52:1637-1642, 2017).

Based on these initial observations and published findings in acute graft-versus-host disease (GvHD), prior to the initiation of the study with narsoplimab, we began measuring CEC counts in a non-study cohort of molecularly confirmed COVID-19 patients randomly selected in our hospital. In this non-study cohort of 33 COVID-19 patients, we found that CEC/mL of peripheral blood (median 110, range 38-877) were significantly increased compared to healthy controls (median 7, range 0-37 (P=0.0004), as shown in FIGURE 42A.

In this study, the number of CEC/ml was measured in COVID-19 patients before and after treatment with narsoplimab. As noted above, interestingly, it was determined that the number of CEC/ml of peripheral blood (median 110, range 38-877) was significantly increased in an independent cohort of COVID-19 patients when compared to

healthy normal subjects (median 7, range 0-37) (see FIG. 42A). An increased number of CEC/ml (median 334, range 0-9315) was also confirmed in the six patients that were selected for the treatment with narsoplimab. After treatment with narsoplimab, a rapid decrease in the number of CEC/ml was documented after the first two doses (median 92 CEC/mL, range 18-460) and confirmed after the fourth dose (median 73, range 0-593), as shown in FIG 42B. It was further confirmed that the number of CEC/mL was also decreased after the sixth dose of narsoplimab (median 59, range 15-276) (data not shown in FIGURE 42B).

FIGURE 42A graphically illustrates the CEC/ml counts in normal healthy controls (n=6) as compared to the CEC/ml counts in COVID-19 patients that were not part of this study (n=33). As shown in FIGURE 42A, when compared to healthy normal subjects, it was determined that the number of CEC/ml was significantly increased in this independent cohort of COVID-19 patients.

FIGURE 42B graphically illustrates the CEC/ml counts in the 6 patients selected for this study before (baseline) and after treatment with narsoplimab, boxes represent values from the first to the third quartile, horizontal line shows the median value and the whiskers indicate the min and max value. As shown in FIGURE 42B, an increased CEC/ml was also confirmed in the six patients that were selected for the treatment with narsoplimab, which rapidly decreased after treatment with narsoplimab.

Because our hospital established guidelines implementing standard steroid use for COVID-19 patients 16 days after the initiation of this study, steroid treatment was given to five of the six patients as part of the supportive therapy, beginning 2 to 10 days following initiation of narsoplimab. For this reason, the number of CEC/ml were also evaluated in a separate group of four patients (all female, median age 83 years with a range of 62 to 90 years, three requiring oxygen by mask and one on CPAP) who received only steroids. In these four patients, the CEC counts evaluated after 48 hours were found to be unaffected by steroid administration (p=0·38). In two additional patients receiving

only steroids, CEC counts were evaluated at baseline and after 4 weeks of steroid-inclusive supportive treatment. In the first patient, whose clinical course progressively worsened, CEC counts remained unaffected (271/mL vs. 247/mL) while, in the second, clinical improvement was accompanied by a simultaneous decrease of CEC (165 vs. 65/mL).

In the six narsoplimab-treated patients, CEC/mL were markedly increased at baseline (median 334, range 0-9315). With narsoplimab, CEC counts rapidly decreased after the second (median 92 CEC/mL, range 18-460), fourth (median 72·5, range 0-593) and sixth (median 59, range 15-276) doses of treatment (p=0·01). Serum concentrations of IL-6, IL-8, CRP and LDH also markedly decreased with narsoplimab treatment as further described below.

Serum levels of C Reactive Protein (CRP), Lactate Dehydrogenase (LDH) and cytokines

FIGURE 43 graphically illustrates the serum level of C Reactive Protein (CRP) (median; interquartile range (IQR)) in 6 patients with COVID-19 at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab. As shown in Table 13, the serum level of CRP in healthy subjects is in the range of (0.0-1.0 mg/dl) and the median level of CRP in the 6 COVID-19 patients prior to start of treatment was 14 mg/dl. As shown in FIGURE 43, after 2 weeks of treatment with narsoplimab, the level of CRP in the 6 COVID-19 patients was reduced to a median level of nearly 0.0 mg/dl, which is within the normal range of healthy subjects.

FIGURE 44 graphically illustrates the serum level of Lactate Dehydrogenase (LDH) (median; IQR) in 6 patients with COVID-19 at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab. As shown in Table 13, the serum level of LDH in healthy subjects is in the range of (120-246 U/l) and the median level of LDH in the 6 COVID-19 patients prior to start of treatment was 518 U/l. As

shown in FIGURE 44, after 2 weeks of treatment with narsoplimab, the level of LDH in the COVID-19 patients was reduced to a median level of about 200 U/l, which is within the normal range of healthy subjects.

FIGURE 45 graphically illustrates the serum level of Interleukin 6 (IL-6) pg/mL (median; interquartile range (IQR)) in 6 patients with COVID-19 at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab. As shown in FIGURE 45, the median level of IL-6 in the COVID-19 patients at baseline prior to treatment was about 180 pg/mL. After 1 dose of narsoplimab (pre-dose 2), the median level of IL-6 in the COVID-19 patients was reduced to about 40 pg/mL and after 2 doses of narsoplimab (pre-dose 3), the median level of IL-6 in the COVID-19 patients was further reduced to about 10 pg/mL.

FIGURE 46 graphically illustrates the serum level of Interleukin 8 (IL-8) pg/mL (median; interquartile range (IQR)) in 6 patients with COVID-19 at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab. Treatment with narsoplimab was given on Day 1, Day 4, Day 7, Day 11 and Day 14. As shown in FIGURE 46, the median level of IL-8 in the COVID-19 patients at baseline prior to treatment was about 30 pg/mL. After 1 dose of narsoplimab (pre-dose 2), the median level of IL-8 in the COVID-19 patients was reduced to about 20 pg/mL and after 2 doses of narsoplimab (pre-dose 3), the median level of IL-8 in the COVID-19 patients was further reduced to about 15 pg/mL.

Clinical Outcomes After Treatment with narsoplimab

The clinical characteristics of the 6 patients selected for treatment with narsoplimab are summarized in Table 13. The median age was 56.5 years and most of the patients were males (83%). All patients were overweight or obese based on a body mass index (BMI) $\geq$ 25 and $\geq$ 30, respectively. At enrollment, all patients had pneumonia/ARDS requiring CPAP, with two patients rapidly deteriorating and requiring intubation soon after enrollment. Treatment with narsoplimab started within 48 hours

from the beginning of non-invasive ventilation with CPAP. A summary of the clinical outcome observed in these patients treated with narsoplimab is presented below in Table 14, which has been updated to reflect the patient status after treatment. Patients received narsoplimab administration twice weekly. Following treatment, the respiratory distress of 4 patients (67%) improved and they reduced the ventilatory support from CPAP to high flow oxygen after a median of 3 narsoplimab doses (range 2-3). Oxygen support was then decreased and stopped until discharge in 3 patients. As documented by a contrast enhanced CT scan, patient #4 developed a massive pulmonary embolism at day 4 after treatment start. For this reason, low molecular weight heparin was added on the top of the ongoing narsoplimab and a rapid improvement of the clinical and CT scan picture was documented after 7 days. In the last two patients (#5 and #6) a rapid and progressive worsening of severe ARDS was documented soon after the enrolment. In case #5 the severe ARDS (with a PiO_2/FiO_2 value of 57) lead the patient to be intubated at day 4. Nonetheless, the subsequent clinical outcome was rapidly favorable and the patient was discharged from the ICU after 3 days. After 2 days of CPAP he is now stable with low flow oxygen support. In case #6, severe ARDS developed 4 days after the enrolment and the patient required intubation. Similar to the previous case, she was placed back in CPAP and subsequently in high flow oxygen due to a rapid clinical improvement and later discharged.

No treatment-related adverse events were reported in this study.

TABLE 14: Patient Outcomes to Date

Patient	**Dosing of narsoplimab**	**Outome to Date**
1	6 doses	Discontinued CPAP within 1 week of treatment initiation, discharged on Day 18, no steroids
2	5 doses	Discontinued CPAP within 1 week of treatment initiation, steroids started on day 10, discharged on Day 16

3	5 doses *updated: 7 doses	Was at home greater than 1 week with rapidly progressive respiratory distress before admission; discontinued CPAP within 11 days after treatment initiation, started steroids on day 10, discharged on Day 22*
4	4 doses so far, dosing continues ** updated: 8 doses	Course complicated by multiple pulmonary emboli determined to predate narsoplimab treatment; started steroids on day 1, developed pulmonary embolism on day 4, improved by day 7 with improved CT scan, discontinued CPAP within 12 days, stabilized with narsoplimab, still improving and dosing continues**updated: nasal cannula on day 26, room air on day 28 and discharged on day 33.
5	3 doses so far, dosing continues ***updated: 8 doses	Started steroids on day 3, was able to receive only 2 doses of narsoplimab before requiring intubation and transfer to ICU on day 4; stabilized with narsoplimab, improved, extubated and transferred to step-down unit back to CPAP on Day 7, discontinued CPAP on day 9, still improving and dosing continues*** updated: nasal cannula on day 25, room air on day 27 and discharged on day 33.
6	3 doses so far, dosing continues ****updated: 8 doses	Started steroids on day 1, was able to receive only 1 dose of narsoplimab before requiring intubation and transfer to ICU on day 3, stabilized on narsoplimab and remains intubated; improving and dosing continues****updated: extubated and transferred to CPAP on day 20, discontinued CPAP on day 82 and moved to nasal cannula, room air on day 85, discharged on day 90.

*, **, ***, **** see updates on patients 3-6 described below.

DISCUSSION

The findings in this study indicate that endothelial injury and thrombosis are central to the pathophysiology of COVID-19-related lung injury. Patients with severe respiratory failure demonstrated not only markedly elevated levels of CRP, LDH, IL-6 and IL-8 but also of circulating endothelial cells (CEC). This novel observation is in keeping with the histopathological findings detected in the lung and the liver that showed a marked endothelial injury and thrombosis in COVID-19 patients. The multi-organ microvascular histopathological changes, specifically the formation of microvascular thrombi, resemble those of HSCT-TMA, further supporting the role of endothelial injury in COVID-19-related pulmonary injury. Endothelial injury is known to be a central component of the pathophysiology of complement activation that is present in ARDS (Thompson BT, et al., *N Engl J Med*, 377(6):562–572, 2017). Complement activation has also been reported in models of SARS and MERS and is important in other conditions characterized by endothelial injury. Endothelial injury, the underlying cause of increased capillary permeability and pulmonary edema in ARDS, can also cause microvascular angiopathy and thrombosis.

The complement system is an important part of the immune system. Three pathways activate complement in response to distinct initiating events: the classical, lectin, and alternative pathways. The lectin pathway of complement is part of the innate immune response. A pattern-recognition system, activation of the lectin pathway is initiated by members of the MASP enzyme family (MASP-1, MASP-2 and MASP-3). These proteases are synthesized as proenzymes that form a complex in blood with lectins, specifically mannan-binding lectin (MBL), the ficolins, and collectins. These lectins recognize and bind to carbohydrate patterns found on the surfaces of pathogenic microorganisms or injured host cells, targeting MASPs to their site(s) of action and leading to their activation. In this way, lectin pathway activation occurs on the surface of damaged endothelial cells. As described in Example 20, the lectin pathway activation was expected to occur in the setting of COVID-19-related endothelial injury.

MASP-2 is the key enzyme responsible for activation of the lectin pathway, Once activated, MASP-2 cleaves complement component 2 (C2) and C4, initiating a series of enzymatic steps that result in the activation of C3 and C5, yielding the anaphylatoxins C3a and C5a, and the formation of C5b-9 (the membrane attack complex). Preclinically, C3a and C5a have induced endothelial activation associated with endothelial injury and pro-inflammatory changes, leukocyte recruitment, and endothelial apoptosis. Membrane-bound C5b-9 also can cause cell lysis. Even when sub-lytic, C5b-9 causes additional cell injury that induces secretion of prothrombotic factors, platelet activation, upregulation of adhesion molecules, and dysfunctional morphological changes in the endothelium (Kerr H, Richards A. *Immunobiology* 217(2):195–203, 2012). These complement-mediated activities can amplify endothelial injury and dysfunction, causing or worsening clinical condition. A recent publication by Gao et al. reports the core involvement of MASP-2 and the lectin pathway in the pathophysiology of SARS and MERS in animal models. MASP-2, the key enzyme responsible for lectin pathway activation, binds and undergoes activation by the COVID-19 N protein (Gao et al., *medRxiv* 2020, 2020.03.29.20041962) and has been found in the microvasculature of lung tissue in patients with severe COVID-19 (Magro C., et al., *Transl Res* 2020; doi.org/10.1016/j.trsl.2020.04.007). Activated MASP-2 initiates a series of enzymatic steps that results in production of the anaphylatoxins C3a and C5a and in formation of the membrane attack complex C5b-9 (Dobo et al., *Front Immunol* 9:1851, 2018), which can induce proinflammatory responses and cause cell lysis and death. MASP-2 can also cleave C3 directly through the C4 bypass (Yaseen S. et al., *FASEB J* 31(5):2210-9, 2017). Importantly, MASP-2 is located upstream in the lectin pathway, so inhibition of MASP-2 does not interfere with the lytic arm of the classical pathway (i.e., C1r/C1s-driven formation of the C3 and C5 convertases), preserving the adaptive immune response needed to fight infection (Schwaeble et al., *Proc Natl Acad Sci* 108(18):7523-8, 2011).

In addition to its role in complement, MASP-2 acts directly on the coagulation cascade and the contact system, cleaving prothrombin to thrombin and forming fibrin

clots (Gulla K.C., *Immunology* 129(4):482-95, 2010; Krarup A. et al., *PLoS One* 2(7):e623, 2007). Narsoplimab not only inhibits lectin pathway activation but also blocks microvascular injury-associated thrombus formation as well as MASP-2-mediated activation of kallikrein and factor XII, as described in WO2019246367, hereby incorporated herein by reference. These activities could contribute to beneficial effects by inhibiting microvascular thrombosis, which may have played an important therapeutic role in the narsoplimab-treated patients, particularly those who suffered massive pulmonary thromboses. Narsoplimab does not prolong bleeding time nor does it affect prothrombin or activated partial thromboplastin times, and no bleeding was observed in the narsoplimab-treated patients. While not wishing to be bound by any particular theory, it is believed that narsoplimab may block coagulation resulting from endothelial damage (associated with factor XII activation) but not extracellular matrix related (factor VII-driven) coagulation.

Lectin pathway inhibition has not previously been investigated as a treatment for COVID-19. All patients in this study had COVID-19-related respiratory failure. In the current study, inhibition of MASP-2 and the lectin pathway by narsoplimab was associated with clinical improvement and survival in all COVID-19 patients treated with the drug. Following treatment with the MASP-2 inhibitor narsoplimab, all six patients recovered and were able to be discharged from the hospital. The clinical improvement observed in patients suffering from COVID-19-related respiratory failure following treatment with narsoplimab, which inhibits MASP-2 and lectin pathway activation, further supports an important role of the lectin pathway in COVID-19 pathophysiology. As described in this Example, all six COVID-19 patients demonstrated clinical improvement following narsoplimab treatment. In each case, COVID-19 lung injury had progressed to ARDS prior to narsoplimab treatment and all patients were receiving non-invasive mechanical ventilation, initiated for each at the time of hospital admission. Two patients experienced continued deterioration following the first dose of narsoplimab and required invasive mechanical ventilation. Both of these patients were subsequently able

to discontinue mechanical ventilation entirely with continued narsoplimab treatment. Two patients (one intubated and the other on CPAP) experienced massive bilateral pulmonary thromboses, and both patients completely recovered with narsoplimab, possibly benefitting from the drug's anticoagulant effects. The temporal patterns of laboratory markers (CEC, IL-6, IL-8, CRP and LDH) were consistent with the observed clinical improvement and with the proposed mechanism of action of narsoplimab. In particular, CEC counts appear to be a reliable tool to evaluate endothelial damage and treatment response in this disease. Notably, improvement in IL-6 levels and IL-8 levels also correlated temporally with narsoplimab treatment, suggesting that lectin pathway activation may precede cytokine storm elevation in COVID-19 and that lectin pathway inhibition has a potential beneficial effect on the cytokine storm described in patients with COVID-19 infection (Xiong Y, et al., *Emerg Microbes Infect* 9(1):761–770, 2020). Two weeks of narsoplimab dosing was planned initially but was increased to 3 to 4 weeks following the rise in CEC in patient #1 when dosing was first discontinued. Rebound pulmonary signs and symptoms have not been observed following 3 to 4 weeks of narsoplimab treatment. We saw no evidence of impaired viral defense in the narsoplimab-treated patients, and no narsoplimab-related adverse events were observed Notably, narsoplimab does not inhibit the alternative or classical complement pathways and does not interfere with the adaptive immune response or antigen-antibody complexing. No evidence of narsoplimab-related infection risk has been observed in clinical trials. In addition to inhibiting lectin pathway activation, narsoplmab has been demonstrated to block MASP-2-mediated cleavage of prothrombin to thrombin (Krarup A, et al., *PLoS One* ;2(7):e623, 2007), activation of kallikrein, and autoactivation of factor XII to XIIa. These activities could contribute to beneficial effects by inhibiting microvascular thrombosis. Narsoplimab does not prolong bleeding time nor does it affect either prothrombin or activated partial thromboplastin times. (Krarup PLoS One 2007)

The results described in this Example strongly implicate MASP-2-mediated lectin pathway activation caused by endothelial injury in the pathophysiology of COVID-19-

related lung injury. The improvement in the clinical status and laboratory findings following narsoplimab treatment is notable. These findings strongly suggest meaningful clinical efficacy with supportive evidence related to the drug's mechanism of action and the pathophysiology of the disease. Lectin pathway inhibition by narsoplimab appears to be a promising potential treatment of COVID-19-related lung injury.

Supplemental Data from the Clinical Study described in this Example

As described above in this Example, six patients with laboratory-confirmed COVID-19 and ARDS (per the Berlin criteria) were treated with narsoplimab (4 mg/kg intravenously (IV) twice weekly for 3 to 4 weeks. All patients received standard supportive care including prophylactic enoxaparin (Clexane, Sanofi Aventis) 4,000 IU/0.4 mL, azithromycin (Zitromax, Pfizer SpA, Italy) 500 mg once daily, hydroxychloroquine (Plaquenil, Sanofi Aventis) 200 mg twice daily, and darunavir and cobicistat (Rezolsta, Janssen-Cilag S.p.A., Italy) 800/150 mg once daily. Beginning March 27, per updated institutional guidelines, all Covid-19 patients in our hospital received methylprednisolone (1 mg/kg), which was administered to 5 of the 6 narsoplimab-treated patients.

Histopathological evaluation was performed on deceased COVID-19 patients who were not treated with narsoplimab. Clinical and laboratory measures, including blood counts, LDH and CRP levels, were collected per standard practice on narsoplimab-treated and patients not treated with narsoplimab. Routine blood examinations were collected prior to each narsoplimab dose and then twice weekly. Circulating endothelial cell counts and IL-6 and IL-8 levels were serially assessed by flow cytometry. Respiratory function was evaluated daily. All patients received chest computed tomography (CT) at hospital admission to document interstitial pneumonia, and if clinically indicated, during hospitalization to document pulmonary embolism. Chest radiography was also performed as clinically indicated.

Data are presented as frequency with percentage for categorical variables and median with range for continuous variables. Differences in clinical and laboratory

measures between time points were evaluated with non-parametric Friedman test. Pairwise-comparisons were performed using paired Wilcoxon signed-rank test. Significance at 5% was fixed. Analysis was performed using R software (version 3.6.2).

As described in this Example, autopsies were performed on an initial group of 20 deceased COVID-19 patients. Consistent with the clinical picture of frequently lethal pulmonary thromboembolism, the lungs and liver of most patients were found to be extensively affected by thromboses. Histologically, arterial thromboses were evident in septal vessels of the lung, including areas unaffected by the destructive inflammatory process. Immunohistochemical staining for CD34 (an endothelial cell marker) demonstrated severe endothelial damage with cell shrinkage, degenerated hydropic cytoplasm and adhesion of lymphocytes to endothelial cells, as shown in FIGURES 41A-D.

As described in this Example, inhibition of the lectin pathway of complement by narsoplimab was associated with clinical improvement in this study. Treatment with narsoplimab was associated with a rapid and sustained reduction of CEC paralleled by a concomitant reduction of serum IL-6, IL-8, CRP and LDH. In particular, CEC counts appear to be a reliable tool to evaluate the endothelial damage and treatment response in this disease. The temporal improvement of IL-6 and IL-8 with narsoplimab treatment suggests a potential beneficial effect on the cytokine storm described in patients with Covid-19 infection ((Xiong Y, et al., *Emerg Microbes Infect* 9(1):761–770, 2020). This study's findings indicate that endothelial injury is central to the pathophysiology of COVID-19-related lung injury. Patients with severe respiratory failure demonstrated not only markedly elevated levels of C-reactive protein (CRP) and lactate dehydrogenase (LDH), but also IL-6, IL-8 and circulating endothelial cells (CEC). This novel observation is consistent with the histopathological finding in the lung and liver showing marked endothelial injury and thrombosis in COVID-19 patients. The microvascular histopathological changes are very similar to those of the endothelial injury syndrome

HSCT-TMA, further supporting the role of endothelial injury in COVID-19-related pulmonary injury.

Two weeks of dosing was planned initially but was increased to 3-4 weeks following the rise in CEC in patient #1 when dosing was first discontinued. With the third week of dosing, the patient's CEC counts again improved. Rebound pulmonary signs and symptoms have not been observed following 4 weeks of narsoplimab treatment. We saw no evidence of impaired viral defense in the narsoplimab-treated patients. Notably, narsoplimab does not inhibit the classical or alternative complement pathways and does not interfere with the adaptive immune response or antigen-antibody complexing. No evidence of narsoplimab-related infection risk has been observed in clinical trials. In addition to inhibiting lectin pathway activation, narsoplimab has been shown to block MASP-2-mediated cleavage of prothrombin to thrombin, activation of kallikrein, and autoactivation of factor XII to XIIa. These activities could contribute to beneficial effects by inhibiting microvascular thrombosis, and this could have played an important therapeutic role, particularly in those patients who suffered massive pulmonary thrombosis. Narsoplimab does not prolong bleeding time nor does it affect prothrombin or activated partial thromboplastin times, and no bleeding was observed in the patients we treated.

Our findings strongly implicate lectin pathway activation caused by endothelial injury in the pathophysiology of Covid-19-related lung injury. Inhibition of the lectin pathway of complement by narsoplimab was associated with clinical improvement in all patients in this study. Narsoplimab was well tolerated, and no adverse drug reactions were reported. All patients improved during treatment and survived. The improvements in clinical status and laboratory findings following narsoplimab treatment are notable. These findings strongly suggest meaningful clinical efficacy with supportive evidence related to the drug's mechanism of action and the pathophysiology of the disease. Lectin pathway inhibition by narsoplimab appears to be a promising potential treatment of Covid-19-related lung injury.

Additional data is provided from the clinical study described in this Example.

The clinical characteristics of the 6 narsoplimab-treated patients are summarized in Table 13. Narsoplimab 4 mg/kg was administered intravenously twice weekly for 3 to 4 weeks. Following treatment, all patients improved clinically.

In 4 patients, enoxaparin was given at therapeutic doses (100 IU/kg twice daily) due to CT scan-documented pulmonary embolism (patients #4 and #6), medical decision (patient #3) and rapid deterioration of respiratory function requiring intubation (patient #5). Median follow-up was 27 days (16-90), and patients were administered narsoplimab twice weekly with a median of 8 total narsoplimab doses (range 5-8). Following treatment, all patients improved clinically. Four patients (67%) reduced ventilatory support from CPAP to high-flow oxygen (non-rebreather or Venturi oxygen mask) after a median of 3 narsoplimab doses (range 2-3).

FIGURE 50 graphically illustrates the clinical outcome of six COVID-19 infected patients treated with narsoplimab.

As shown in FIGURE 50, in 3 of these patients, oxygen support was weaned and then discontinued, and they were discharged following a median of 6 (5-8) total narsoplimab doses.

In patient #4, massive bilateral pulmonary emboli were documented by contrast-enhanced CT scan 4 days following enrollment. Enoxaparin was added to the ongoing narsoplimab dosing, and rapid clinical and radiographic (repeat CT scan) improvement was documented 11 days later (FIGURE 47A and FIGURE 47B) and was subsequently discharged.

In the 2 remaining patients (#5 and #6), rapid and progressively worsening severe ARDS was documented soon after enrollment.

In patient #5, severe ARDS (PaO_2/FiO_2 of 55) led to intubation at day 4. Nonetheless, the subsequent clinical outcome was rapidly favorable, and the patient was discharged from the intensive care unit after 3 days. Following 2 days of CPAP, he

stabilized with low-flow oxygen support. He subsequently required no oxygen and was discharged.

Patient #6 had PaO2/FiO2 of 60 and severe ARDS at enrollment and required intubation 2 days later. Her course was complicated by massive bilateral pulmonary thrombosis and nosocomical methicillin-resistant Staphylococcus aureus (MRSA) infection. Her condition improved and, after 18 days, she was extubated, tracheostomized (due to claustrophobia) and supported with low-flow oxygen. Her condition improved, oxygen support was removed and, at day 90, she was discharged. (day 33 to day 90 not shown in FIGURE 50)

No treatment-related adverse events were reported in this study.

As described above, in patient #4, massive bilateral pulmonary emboli were documented by contrast-enhanced CT scan 4 days following enrollment. Enoxaparin was added to the ongoing narsoplimab dosing, and rapid clinical and radiographic (repeat CT scan) improvement was documented 11 days later as shown in FIGURE 47A,B.

FIGURE 47A and FIGURE 47B are images from CT-scans taken of the lungs of patient #4 with COVID-19 pneumonia treated with narsoplimab.

FIGURE 47A shows the CT-scan of patient #4 on Day 5 since enrollment (i.e., after treatment with narsoplimab) wherein the patient is observed to have severe interstitial pneumonia with diffuse ground-glass opacity involving both the peripheral and central regions. Consolidation in lower lobes, especially in the left lung. Massive bilateral pulmonary embolism with filling defects in interlobar and segmental arteries (not shown).

FIGURE 47B shows the CT-scan of patient #4 on Day 16 since enrollment (i.e., after treatment with narsoplimab) in which the ground-glass opacity is significantly reduced with almost complete resolution of parenchymal consolidation. “Crazy-paving” pattern is observed with peripheral distribution, especially in the lower lobes. Evident

pneumomediastinum. Minimal filing defects in subsegmental arteries of the right lung (not shown).

FIGURE 48 graphically illustrates the serum levels of IL-6 (pg/mL) at baseline and at different time points after narsoplimab treatment (after 2 doses, after four doses) in the patients treated with narsoplimab. Boxes represent values from the first to the third quartile, horizontal line shows the median value, and dots show all patient values. FIGURE 48 provides an update of the IL-6 data presented in FIGURE 45.

FIGURE 49 graphically illustrates the serum levels of IL-8 (pg/mL) at baseline and at different time points after narsoplimab treatment (after two doses, after 4 doses) in the patients treated with narsoplimab. Boxes represent values from the first to the third quartile, horizontal line shows the median value, and dots show all patient values. FIGURE 49 provides an update of the IL-8 data presented in FIGURE 46.

FIGURE 50 graphically illustrates the clinical outcome of six COVID-19 infected patients treated with narsoplimab. The bar colors indicate the different oxygen support (CPAP: yellow; mechanical ventilation with intubation: red; non-rebreather oxygen mask: green; low-flow oxygen by nasal cannula: light green; room air: blue). Narsoplimab doses are marked by blue arrows. Black circle indicates the beginning of steroid treatment. Diamond symbol indicates TEP. Astericks (*) indicate discharged from the hospital. CPAP=continuous positive airway pressure. NRM=non-rebreather oxygen mask. VM=Venturi mask. TEP=pulmonary thromboembolism.

FIGURE 51A graphically illustrates the serum levels of Aspartate aminotransferase (AST) (Units/Liter, U/L) values before and after narsoplimab treatment. Black lines represent median and interquartile range (IQR). The red line represents normality level and dots show all patient values.

FIGURE 51B graphically illustrates the serum levels of D-Dimer values (ng/ml), in the four patients in whom base line values were available before treatment with narsoplimab started. Black circles indicate when steroid treatment was initiated. The red line represents normality level.

In summary, in this study, the first time a lectin-pathway inhibitor was used to treat COVID-19, six COVID-19 patients with ARDS requiring continuous positive airway pressure (CPAP) or intubation received narsoplimab. The median age of the patients was 57 years (range 47 – 63 years), 83 percent were men, and all had comorbidities. At baseline, circulating endothelial cell (CEC) counts and serum levels of interleukin-6 (IL-6), interleukin-8 (IL-8), C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer and aspartate transaminase (AST) – all markers of endothelial/cellular damage and/or inflammation – were significantly elevated. Narsoplimab treatment was begun within 48 hours of initiation of mechanical ventilation. Dosing was twice weekly for two to four weeks.

Study Results

- All narsoplimab-treated patients fully recovered, survived and were discharged from the hospital
- Narsoplimab treatment was associated with rapid and sustained reduction/normalization across all assessed markers of endothelial/cellular damage and/or inflammation – CEC, IL-6, IL-8, CRP LDH, D-dimer and AST
    - Temporal patterns of laboratory markers were consistent with the observed clinical improvement
    - In particular, CEC counts appear to be a reliable tool to evaluate endothelial damage and treatment response in this disease
    - The temporal improvement of IL-6 and IL-8 with narsoplimab treatment suggests that lectin pathway activation may precede cytokine elevation in COVID-19 and that lectin pathway inhibition has a beneficial effect on the cytokine storm described in patients with COVID-19 infection
- The courses of two patients (one intubated and the other on CPAP) were further complicated by massive bilateral pulmonary thromboses, and both patients completely recovered with narsoplimab, possibly benefitting from the drug's anticoagulant effects
- Narsoplimab was well tolerated in the study and no adverse drug reactions were reported
- Two control groups with similar entry criteria and baseline characteristics were used for retrospective comparison, both showing substantial mortality rates at 32 percent and 53 percent.

Conclusion

As demonstrated in this Example, inhibiting the lectin pathway of complement with narsoplimab may represent an effective treatment for Covid-19 patients by reducing Covid-19-related endothelial cell damage and thus the inflammatory status and thrombotic risk. Lectin pathway inhibition has not previously been investigated as a treatment for COVID-19. All patients in this study had COVID-19-related respiratory failure. Following treatment with the MASP-2 inhibitor narsoplimab, all patients recovered and were able to be discharged from the hospital, further supporting the importance of the lectin pathway in COVID-19 pathophysiology.

Use of other complement inhibitors in COVID-19 have been reported. AMY-101, a compstatin-based C3 inhibitor (Mastaglio S. et al., *Clin Immunol* 215:108450, 2020) was used in one patient and eculizumab was administered together with antiviral and anticoagulant therapy to four patients (Diurno F. et al., *Eur Rev Med Pharmacol Sci* 24(7):4040-7, 2020. These five patients were on CPAP and survived. Two COVID-19 patients on high-flow nasal oxygen received a C5a antibody in conjunction with supportive therapy, including antiviral therapy, following steroid treatment and these two patients also survived. Collectively, these reports support our findings with narsoplimab. However, unlike C3 and C5 inhibitors, the MASP-2 antibody narsoplimab fully maintains classical complement pathway function and does not interfere with the adaptive immune response or the antigen-antibody complex-mediated lytic response (Schwaeble W. et al., *Proc Natl Acad Sci* 108(18):7523-8, 2011). No evidence of narsoplimab-related infection risk has been observed in narsoplimab clinical trials.

While this was a compassionate use, single-arm study, two different control groups provide a retrospective comparison. The first was described in a recently published article by Gritti et al (*medRxiv* 2020:2020.04.01.20048561) evaluating the use of siltuximab, an IL-6 inhibitor, in COVID-19 patients. The siltuximab study and our narsoplimab study share the same lead investigators (G.G. and A.R.), entry criteria and patient characteristics (i.e., demographics, symptoms, comorbidities, ARDS severity,

laboratory values and respiratory support at enrollment). In that study, mortality rates in the siltuximab-treated and the control groups were 33% and 53%, respectively. The second retrospective comparator is represented by the 33 patients who were randomly selected within our hospital to assess the viability of CEC measurements in COVID-19 patients. Of these 33 patients, 22 met the same entry criteria and had similar baseline characteristics as the narsoplimab-treated patients. Median baseline CEC count, however, in the control group compared to that in the narsoplimab-treated group was 101/mL versus 334/mL, respectively. Interestingly, 20 of these 22 patients (91%) were treated with IL-6 inhibitors (tocilizumab or siltuximab) and/or steroids, and the group had an overall 30-day mortality of 32%. The mortality rate was still 31% when the outcome analysis was restricted to 16 patients matched for age to narsoplimab-treated patients (median 58 years, range 51-65 years). In this latter group, 94% received IL-6 and/or steroid therapy and the median baseline CEC count at 55/mL was six-fold lower than in the narsoplimab-treated patients.

The use of steroids in COVID-19 has resulted in reports of mixed outcomes (Veronese N. et al., Front Med (Lausanne) 7:170, 2020). Most recently, the Randomised Evaluation of COVID-19 therapy (RECOVERY) trial, demonstrated that dexamethasone reduced 28-day mortality in patients on invasive mechanical ventilation by 28.7% (29.0% versus 40.7% with usual care), by 14% (21.5% versus 25.0% with usual care) in those receiving oxygen support without invasive mechanical ventilation and had no effect on mortality in patients not receiving respiratory support at randomization (17.0% versus 13.2% with usual care) (Horby P. et al., *medRxiv* 2020:2020.06.22.20137273). Based on these data and the experience at our hospital, we believe that steroids have a role to play in treating COVID-19 patients with respiratory dysfunction, acting to tamp down the inflammatory response. In the narsoplimab-treated group, one (patient #1) of the six patients did not receive steroids. Subsequently, in late March, institutional guidelines were updated, requiring that all patients in our hospital receive steroids. Of the five narsoplimab-treated patients who received steroids, two (patients #2 and #3) initiated

them after already improving such that CPAP was no longer required or was discontinued the following day. As described previously, we evaluated CEC counts in a separate group of four patients receiving only steroids for a short duration, and the counts were found to be unaffected by steroid administration. This suggests that any beneficial effect of steroids on COVID-19-associated endothelial damage may be delayed and had little effect on the recovery course of patients #2 and #3.

In conclusion, our findings strongly suggest that endothelial injury-induced activation of MASP-2 and the lectin pathway play a central role in the pathophysiology of COVID-19-related lung injury. The improvements in clinical status and laboratory findings following narsoplimab treatment are notable. There findings strongly suggest meaningful clinical efficacy and provide supportive evidence related to the drug's mechanism of action and the pathophysiology of the disease. Lectin pathway inhibition by narsoplimab appears to be a promising treatment of COVID-19-related lung injury and endothelial damage-associated thromboses.

Further Supplemental Data from the Clinical Study described in this Example

As described above in this Example, six patients with laboratory-confirmed COVID-19 and ARDS (per the Berlin criteria) were treated with narsoplimab (4 mg/kg intravenously (IV) twice weekly for 3 to 4 weeks. As described in this Example, all six patients in this study had COVID-19-related respiratory failure. Following treatment with the MASP-2 inhibitor narsoplimab, all patients recovered and were able to be discharged from the hospital. These patients have been monitored since discharge from the hospital. As of October 22, 2020, (5 to 6 months following treatment with narsoplimab), all six patients are clinically normal with no evidence of any long-term sequelae that has been reported in COVID-19 patients not treated with narsoplimab. The clinical laboratory measures for all six patients are also normal as of October 22, 2020, including serum levels of D-Dimers, were all found to be in the normal range (see Table 15 below).

Table 15 shows the baseline laboratory measures taken from the six COVID-19 infected patients at hospital admission (baseline) prior to treatment with narsoplimab, as compared to the laboratory measures taken in October, 2020, five to six months later.

Table 15 summarizes the clinical characteristics of the six narsoplimab-treated patients at baseline (prior to treatment, see also Table 13) and as measured in October 2020, five to six months post-treatment.

TABLE 15: Laboratory Measures of COVID-19 Patients Treated with narsoplimab

Laboratory Findings	Baseline:All Patients Prior to narsoplimab treatment (March-June, 2020) (N=6)	Last Evaluation (October 2020) (N=6)
White cell count- – per mm^3, median (range)	8335 (6420-10,120)	7320 (3200-8770)
……>10,000 per mm^3 – number (%)	2 (33)	0 (0)
……<4000 per mm^3 – number (%)	0 (0)	1 (17)
Lymphocyte count- per mm^3 median (range)	875 (410-1290)	2815 (810-3780)
Platelet count x 10^3 per mm^3 median (range)	282 (199-390)	238 (170-354)
Hemoglobin – g/dL, median (range)	13.4 (13.2-14.1)	14.8 (13.4-15.8)
Distribution of other findings (laboratory reference ranges)		
C-reactive protein (0.0-1.0 mg/dL)	14 (9.5-31.3)	0.15 (0-0.5)
Lactate dehydrogenase (120/246 U/L)	518.5 (238-841)	212 (119-249)
Aspartate aminotransferase (13-40 U/L)	78.5 (51-141)	18 (12-29)
Alanine aminotransferase (7-40 U/L)	73 (37-183)	22.5 (20-67)
Creatinine (0.3-1.3 mg/dL)	0.85 (0.38-1.33)	0.94 (0.51-1.07)
D-dimer (<500 ng/mL)		
<190 – no. (%)	0 (0)	3 (50)
>190 – median (range)	1250.5 (943-1454)	324 (202-390)

These results demonstrate that treatment of COVID-19 infected patients with narsoplimab in these six patients has led to a complete recovery in these patients with no evidence of any long-term sequelae.

As widely reported, many COVID-19 infected patients, including those with mild symptoms as well as those with severe COVID-19 related lung injury such as ARDS and/or thrombosis, suffer from immediate complications from COVID-19 infection as well as long-term sequelae even after recovery from the initial infection, also referred to as "long-haulers." As described in Marshall M., ("The lasting misery of coronavirus long-haulers," *Nature* Vol 585, 9/17/2020, page 339-341) people with more severe COVID-19 infections may experience long-term damage in their lungs, heart, immune system, brain, central nervous system, kidneys, gut and elsewhere, and even mild cases of COVID-19 infection can cause a lingering malaise similar to chronic fatigue syndrome. As further described in Marshall (2020), immediate and long-term sequelae from COVID-19 infection include cardiovascular complications (including myocardial injury, cardiomyopathy, myocarditis, intravascular coagulation, stroke, venous and arterial complications, and pulmonary thrombosis); neurological complications (including cognitive difficulties, confusion, memory loss, also referred to as "brain fog", headache, stroke, dizziness, syncope, seizure, anorexia, insomnia, anosmia, ageusia, myoclonus, neuropathic pain, myalgias; development of neurological disease such as Alzheimer's disease, Guillian Barre Syndrome, Miller-Fisher Syndrome, Parkinson's disease); kidney injury (such as acute kidney injury (AKI), pulmonary complications including lung fibrosis, dyspnea, pulmonary embolism); inflammatory conditions such as Kawasaki disease, Kawasaki-like disease, multisystem inflammatory syndrome in children; and multi-system organ failure. See also Troyer A. et al., *Brain, Behavior and Immunity* 87:43-39, 2020; Babapoor-Farrokhram S. et al., *Life Sciences* 253:117723, 2020; and Heneka M. et al., *Alzheimer's Research & Therapy*, vol 12:69, 2020. As further described in Yelin D. et al., *Lancet Infect Dis* 2020, 9/1/2020, long-term complaints of people recovering from acute COVID-19 include: extreme fatigue, muscle weakness, low

grade fever, inability to concentrate, memory lapses, changes in mood, sleep difficulties, needle pains in arms and legs, diarrhea and vomiting, loss of taste and smell, sore throat and difficulties in swallowing, new onset of diabetes and hypertension, skin rash, shortness of breath, chest pains and palpitations.

As described in this Example, treatment of COVID-19 infected patients with narsoplimab in these six patients has led to a complete recovery in these patients with no evidence of any long-term sequelae from COVID-19 infection.

EXAMPLE 22

OMS646 (narsoplimab) Treatment in COVID-19 Infected Patient #7

This Example describes the use of narsoplimab (OMS646) in the treatment of a seventh COVID-19 infected patient (patient #7) using the methods described in Example 20 and Example 21. The results described in this Example are consistent with the results observed with the six COVID-19 infected patients in Example 21 and further confirm the efficacy of narsoplimab in the treatment of COVID-19-infected patients.

Methods and Results:

Patient #7 is a COVID-19 infected 76-year-old obese, diabetic man with a long history of smoking and COPD who had also undergone surgery for prostate cancer (i.e., a patient classified as "high risk" for COVID-19 related complications). The patient entered the hospital in Bergamo initially requiring oxygen by nasal cannulae. His respiratory status quickly deteriorated, first requiring oxygenation by mask followed by mechanical ventilation with continuous positive airway pressure and then intubation. After intubation, treatment was initiated with narsoplimab (OMS646), a fully human monoclonal antibody comprised of immunoglobulin gamma 4 (IgG4) heavy-chain and lambda light-chain constant regions. Narsoplimab binds to and inhibits MASP-2 with sub-nanomolar affinity. Treatment of patient #7 with narsoplimab was carried out in accordance with the methods described in Example 20 and Example 21 at a dosage of 4 mg/kg administered intravenously twice weekly for 2 to 4 weeks, with a maximum of 6 to 8 doses (i.e., a dosing duration of two weeks, three weeks or four weeks). To date,

patient #7 has received 4 doses of narsoplimab. After treatment with narsoplimab patient #7 rapidly improved and he was extubated after the second dose. His laboratory findings are show in FIGURE 52A-E, described below, with the dosing denoted by the vertical arrows on each graph.

FIGURE 52A graphically illustrates the serum level of D-dimer values (ng/mL) in patient #7, critically ill with COVID-19, at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab. Dosing with narsoplimab is indicated by the vertical arrows. The red horizontal line represents normality level.

FIGURE 52B graphically illustrates the serum level of C reactive protein (CRP) in patient #7, critically ill with COVID-19. at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab. Dosing with narsoplimab is indicated by the vertical arrows. The red horizontal line represents normality level

FIGURE 52C graphically illustrates the serum level of aspartate aminotransferase (AST) (Units/Liter, U/L) in patient #7, critically ill with COVID-19, at baseline prior to treatment (day 0) and at different time points after narsoplimab treatment. Dosing with narsoplimab is indicated by the vertical arrows. The red horizontal line represents normality level.

FIGURE 52D graphically illustrates the serum level of alanine transaminase (ALT) (Units/Liter, U/L) in patient #7, critically ill with COVID-19, at baseline prior to treatment (day 0) and at different time points after narsoplimab treatment. Dosing with narsoplimab is indicated by the vertical arrows. The red horizontal line represents normality level.

FIGURE 52E graphically illustrates the serum level of lactate dehydrogenase (LDH) in patient #7 with severe COVID-19 at baseline prior to treatment (day 0) and at different time points after treatment with narsoplimab. Dosing with narsoplimab is indicated by the vertical arrows. The red horizontal line represents normality level.

Summary of Results:

As shown in FIGURES 52A to 52E, at the time of hospital admission and prior to treatment with narsoplimab, patient #7 had a high serum level of D-dimer (considered the

premier marker of coagulability in COVID-19), a high serum level of C-reactive protein (a marker of inflammation), a high serum level of aspartate aminotransferase (an enzyme marker of critical illness in COVID-19), a high serum level of alanine transaminase (a marker of liver function), and a high serum level of lactate dehydrogenase (a marker of cellular death). As further shown in FIGURES 52A to 52E, patient #7 improved following the first dose of narsoplimab, with all the above laboratory measures dropping near or to normal levels after the fourth dose. He was extubated after the second dose of narsoplimab. The ICU staff were amazed with his rapid improvement following treatment with narsoplimab. The rapid improvement of patient #7 reported in this Example is consistent with the recovery of COVID-19 infected patients #1-6 after treatment with narsoplimab as described in Example 21.

Additional data are provided from the clinical study described in this Example:

As described in this Example, patient #7 improved following the first dose of narsoplimab, he was extubated after the second dose, and all the above laboratory measures dropped near or to normal levels after the fourth dose. As an update, patient #7 received a total of 6 narsoplimab doses and was discharged from the hospital. As shown in FIGURE 53, serology data from patient #7 over time indicate that appropriately high titers of anti-SARS-CoV-2 antibodies were generated during treatment with narsoplimab, indicating that narsoplimab does not impede effector function of the adaptive immune response.

In addition to patients #1-7 described herein, numerous additional COVID-19 patients suffering from ARDS (total of n=19) have been treated with narsoplimab under compassionate use in accordance with the methods described in Example 20 and Example 21 at a dosage of 4 mg/kg administered intravenously twice weekly for 2 weeks to 4 weeks or 5 weeks, with a maximum of 4 to 10 doses (for two weeks, three weeks, four weeks or 5 weeks). All the additional patients described in this example were severely ill with COVID-19-associated ARDS prior to treatment, all were intubated, with the majority initiating narsoplimab multiple days after intubation and all had failed other

therapies prior to initiating narsoplimab. Strikingly positive outcomes were observed in most patients treated with narsoplimab, similar to those observed with patients #1-7 described herein. Most COVID-19 patients treated with narsoplimab showed rapid and marked improvement in clinical symptoms and laboratory values and were subsequently discharged from the hospital. Importantly, narsoplimab-treated COVID-19 patients for whom follow-up data (5-6 months after cessation of narsoplimab treatment) are available show no observed clinical or laboratory evidence of long-term sequelae. It was also observed that COVID-19 patients treated with narsoplimab that survived developed appropriately high anti-SARS-CoV-2 antibodies as described above for patient #7. These results demonstrate that treatment with narsoplimab, which specifically inhibits the lectin pathway and leaves the alternative pathway and the classical pathway of complement fully functional, preserves the infection-fighting effector function of the adaptive immune response and maintains the antigen-antibody complex-mediated lytic response that plays an important role in killing virus-infected cells.

A brief description of the treatment course of the critically ill COVID-19 patients #8-15 treated with narsoplimab in Bergamo, Italy and patients #1-4 treated with narsoplimab in the U.S. are provided below:

Patient #8 (Bergamo, Italy)

Patient #8 was a 76-year-old obese man with congestive heart failure, hypertension, dyslipidemia and severe COVID-19. He began narsoplimab treatment 3 days after intubation and died of complications of pre-existing cardiomyopathy following the 3rd dose. His D-dimer and LDH levels were improved after 1 to 2 doses of narsoplimab. Serology data indicate that he did not develop a high titer of anti-SARS-CoV-2 antibodies.

Patient #9 (Bergamo, Italy)

Patient #9 is a 41-year-old overweight man with severe COVID-19. He began narsoplimab treatment 2 days after intubation and was extubated after the 2nd dose. He received a total of 6 doses and was discharged from the hospital. His D-dimer levels and LDH levels were improved after 1 to 2 doses of narsoplimab. Serology data indicate that he developed appropriately high titers of anti-SARS-CoV-2 antibodies during the course of treatment with narsoplimab.

Patient #10 (Bergamo, Italy)

Patient #10 is a 65-year-old overweight man with severe COVID-19. He began narsoplimab treatment 3 days after intubation and was extubated after the 4th dose. He received a total of 9 doses of narsoplimab and was discharged from the hospital. His D-dimer levels and LDH levels were improved after 1 to 2 doses of narsoplimab. Serology data indicate that he developed appropriately high titers of anti-SARS-CoV-2 antibodies during the course of treatment with narsoplimab.

Patient #11 (Bergamo, Italy)

Patient #11 was a 68-year-old overweight man with hypertension, dyslipidemia and severe COVID-19. He began narsoplimab treatment 13 days after intubation. He received a total of 7 doses and died of multi-organ failure. Serology data indicate that he did not develop a high titer of anti-SARS-CoV-2 antibodies.

Patient #12 (Bergamo, Italy)

Patient #12 is a 62-year-old overweight man with diabetes, hypertension, dyslipidemia and severe COVID-19. He began narsoplimab treatment 2 days after intubation. He developed a nosocomial infection requiring re-intubation followed by tracheostomy. He received a total of 6 doses of narsoplimab and was discharged to a rehabilitation facility. Serology data indicate that he developed appropriately high titers of anti-SARS-CoV-2 antibodies during the course of treatment with narsoplimab.

Patient #13 (Bergamo, Italy)

Patient #13 is a 62-year-old man with hypertension and severe COVID-19. He began narsoplimab treatment 3 days after intubation and was extubated after 7 doses. He received a total of 8 doses of narsoplimab and is breathing spontaneously. Serology data indicate that he developed appropriately high titers of anti-SARS-CoV-2 antibodies during the course of treatment with narsoplimab.

Patient #14 (Bergamo, Italy)

Patient #14 is a COVID-19 infected 64-year-old man with hypertension. He began narsoplimab treatment 6 days after intubation and was extubated after 7 doses. He received a total of 8 doses of narsoplimab, began breathing spontaneously and was discharged to a rehabilitation facility. Serology data indicate that he developed appropriately high titers of anti-SARS-CoV-2 antibodies during the course of treatment with narsoplimab.

Patient #15 (Bergamo, Italy)

Patient #15 is a 79-year-old man with hypertension and severe COVID-19. He began narsoplimab treatment 3 days after intubation. He was extubated after 3 doses of narsoplimab and is continuing to improve. Serology data are not yet available.

Patient #1 (U.S.)

Patient #1 is a 53-year-old man with severe COVID-19 who had been intubated for about 2 weeks after failing other therapy regimens including remdesivir, tocilizumab, initial steroid therapy and convalescent plasma. He began treatment with narsoplimab and concurrently received enoxaparin and methylprednisolone. He responded quickly and was extubated soon after the 5th dose of narsoplimab. He was discharged to a rehabilitation facility for physical therapy, continued to improve and returned to work last month. He reportedly has no longer-term sequelae of COVID-19.

Patient #2 (U.S.)

Patient #2 is a 55-year-old African American woman with rapidly deteriorating respiratory function as a result of severe COVID-19. She began treatment with narsoplimab several days after intubation. Her oxygen requirement was successfully weaned but, due to mask intolerance, a tracheostomy was placed for low-level oxygen support and a feeding tube was inserted. She was discharged to an acute care facility and then to home. The tracheostomy and feeding tube were removed and she is reportedly doing well without evidence of longer-term clinical sequelae.

Patient #3 (U.S.)

Patient #3 was an 80-year-old man with severe COVID-19. He began treatment with narsoplimab several days after intubation. He died after the 3rd or 4th dose of narsoplimab. His death was reportedly associated with barotrauma and related complications secondary to mechanical ventilation. His family declined extracorporeal membrane oxygenation (ECMO) treatment for religious reasons.

Patient #4 (U.S.)

Patient #4 is a 61-year-old man with hypertension and severe COVID-19. Prior to initiation of narsoplimab treatment, he had been intubated for 8 days and undergoing ECMO. He had failed treatment with remdesivir, baricitinib and high-dose steroids. He has received several doses of narsoplimab to date and his clinical status remains stable.

Influenza Virus

As described in Examples 20, 21 and 22, it has been demonstrated that the lectin pathway contributes to the pulmonary injury in COVID-19 infection and that a representative MASP-2 inhibitory antibody, narsoplimab, is effective to alleviate the pulmonary symptoms in COVID-19 infected patients. Complement activation has also been demonstrated to contribute to pulmonary injury in a model of Influenza H5N1 virus infection. Pulmonary histopathological changes are very similar in patients with H5N1 infection and SARS-CoV infection In the H5N1 murine model, expression of MASP-2 RNA, C3a receptor RNA and C5a receptor RNA were all increased by the first day following infection Complement inhibition with the use of a C3aR antagonist or cobra

venom factor attenuated lung injury and clinical signs. Survival was also increased (see Sun et al., *Am J Respir Cell Mol Biol* 49(2):221-30, 2013). Accordingly, it is expected that a MASP-2 inhibitory agent will also be effective for use in methods for treating, inhibiting, alleviating or preventing acute respiratory distress syndrome or other manifestation of the disease in a mammalian subject infected with influenza virus.

In accordance with the foregoing, in one aspect, the present invention provides a method for treating, inhibiting, alleviating or preventing acute respiratory distress syndrome or other manifestation of the disease, such as thrombosis, in a mammalian subject infected with coronavirus or influenza virus, comprising administering to the subject an amount of a MASP-2 inhibitory agent effective to inhibit MASP-2-dependent complement activation (i.e., inhibit lectin pathway activation). In some embodiments, the subject is suffering from one or more respiratory symptoms and/or thrombosis and the method comprises administering to the subject an amount of a MASP-2 inhibitory agent effective to improve at least one respiratory symptom (i.e., improve respiratory function) and/or alleviate thrombosis.

In one embodiment, the method comprises administering the composition to a subject infected with COVID-19. In one embodiment, the method comprises administering the composition to a subject infected with SARS-CoV. In one embodiment, the method comprises administering the composition to a subject infected with MERS-CoV. In one embodiment, the subject is identified as having coronavirus (i.e., COVID-19, SARS-CoV or MERS-CoV) prior to administration of the MASP-2 inhibitory agent. In one embodiment, the subject is identified as being infected with COVID-19 and is in need of supplemental oxygen and the MASP-2 inhibitory agent, such as a MASP-2 inhibitory antibody, such as, for example, narsoplimab, is administered to the subject at a dosage and time period effective to eliminate the need for supplemental oxygen.

In one embodiment, the subject is identified as having COVID-19 and is suffering from, or at risk for developing, COVID-19-induced thrombosis and the method comprises administering a composition comprising a MASP-2 inhibitory agent (e.g., a MASP-2 inhibitory antibody such as narsoplimab) in a therapeutically effective amount to treat, prevent or reduce the severity of coagulation or thrombosis in said subject. In some embodiments, the methods of the invention provide anticoagulation and/or antithrombosis and/or antithrombogenesis without affecting hemostasis. In one embodiment, the level of

D-Dimer is measured in a subject suffering from COVID-19 to determine the presence or absence of thrombosis in said subject, wherein a D-Dimer level higher than the standard range is indicative of the presence of thrombosis and the subject is treated with a MASP-2 inhibitory agent (e.g., a MASP-2 inhibitory antibody such as narsoplimab) in a therapeutically effective amount to treat, prevent or reduce the severity of coagulation or thrombosis in said subject, which can be measured, for example, by a reduction in the level of D-Dimer level into the normal range of a healthy subject.

In one embodiment, the method comprises administering the composition to a subject infected with influenza virus, such as influenza A virus (H1N1 (caused the "Spanish Flu" in 1918 and "Swine Flu" in 2009); H2N2 (caused the "Asian Flu" in 1957), H3N2 (caused the "Hong Kong Flu" in 1968), H5N1 (caused the "Bird Flu in 2004), H7N7, H1N2, H9N2, H7N2, H7N3, H10N7, H7N9 and H6N1); or influenza B virus, or influenza virus C virus. In one embodiment, the subject is identified as having influenza virus prior to administration of the MASP-2 inhibitory agent.

In one embodiment, the subject is determined to have an increased level of circulating endothelial cells in a blood sample obtained from the subject prior to treatment with the MASP-2 inhibitory agent as compared to the level of circulating endothelial cells in a control healthy subject or population. In some embodiments, the method comprises administering an amount of a MASP-2 inhibitory agent in an amount sufficient to reduce the number of circulating endothelial cells in a subject infected with coronavirus or influenza virus.

In one embodiment, the MASP-2 inhibitory agent is a small molecule that inhibits MASP-2-dependent complement activation.

In one embodiment, the MASP-2 inhibitory agent is an expression inhibitor of MASP-2.

In one embodiment, the MASP-2 inhibitory antibody is a monoclonal antibody, or fragment thereof that specifically binds to human MASP-2. In one embodiment, the MASP-2 inhibitory antibody or fragment thereof is selected from the group consisting of a recombinant antibody, an antibody having reduced effector function, a chimeric antibody, a humanized antibody, and a human antibody. In one embodiment, the MASP-2 inhibitory antibody does not substantially inhibit the classical pathway. In one embodiment, the MASP-2 inhibitory antibody inhibits C3b deposition in 90% human serum with an IC_{50} of 30 nM or less.

In one embodiment, the MASP-2 inhibitory antibody or antigen-binding fragment thereof, comprises a heavy chain variable region comprising CDR-H1, CDR-H2 and CDR-H3 of the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising CDR-L1, CDR-L2 and CDR-L3 of the amino acid sequence set forth as SEQ ID NO:69. In one embodiment, the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising the amino acid sequence set forth as SEQ ID NO:69.

In some embodiments, the method comprises administering to a subject infected with coronavirus or influenza virus a composition comprising a MASP-2 inhibitory antibody, or antigen binding fragment thereof comprising a heavy-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:67 and a light-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:69 in a dosage from 1 mg/kg to 10 mg/kg (i.e., 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg or 10 mg/kg) at least once weekly (such as at least twice weekly or at least three times weekly) for a period of at least 2 weeks (such as for at least 3 weeks, or for at least 4 weeks, or for at least 5 weeks, or for at least 6 weeks, or for at least 7 weeks, or for at least 8 weeks, or at least 9 weeks, or at least 10 weeks, or at least 11 weeks, or at least 12 weeks).

In one embodiment, the dosage of MASP-2 inhibitory antibody is about 4 mg/kg (i.e., from 3.6 mg/kg to 4.4 mg/kg).

In one embodiment, the dosage of MASP-2 inhibitory antibody (e.g., narsoplimab) is administered to a subject suffering from COVID-19 at a dosage of about 4 mg/kg (i.e., from 3.6 mg/kg to 4.4 mg/kg) at least twice a week for a time period of at least two weeks, or at least three weeks, or at least four weeks (e.g., from two weeks to four weeks).

In one embodiment, dosage of the MASP-2 inhibitory antibody is a fixed dose from about 300 mg to about 450 mg (i.e., from about 300 mg to about 400 mg, or from about 350 mg to about 400 mg), such as about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg,

about 435 mg, about 440 mg, about 445 mg or about 450 mg). In one embodiment, the dosage of the MASP-2 inhibitory antibody is a fixed dose of about 370 mg (±10%).

In one embodiment, the method comprises administering a fixed dosage of MASP-2 inhibitory antibody at about 370 mg (±10%) to a subject infected with coronavirus or influenza virus twice weekly intravenously for a treatment period of at least 8 weeks.

In one embodiment, the MASP-2 inhibitory agent is delivered to the subject systemically. In one embodiment, the MASP-2 inhibitory agent is administered orally, subcutaneously, intraperitoneally, intra-muscularly, intra-arterially, intravenously, or as an inhalant.

In one embodiment, the subject is suffering from COVID-19-induced pneumonia or ARDS and the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered for a time sufficient to alleviate one or more symptoms of pneumonia or ARDS. In one embodiment, the subject is on a mechanical ventilator and the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered at a dosage and for a time period sufficient to discontinue the need for mechanical ventilation. In one embodiment the subject is on an invasive mechanical ventilator. In one embodiment, the subject is on a non-invasive mechanical ventilator. In one embodiment, the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered at a dosage and for a time period sufficient to discontinue the use of supplemental oxygen.

In one embodiment, the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered to a subject infected with coronavirus or influenza virus as a monotherapy. In some embodiments, the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered to a subject infected with coronavirus or influenza virus in combination with one or more additional therapeutic agents, such as in a pharmaceutical composition comprising a MASP-2 inhibitory agent and one or more antiviral agents, or one or more anti-coagulants, or one or more therapeutic antibodies or one or more therapeutic small molecule compounds. In some embodiments, the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered to a subject infected with coronavirus or influenza virus, wherein the subject is undergoing treatment with one or more additional therapeutic agents, such as one or more antiviral agents or one or more anti-coagulants, or one or more therapeutic antibodies or one or more therapeutic small molecule compounds.

In accordance with the foregoing, in another aspect, the present invention provides a method for treating, ameliorating, preventing or reducing the risk of developing one or more long-term sequelae in a mammalian subject infected with coronavirus or influenza virus, comprising administering to the subject an amount of a MASP-2 inhibitory agent effective to inhibit MASP-2-dependent complement activation (i.e., inhibit lectin pathway activation). In some embodiments, the subject is suffering from one or more respiratory symptoms and/or thrombosis and the method comprises administering to the subject an amount of a MASP-2 inhibitory agent effective to improve at least one respiratory symptom (i.e., improve respiratory function) and/or alleviate thrombosis.

In one embodiment, the method comprises administering the composition to a subject infected with COVID-19. In one embodiment, the method comprises administering the composition to a subject infected with SARS-CoV. In one embodiment, the method comprises administering the composition to a subject infected with MERS-CoV. In one embodiment, the subject is identified as having coronavirus (i.e., COVID-19, SARS-CoV or MERS-CoV) prior to administration of the MASP-2 inhibitory agent. In one embodiment, the subject is identified as being infected with COVID-19 and is in need of supplemental oxygen and the MASP-2 inhibitory agent, such as a MASP-2 inhibitory antibody, such as, for example, narsoplimab, is administered to the subject at a dosage and time period effective to eliminate the need for supplemental oxygen.

In one embodiment, the subject is identified as being infected with COVID-19 and experiences mild symptoms and the MASP-2 inhibitory agent, such as a MASP-2 inhibitory antibody, such as, for example, narsoplimab, is administered to the subject at a dosage and time period effective to treat, ameliorate, prevent or reduce the risk of developing one or more COVID-19 related long-term sequelae in said subject. In some embodiments, the method is useful for treating, ameliorating, preventing or reducing the risk of developing one or more COVID-19 related long term sequelae in a subject suffering from, or previously infected with COVID-19, wherein the long term sequelae are selected from the group consisting of cardiovascular complications (including myocardial injury, cardiomyopathy, myocarditis, intravascular coagulation, stroke, venous and arterial complications and pulmonary thrombosis); neurological complications (including cognitive difficulties, confusion, memory loss, also referred to

as "brain fog" headache, stroke, dizziness, syncope, seizure, anorexia, insomnia, anosmia, ageusia, myoclonus, neuropathic pain, myalgias; development of neurological disease such as Alzheimer's disease, Guillian Barre Syndrome, Miller-Fisher Syndrome, Parkinson's disease); kidney injury (such as acute kidney injury (AKI); pulmonary complications including lung fibrosis, dyspnea, pulmonary embolism) and inflammatory conditions such as Kawasaki disease, Kawasaki-like disease, multisystem inflammatory syndrome in children (MIS-C) and multi-system organ failure in a subject that has been infected with COVID-19. Recently published data show that SARS-CoV-2 infection in children results in high incidence of TMA, independent of clinical severity (see Diorio C. et al., *Blood Advances* vol 4(23), Dec 8, 2020). It has also been reported that SARS-CoV-2 infection in children can result in multi-system inflammatory syndrome (MIS-C) (see Radia T. et al., *Paediatr Respri Rev* Aug 11, 2020).

Multiple international groups have recently published reports that more than 60% of "recovered" COVID-19 patients have serious sequelae, including cognitive/CNS, pulmonary, cardiac, hepatic and other abnormalities (see e.g., Bonow et al., *JAMA Cardiology* vol 5(7) July 2020; Del Rio et al., *JAMA* vol 324 (17), November 2020; Lindner et al., *JAMA Cardiology* vol 5(11), November 2020; Marchiano S. et al., *bioRxiv*, August 30, 2020; Puntmann V. et al., *JAMA Cardiology* vol 5 (11), November 2020; Xiong Q. et al., *Clin Microbial Infect* 2020). For example, as described in Yelin D. et al., *Lancet Infect Dis* 2020, 9/1/2020, long-term complaints of people recovering from acute COVID-19 include: extreme fatigue, muscle weakness, low grade fever, inability to concentrate, memory lapses, changes in mood, sleep difficulties, needle pains in arms and legs, diarrhea and vomiting, loss of taste and smell, sore throat and difficulties in swallowing, new onset of diabetes and hypertension, skin rash, shortness of breath, chest pains and palpitations. Remarkably, as described in Examples 21 and 22 herein, 5- to 6-month follow-up on the initial 6 Bergamo study COVID-19 patients treated with narsoplimab showed no clinical or laboratory evidence of longer-term COVID-19 sequelae.

In one embodiment, the subject is determined to have an increased level of circulating endothelial cells in a blood sample obtained from the subject prior to

treatment with the MASP-2 inhibitory agent as compared to the level of circulating endothelial cells in a control healthy subject or population. In some embodiments, the method comprises administering an amount of a MASP-2 inhibitory agent in an amount sufficient to reduce the number of circulating endothelial cells in a subject infected with coronavirus or influenza virus.

In one embodiment, the MASP-2 inhibitory agent is a small molecule that inhibits MASP-2-dependent complement activation.

In one embodiment, the MASP-2 inhibitory agent is an expression inhibitor of MASP-2.

In one embodiment, the MASP-2 inhibitory antibody is a monoclonal antibody, or fragment thereof that specifically binds to human MASP-2. In one embodiment, the MASP-2 inhibitory antibody or fragment thereof is selected from the group consisting of a recombinant antibody, an antibody having reduced effector function, a chimeric antibody, a humanized antibody, and a human antibody. In one embodiment, the MASP-2 inhibitory antibody does not substantially inhibit the classical pathway. In one embodiment, the MASP-2 inhibitory antibody inhibits C3b deposition in 90% human serum with an IC_{50} of 30 nM or less.

In one embodiment, the MASP-2 inhibitory antibody or antigen-binding fragment thereof, comprises a heavy chain variable region comprising CDR-H1, CDR-H2 and CDR-H3 of the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising CDR-L1, CDR-L2 and CDR-L3 of the amino acid sequence set forth as SEQ ID NO:69. In one embodiment, the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising the amino acid sequence set forth as SEQ ID NO:69.

In some embodiments, the method comprises administering to a subject infected with coronavirus or influenza virus a composition comprising a MASP-2 inhibitory antibody, or antigen binding fragment thereof comprising a heavy-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:67 and a light-chain variable region comprising the amino acid sequence set forth as SEQ ID NO:69 in a dosage from 1 mg/kg to 10 mg/kg (i.e., 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg or 10 mg/kg) at least once weekly (such as at least twice weekly or at least three times weekly) for a period of at least 2 weeks (such as for at least

3 weeks, or for at least 4 weeks, or for at least 5 weeks, or for at least 6 weeks, or for at least 7 weeks, or for at least 8 weeks, or at least 9 weeks, or at least 10 weeks, or at least 11 weeks, or at least 12 weeks).

In one embodiment, the dosage of MASP-2 inhibitory antibody is about 4 mg/kg (i.e., from 3.6 mg/kg to 4.4 mg/kg).

In one embodiment, the dosage of MASP-2 inhibitory antibody (e.g., narsoplimab) is administered to a subject suffering from COVID-19 at a dosage of about 4 mg/kg (i.e., from 3.6 mg/kg to 4.4 mg/kg) at least twice a week for a time period of at least two weeks, or at least three weeks, or at least four weeks or at least five weeks or at least 6 weeks or at least 7 weeks or at least 8 weeks (e.g., from two weeks to four weeks, or from two weeks to five weeks or from two to six weeks or from two weeks to seven weeks or from two weeks to eight weeks).

In one embodiment, dosage of the MASP-2 inhibitory antibody is a fixed dose from about 300 mg to about 450 mg (i.e., from about 300 mg to about 400 mg, or from about 350 mg to about 400 mg), such as about 300 mg, about 305 mg, about 310 mg, about 315 mg, about 320 mg, about 325 mg, about 330 mg, about 335 mg, about 340 mg, about 345 mg, about 350 mg, about 355 mg, about 360 mg, about 365 mg, about 370 mg, about 375 mg, about 380 mg, about 385 mg, about 390 mg, about 395 mg, about 400 mg, about 405 mg, about 410 mg, about 415 mg, about 420 mg, about 425 mg, about 430 mg, about 435 mg, about 440 mg, about 445 mg or about 450 mg). In one embodiment, the dosage of the MASP-2 inhibitory antibody is a fixed dose of about 370 mg (±10%).

In one embodiment, the method comprises administering a fixed dosage of MASP-2 inhibitory antibody at about 370 mg (±10%) to a subject infected with coronavirus or influenza virus twice weekly intravenously for a treatment period of at least 8 weeks.

In one embodiment, the MASP-2 inhibitory agent is delivered to the subject systemically. In one embodiment, the MASP-2 inhibitory agent is administered orally, subcutaneously, intraperitoneally, intra-muscularly, intra-arterially, intravenously, or as an inhalant.

In one embodiment, the subject is suffering from COVID-19-induced pneumonia or ARDS and the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered for a time sufficient to alleviate one or more symptoms of pneumonia or ARDS and to alleviate or prevent COVID-19-related long term sequelae. In one

embodiment, the subject is on a mechanical ventilator and the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered at a dosage and for a time period sufficient to discontinue the need for mechanical ventilation. In one embodiment the subject is on an invasive mechanical ventilator. In one embodiment, the subject is on a non-invasive mechanical ventilator. In one embodiment, the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered at a dosage and for a time period sufficient to discontinue the use of supplemental oxygen.

In one embodiment, the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered to a subject infected with coronavirus or influenza virus as a monotherapy. In some embodiments, the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered to a subject infected with coronavirus or influenza virus in combination with one or more additional therapeutic agents, such as in a pharmaceutical composition comprising a MASP-2 inhibitory agent and one or more antiviral agents, or one or more anti-coagulants, or one or more therapeutic antibodies or one or more therapeutic small molecule compounds. In some embodiments, the MASP-2 inhibitory agent (e.g., MASP-2 inhibitory antibody) is administered to a subject infected with coronavirus or influenza virus, wherein the subject is undergoing treatment with one or more additional therapeutic agents, such as one or more antiviral agents or one or more anti-coagulants, or one or more therapeutic antibodies or one or more therapeutic small molecule compounds.

EXAMPLE 23

SARS-Cov-2 Nucleocapsid (N) protein binds to MASP-2 and activates complement C4 and a representative MASP-2 inhibitory antibody HG4 inhibits this activation.

Background/Rationale:

As described in Examples 21 and 22, treatment of COVID-19 patients suffering from ARDS with the MASP-2 inhibitory antibody narsoplimab resulted in rapid improvements. This Example demonstrates that SARS-Cov-2 Nucleocapsid (N) protein binds to MASP-2 and activates complement C4 and a representative MASP-2 inhibitory antibody HG4 inhibits this activation, further confirming that MASP-2-mediated lectin pathway is activated after infection with SARS-Cov-2.

1. SARS-Cov-2 Nucleocapsid Protein binds to MASP-2

Methods:

Microtiter plates were coated with 1 μg/well recombinant SARS-Cov-2 nucleocapsid protein (NP2) or control substrate (BSA). Residual binding sites were blocked using 1% BSA. Serial dilutions of recombinant MASP-2 (rMASP-2) were added and binding was detected using an anti-MASP-2 mAb.

Results:

FIGURE 54 graphically illustrates concentration-dependent binding of recombinant MASP-2 to SARS-Cov-2 nucleocapsid protein (NP2) as compared to the BSA control.

2. MASP-2 binds Directly to SARS-Cov-2 N-protein and Mediates Complement C4 Activation

Methods:

Mictrotiter plates were coated with 2.5 μg/well SARS-Cov-2 recombinant nucleocapsid protein (NP2). Residual binding sites were blocked using 1% BSA. rMASP-2 (1 μg) in barbital buffered saline (BBS) was added. Control wells received buffer only. After 1 hour incubation at 37°C, wells were washed with TBS/Tween. Purified human C4 (1 μg) was added to each well. MASP-2 inhibitory antibody HG4 (0.1 μM) (also referred to as OMS646-SGMI-2 as described in Example 13) was added to certain wells coated with NP2 containing rMASP-2 and C4. After a 1 hour incubation at 37°C, the supernatant was aspirated and separated on SDS-PAGE under reducing conditions and loaded on a Western blot as follows:

Lane 1: C4 only control

Lane 2: NP2 plus rMASP-2 plus C4:

Lane 3: NP2 plus rMASP-2 plus C4 plus HG4 (0.1 mM)

Lane 4: NP2 plus C4

Lane 5: BSA plus rMASP-2 plus C4

Results:

FIGURE 55 depicts an SDS-PAGE Western blot gel with wherein: Lane 1 contains purified C4 as a control showing the bands corresponding to C4α, C4β and C4γ. Lane 2 contains NP2 plus rMASP-2 plus C4, showing C4α, C4β, C4γ and a new band

corresponding to C4'α, indicating that MASP-2 directly binds to NP2 and cleaves C4. Lane 3 contains NP2 plus rMASP-2 plus C4 plus HG4 (0.1 μM), showing that the addition of the MASP-2 inhibitory antibody HG4 inhibited NP2/MASP-2-mediated C4 cleavage. Lane 4 contains NP2 plus C4, indicating that there is no C4 cleavage in the absence of MASP-2. Lane 5 contains BSA plus MASP-2 plus C4 showing no C4 cleavage in the absence of NP2.

Discussion

This Example demonstrates that SARS-Cov-2 Nucleocapsid protein (NP2) binds to MASP-2 and activates complement C4 and a representative MASP-2 inhibitory antibody HG4 inhibits this activation, further confirming that MASP-2-mediated lectin pathway is activated after infection with SARS-Cov-2.

EXAMPLE 24

Longitudinal Study to Measure Complement Activation in Acute COVID-19 patients as Compared to Healthy Volunteers

Background/Rationale:

Infection with a new strain of coronavirus, SARS-CoV-2, usually passes without symptoms or with mild disease exacerbations. However, in a minority of those infected, SARS-CoV-2 can cause severe to life-threatening disease, with mild to severe long-term morbidity and mortality. What determines the susceptibility to severe exacerbations is not fully understood and, besides co-morbidities, it is considered that genetic factors, epigenetic phenomena, and age and sex differences can affect the risk of developing severe to fatal pathology. Experimental evidence provided herein and reported in Rambaldi A. et al., *Immunobiology* 225(6):152001, 2020 and elsewhere make it clear that the complement system is a key driver of the inflammatory response in both the initiation and the maintenance of endothelial pathology in acute COVID-19. As described herein in Examples 21 and 22 and reported in Rambaldi A. et al., *Immunobiology* 225(6):152001, 2020, treatment of severely ill COVID-19 patients with narsoplimab, a MASP-2 inhibitory antibody, achieved a therapeutic breakthrough with rapid improvements of disease manifestations following infusions. As further described in Example 23, consistent with the therapeutic efficacy of narsoplimab, it was demonstrated

that SARS-Cov-2 nucleocapsid protein (NP2) directly binds to MASP-2 resulting in C4 cleavage which is blocked by a MASP-2 antibody HG4.

In this Example, further investigation of each of the three complement activation pathways is examined in donors at defined stages and severity of COVID-19 in order to identify clinical and prognostic markers for acute COVID-19 in order to identify windows of therapeutic opportunities for treatment as well as further insight into the molecular events that cause acute COVID-19. The results may also deliver predictive clinical markers for disease severity and ongoing pro-inflammatory events leading to the unfavorable outcomes of Long-COVID-19 syndrome.

Methods:

This example describes initial results from a study in progress to measure complement activation in various categories of subjects in which longitudinal plasma and serum samples are taken from various categories of donors as follows:

Categories:

(1) donors with acute or post-acute COVID-19 (also referred to as Long-term COVID-19),

Acute patients: samples from COVID-19 patients taken within 15 days after hospital admission (0-4 days; 5-10 days and 11-15 days).

Recovered/Convalescent patients: subjects 3 months after recovery from acute COVID-19 (i.e., patients that survived acute COVID and were discharged from the hospital).

(2) donors that tested positive for SARS-CoV-2 and were either asymptomatic or with mild symptoms not requiring hospitalization.

(3) uninfected health care workers (HCW) (i.e. sero-negative for SARS-CoV-2)

Complement Assays (CH50, C5a, Bb)

Complement activation results in the release of the pro-inflammatory anaphylatoxin C5a, together with factor Bb, a marker of alternative pathway activation, which were measured in the various populations of subjects as described below.

CH50 Assay

The CH50 assay measures total complement hemolysis of sheep erythrocytes coated with anti-sheep erythrocyte antibody.

FIGURE 56 graphically illustrates the CH50 values in samples obtained from various populations of subjects in the longitudinal study, where each "x" symbol on the graph represents an individual subject.

As shown in **FIGURE 56**, total complement hemolysis is compromised by consumption of complement early in SARS-CoV-2 infection, as evidenced by very low CH50 values in the acute COVID-19 patients at days 0-4 days after hospital admission; 5-10 days after hospital admission; and 11-15 days after hospital admission as compared to CH50 values from convalescent patients (2-3 months after discharge from the hospital); SARS-Cov-2 positive staff; and sero-negative staff (i.e., SARS-Cov-2 negative). As further shown in **FIGURE 56**, most of the convalescent patients show an increase in CH50 values back into the normal range.

C5a Assay

The C5a assay measures the pro-inflammatory complement activation product C5a, shared between all three complement pathways. The assay is a commercially available sandwich ELISA from R&D systems (cat #DY2037).

FIGURE 57 graphically illustrates the level of C5a (ng/ml) in plasma samples obtained from various populations of subjects in the longitudinal study, where each "x" symbol on the graph represents an individual subject.

As shown in FIGURE 57, the C5a levels in plasma obtained from acute COVID-19 patients at days 0-4 days after hospital admission (n=16); 5-10 days after hospital admission (n=12) and 11-15 days (n=12) after hospital admission are significantly higher than the C5a levels in plasma obtained from convalescent patients (n=36), seropositive staff (n=30) and seronegative staff (n=26).

Bb Assay

The activation state of the alternative pathway (AP) was determined using commercially available sandwich ELISA that detects a neoepitope of the Bb activation product of the AP (Quidel MicroVue Bb Plus EIA).

FIGURE 58 graphically illustrates the level of Bb (ug/mL) in plasma obtained from various populations of subjects in the longitudinal study, where each "x" symbol on the graph represents an individual subject.

As shown in FIGURE 58, the Bb levels in plasma obtained from acute COVID-19 patients at days 0-4 days after hospital admission; 5-10 days after hospital admission and 11-15 days after hospital admission are significantly higher than the Bb levels in plasma obtained from convalescent patients, seropositive staff and seronegative staff. As further shown in FIGURE 58, the level of Bb in the recovered patients is within the range of the normal healthy controls (seronegative staff).

Results:

As shown in FIGURES 56, 57 and 58, complement activation occurs early in subjects suffering from acute COVID-19, as evidenced by the low CH50 (FIG 56), high C5a level (FIG 57) and high Bb level (FIG 58) in the acute patients within 15 days of hospital admission as compared to convalescent patients and healthy controls. It is further demonstrated that the AP is activated early in infection, as evidenced by the high Bb levels in acute patients within 15 days of hospital admission and returns to normal levels after recovery (see FIGURE 58).

EXAMPLE 25

High Levels of C1-INH/MASP-2 Complex Correlate with Acute COVID-19

Background/Rationale:

SARS-Cov2 is an emerging virus with very high infectivity and risk of death in those with severe endothelial disease and respiratory symptoms. To maximize success in protecting people against this disease, there is an urgent need for biomarkers and highly accurate tests to identify those persons at risk of developing acute and/or long term disease (post-acute COVID-19, otherwise known as Long-COVID-19 syndrome), or has developed a protective immune response versus a COVID-19 disease response. There is also a need for tests to determine the efficacy of therapeutics to treat and/or prevent COVID-19-related complications, including those suffering from, or at risk of developing Long-COVID-19.

As described in Example 24, complement activation occurs early in subjects suffering from acute COVID-19, as evidenced by the low CH50 (FIG 56), high C5a levels (FIG 57) and high Bb levels (FIG 58) in acute patients within 14 days of hospital admission as compared to healthy controls. It was further demonstrated that the alternative pathway (AP) is activated early in infection, as evidenced by the high Bb

levels in acute COVID-19 patients within 15 days of hospital admission and returns to normal levels after recovery (see FIGURE 58).

This Example describes the development of a sensitive sandwich ELISA assay that is capable of detecting the amount of MASP-2/C1-INH complex in human serum samples. This Example further describes the use of this sensitive sandwich ELISA assay to interrogate the activation state of the lectin pathway (LP) in the various groups of subjects (i.e., acute COVID-19, convalescent patients and healthy control subjects) by measuring the level of fluid-phase MASP-2/CI-INH complex in serum samples obtained from these subjects.

Methods:

1. MASP-2/C1-INH complex ELISA assay

MASP-2 is found in plasma as a zymogen, associated with one of several lectin pathway (LP) pattern recognition molecules. The zymogen form is loosely bound to the serine protease inhibitor, C1-INH. When sufficient LP recognition molecules bind in close proximity on an activating surface, zymogen MASP-2 is cleaved into two disulphide-linked chains, either by another molecule of MASP-2, or by MASP-1. Cleaved MASP-2 is the active form of the enzyme, which cuts its substrates, the downstream complement components C4 and C2. The activity of MASP-2 is regulated by C1-INH, which binds tightly to activated MASP-2, forming a stable 1:1 complex.

To determine the activation state of the LP effector enzyme MASP-2, a feature was utilized that takes advantage of the fact that C1 Inhibitor (C1-INH) which acts as a pseudo-substrate once MASP-2 has been activated, forms a covalent fluid-phase MASP-2/C1-INH complex. Thus, the level of MASP-2/C1-INH complex in a sample of plasma or serum provides a clear measure of recent LP activation.

Human MASP-2 protein (mature form) is set forth as SEQ ID NO:6.

Human C1 esterase inhibitor (C1-INH), Genbank CAA38358, is set forth below as SEQ ID NO:86 (aa 1-21 signal peptide, mature protein aa 22-500)

MASRLTLLTLLLLLLAGDRASSNPNATSSSSQDPESLQDRGEGKVATTVISKMLFVEPILEVSSLPTTNS
TTNSATKITANTTDEPTTQPTTEPTTQPTIQPTQPTTQLPTDSPTQPTTGSFCPGPVTLCSDLESHSTEA
VLGDALVDFSLKLYHAFSAMKKVETNMAFSPFSIASLLTQVLLGAGENTKTNLESILSYPKDFTCVHQAL
KGFTTKGVTSVSQIFHSPDLAIRDTFVNASRTLYSSSPRVLSNNSDANLELINTWVAKNTNNKISRLLDS
LPSDTRLVLLNAIYLSAKWKTTFDPKKTRMEPFHFKNSVIKVPMMNSKKYPVAHFIDQTLKAKVGQLQLS
HNLSLVILVPQNLKHRLEDMEQALSPSVFKAIMEKLEMSKFQPTLLTLPRIKVTTSQDMLSIMEKLEFFD

FSYDLNLCGLTEDPDLQVSAMQHQTVLELTETGVEAAAASAISVARTLLVFEVQQPFLFVLWDQQHKFPV
FMGRVYDPRA

Kajdacsi et al., *Front Immunol* vol 11, 2020, used the anti-human MASP-2 monoclonal rat IgG1 from Hycult Biotech (mAb 8B5) as a capture antibody to measure MASP-2/C1-INH complexes in healthy humans and in hereditary angioedema (HAE) patients in 10% serum concentrations. Hansen et al., *J of Immunol* 195:3596-3604, 2015, also used the anti-human MASP-2 monoclonal rat IgG1 from Hycult Biotech (mAb 8B5) in an ELISA assay to measure MASP-2/C1-INH complexes in HAE patients. Hansen et al. observed that MASP-2/C1-INH complexes were only detected in very high human serum concentrations (20% or greater) as compared to MASP-1/C1-INH complexes and thought that this could be due to the much lower serum concentration of MASP-2 compared with MASP-1 or due to the fact that the commercially available MASP-2 mAb8B5 is less applicable as an assay Ab as compared with the MASP-1 mAb used in their study (see Hansen et al. at page 3602-3603, bridging paragraph).

In order to develop a sensitive ELISA assay suitable for screening individual patient samples at serum concentrations less than 10% (i.e., from 0.3% to 8% serum, such as from 0.3% to 7% serum, such as from 0.3% to 6% serum, such as from 0.3% to 5% serum) for the presence and/or amount of MASP-2/C1-INH complex, a panel of monoclonal antibodies known to bind to MASP-2 (clone C1, C7, D8 and H1) were tested as capture antibodies. These mAbs (clone C1, C7, D8 and H1) were produced from hybridomas obtained from immunized MASP-2 KO mice and were found to bind to MASP-2 but were not capable of inhibiting MASP-2 functional activity (data not shown).

Anti-MASP-2 mAbs: C1, C7, D8, H1 were tested as candidate capture antibodies in an ELISA assay format for detecting MASP-2/C1-INH complex as follows.

(i). Nunc Maxisorb microtiter plates were coated with 100 µl of anti-MASP-2 candidate capture Abs Clones #C1, #C7, #D8 and #H1 (2µg/ml) in carbonate buffer (15mM Na_2CO_3, 35mM $NaHCO_3$, pH 9.6) overnight at 4°C. The microtiter plate was blocked with 280µl/well of 1% (w/v) BSA in TBS buffer for 1hr at RT.

(ii) Activated control serum was prepared by diluting pooled normal human serum (NHS) to 20% v/v in Tris-buffered saline (TBS; 10mM Tris-Cl, 140 mM NaCl, pH 7.4) with 5mM Ca^{2+}. Mannan-agarose (100 uL, Sigma cat. M9917) was washed twice with five volumes of TBS/Ca^{2+}, and resuspended to 100µl in the same buffer. 500µl of 20% serum

was added to the 100µl of mannan-agarose and incubated at room temperature (RT) with gentle shaking or rotation for 30 minutes. EDTA was then added to an end concentration of 10mM and incubated for a further 2 minutes. The agarose was then spun down and the activated serum was aspirated off and stored at -20°C.

(iii) Serial dilutions were prepared from the activated control serum over a range of 0.1% to 20% and the in TBS/Ca^{2+}. The blocking buffer was discarded and 100 ul/well of samples or control sera was added to the plate and incubated for 1 hour at room temperature. The plates were then washed three times with 280µl of TBS/Ca^{2+}/0.05% Tween 20 (wash buffer)

(iv) 100µl/well anti-C1-INH detection Ab (affinity-purified rabbit polyclonal anti-C1-INH, Proteintech cat. 12259-1-AP, diluted 1:2000 in TBS/Ca^{2+}) was added and incubated for 1 hour at room temperature.

(v) The plate was then washed 3x as described above. 100µl/well anti-rabbit HRP (Sigma, 1:5000) was added and incubated for a further 45 min.

(vi) The plate was then washed 3x as described above and 100µl/well TMB substrate was added. When blue color developed, 50µl/well 2N H_2SO_4 was added to stop the reaction and measured at the OD_{450nm}.

Results:

FIGURE 59 graphically illustrates the amount of MASP-2/C1-INH complex detected, based on OD_{450} values, with each of the four candidate anti-MASP-2 mAbs (clone C1, C7, D8 and H1) at various concentrations of activated serum. It is noted that the amount of MASP-2/C1-INH complex in normal, non-activated serum would be at baseline (data not shown).

As shown in FIGURE 59, mAb #C7 was far superior to the other anti-MASP-2 antibodies tested for use as capture antibodies for the MASP-2/C1-INH in an ELISA assay. As shown in FIGURE 59, mAb #7 could detect MASP-2/CI-INH complex in a dilution range of below 5% (i.e., from 0.3% to 5%) in activated human serum. It is noted that the commercially available antibodies from Hycult (clone 8B5 and clone 6G12) were also tested as candidate capture antibodies in this assay format and the results were similar to the mAbs C1 and D8 (i.e., not capable of use in a sensitive ELISA assay). mAb#C7 was chosen for use in the highly sensitive ELISA assay and is described below.

anti-MASP-2 mAb #C7: (CDRs based on the Kabat numbering system are underlined)

Heavy Chain variable region: (SEQ ID NO:87)

EVKLVESGGGLVKPGGSLKLSCAASGFTFSSYLMSWVRQTPEKRLEWVATISGG GGNTYHPDSMKGRFTISRDNAKNTLYLQMSSLRSEDTALYYCARHGDFGNYFD YWGQGTTLTVSS

Light Chain Variable Region: (SEQ ID NO:88)

DIVMSQSPSSLAVSAGEKVTMSCKSSQSLLNSGTQKNYLAWYQQKPGQSPKLLI YWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCKQSYNLFTFGAGTKL ELKR

anti-MASP-2 mAb #C7 CDRs

HC-CDR1 (SEQ ID NO:89): SYLMS

HC-CDR2 (SEQ ID NO:90): TISGGGGNTYHPDSMKG

HC-CDR3 (SEQ ID NO:91): HGDFGNYFDY

LC-CDR1 (SEQ ID NO:92): KSSQSLLNSGTQKNYLA

LC-CDR2 (SEQ ID NO:93): WASTRES

LC-CDR3 (SEQ ID NO:94): KQSYNLFT

#C7_VH (SEQ ID NO:95)

GAGGTGAAGCTGGTGGAGTCTGGGGGAGGCTTGGTGAAGCCTGGAGGGTCCC TAAAACTCTCCTGTGCAGCCTCAGGATTCACTTTCAGTAGTTATCTTATGTCTT GGGTTCGCCAGACTCCGGAGAAGAGGCTGGAGTGGGTCGCAACCATTAGTGG TGGTGGTGGTAACACTTACCATCCAGACAGTATGAAGGGTCGATTCACCATC TCCAGAGACAATGCCAAGAACACCCTGTACCTGCAAATGAGCAGTCTGAGGT CTGAGGACACGGCCTTGTATTACTGTGCAAGACATGGGGACTTTGGTAACTA CTTCGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA

#C7_VK (SEQ ID NO:96)

GACATTGTGATGTCACAGTCTCCATCCTCCCTGGCTGTGTCAGCGGGAGAGA AGGTCACTATGAGCTGCAAATCCAGTCAGAGTCTGCTCAACAGTGGAACCCA

AAAGAACTACTTGGCTTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTG
CTGATCTACTGGGCATCCACTAGGGAATCTGGGGTCCCTGATCGCTTCACAGG
CAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGCAGGCTGAA
GACCTGGCAGTTTATTACTGCAAGCAATCTTATAATCTGTTCACGTTCGGTGC
TGGGACCAAGCTGGAGCTGAAACGG

2. Measurement of MASP-2/C1-INH complex in serum samples obtained from subjects in the longitudinal COVID-19 study

Subjects: As described in Example 24, a study is in progress to measure complement activation in various categories of subjects in which longitudinal plasma and serum samples are taken from various categories of donors as follows:

Categories:

(1) donors with acute or post-acute COVID-19 (also referred to as Long-term COVID-19),

Acute patients: samples from COVID-19 patients taken within 15 days after hospital admission (0-4 days; 5-10 days and 11-15 days).

Recovered/Convalescent patients: subjects 3 months after recovery from acute COVID-19 (i.e., patients that survived acute COVID and were discharged from the hospital).

(2) donors that tested positive for SARS-CoV-2 and were either asymptomatic or with mild symptoms not requiring hospitalization.

(3) uninfected health care workers (HCW) (i.e. sero-negative for SARS-CoV-2)

The assay described below uses anti-MASP-2 mAb #C7, described above, which was immobilized on microtiter plates to capture MASP-2/C1-INH complexes from human serum or plasma, and anti-C1-INH antibodies to detect the captured complexes. A positive control for this assay may be prepared by incubating normal human serum (NHS) with mannan-agarose, artificially activating the LP and releasing MASP-2/C1-INH into the sample. Serial dilutions of the positive control can be used as calibrators/reference standards for the ELISA assay. It is also possible to use naturally activated serum as a calibrator/reference standard, for example, from a pool of COVID-19 patients or other patient group in which MASP-2 is known to be activated.

Methods:

(i). Nunc Maxisorb microtiter plates were coated with 100 µl of anti-MASP-2 capture Ab Clone #C7 (2µg/ml) in carbonate buffer (15mM Na_2CO_3, 35mM $NaHCO_3$, pH 9.6) overnight at 4°C. The microtiter plate was blocked with 280µl/well of 1% (w/v) BSA in TBS buffer for 1hr at RT.

(ii) Activated control serum was prepared by diluting pooled normal human serum (NHS) to 20% v/v in Tris-buffered saline (TBS; 10mM Tris-Cl, 140 mM NaCl, pH 7.4) with 5mM Ca^{2+}. Mannan-agarose (100 uL, Sigma cat. M9917) was washed twice with five volumes of TBS/Ca^{2+}, and resuspended to 100µl in the same buffer. 500µl of 20% serum was added to the 100µl of mannan-agarose and incubated at room temperature (RT) with gentle shaking or rotation for 30 minutes. EDTA was then added to an end concentration of 10mM and incubated for a further 2 minutes. The agarose was then spun down and the activated serum was aspirated off and stored at -20°C.

(iii) Serial dilutions were prepared from the activated control serum (starting at 20%) and the sample sera (5%) in TBS/Ca^{2+}. The blocking buffer was discarded and 100 ul/well of samples or control sera was added to the plate and incubated for 1 hour at room temperature. The plates were then washed three times with 280µl of TBS/Ca^{2+}/0.05% Tween 20 (wash buffer)

(iv) 100µl/well anti-C1-INH detection Ab (affinity-purified rabbit polyclonal anti-C1-INH, Proteintech cat. 12259-1-AP, diluted 1:2000 in TBS/Ca^{2+}) was added and incubated for 1 hour at room temperature.

(v) The plate was then washed 3x as described above. 100µl/well anti-rabbit HRP (Sigma, 1:5000) was added and incubated for a further 45 min.

(vi) The plate was then washed 3x as described above and 100µl/well TMB substrate was added. When blue color developed, 50µl/well 2N H_2SO_4 was added to stop the reaction and measured at the OD_{450nm}.

Results:

FIGURE 60 graphically illustrates the results of the ELISA assay measuring MASP-2/C1-INH complex in 5% serum from acute COVID patients (16 samples from 3 patients <14 days after hospitalization), convalescent patients (n=15), seropositive staff (n=15) and seronegative staff (n=34). The results show the activation of the lectin pathway, measured as the amount of MASP-2/C1-INH complex, as a percentage of that seen in the artificially activated control serum. As shown in FIGURE 60, a significantly higher amount of MASP-2/C1-INH complex (2-3 fold higher) was observed in the serum of

acute COVID-19 patients (<14 days after hospital admission) as compared to convalescent patients, seropositive staff and seronegative staff ($p<0.0001$ ANOVA with Dunnett's post-hoc).

FIGURE 61 graphically illustrates the amount of MASP-2/C1-INH complex present in the 3 acute COVID-19 patients (#2, #3 and #4) upon admission to the hospital and over time up to 14 days after admission. The red line at the bottom of the graph shows the amount of MASP-2/C1-INH detected in pooled normal sero-negative health care workers.

As described above, activation of the lectin pathway (LP) leads to the generation of fluid-phase MASP-2/C1-INH complex. As shown in FIGURES 60 and 61, LP activation in acute COVID-19 patients remains high 14-15 days after admission to the hospital.

EXAMPLE 26

A bead-based immunoassay for measuring MASP-2/C1-INH and C1s/C1-INH complexes

Background/Rationale:

The complement system serine proteases C1s and Mannan-binding lectin associated serine protease-2 (MASP-2) circulate in plasma as zymogens. C1s is a part of the classical pathway (CP) C1 complex, together with another serine protease, C1r, and the recognition component C1q. MASP-2 is associated with one of several lectin pathway (LP) pattern recognition molecules. In both cases, the zymogen forms are loosely bound to the serine protease inhibitor, C1-INH. When the CP or LP are activated, the zymogens are cleaved into two disulphide-linked chains. Cleaved C1s and MASP-2 are the active form of the enzymes, which cut the downstream complement components C4 and C2. Activated MASP-2 and C1s are regulated by C1-INH, which forms a stable 1:1 complex with the serine proteases. Thus, the level of C1s/C1-INH complex and MASP-2/C1-INH complex in a sample provides a clear measure of recent CP or LP activation, respectively.

As described in Example 25, in an ELISA assay that measured MASP-2/C1-INH complex levels in 5% serum from acute COVID patients, convalescent patients, seropositive staff and seronegative staff it was determined that a significantly higher amount of MASP-2/C1-INH complex (2-3 fold higher) was observed in the serum of acute COVID-19 patients (<14 days after hospital admission) as compared to

convalescent patients, seropositive staff and seronegative staff ($p<0.0001$ ANOVA with Dunnett's post-hoc).

This Example describes the development of a sensitive assay suitable for screening individual patient samples at serum concentrations less than 10% (i.e., from 1% to 5%) for the presence and/or amount of MASP-2/C1-INH complex and C1s/C1-INH complex, which involved transferring the sandwich assays to a bead-based fluorescent format and multiplexing them, using the Luminex platform.

Methods:

This Example provides further analysis of MASP-2/C1-INH complex levels and also an analysis of C1s/C1-INH complex levels in samples from acute COVID-19 patients, convalescent patients, seropositive staff and seronegative staff using a high throughput bead-based immunofluorescence assay, carried out using Luminex xMAP (Multi-Analyte Profiling) technology.

Luminex assay for MASP-2/C1-INH and C1s/C1-INH complexes

As described herein, MASP-2/C1-INH and C1s/C1-INH complexes are specific biomarkers of activation of the lectin and classical pathways, respectively. To measure these biomarkers, we devised a multiplexed bead-based fluorescent sandwich assay, in which the capture antibody (bound to the beads) is directed against the serine protease, and the detection antibody is directed against C1-INH.

As illustrated in FIGURE 62, the multiplexed bead-based immunofluorescence assay uses anti-C1s antibodies or anti-MASP-2 antibodies immobilised on polystyrene microspheres, or magnetic polystyrene microspheres (i.e., beads), to capture serine protease/C1-INH complexes (i.e., the analyte) from human serum or plasma, and anti-C1-INH antibodies as a detection antibody to detect the captured complexes.

While the assay described in this example is based on the Luminex xMAP (Multi-Analyte Profiling) technology, it will be understood by those skilled in the art that alternative bead-based immunofluorescence assays could be used to practice the claimed invention.

The bead-based assay can be multiplexed by coating one set of fluorescent beads with anti-MASP-2 monoclonal antibody (mAb), and another, with a different fluorescent spectrum, with an anti-C1s monoclonal antibody (mAb).

A positive control reference standard for MASP-2/C1-INH complexes was prepared using standard sera or plasma pooled from patients with acute COVID-19, as shown in the standard curves presented in FIGURE 63 detecting MASP-2/C1-INH complex with mAb C8 anti-MASP-2 as the capture antibody. As shown in FIGURE 63, the bead-based assay is capable of detecting MASP-2/C1-INH complex in less than 10% plasma or serum from patients with acute COVID-19 (i.e., from 0.1% to 10% plasma or serum, such as from 0.5% to 8%, or from 0.5% to 7.5%, such as 1% to 5% serum or plasma).

Alternatively, a positive control for MASP-2/C1-INH complexes can be prepared by incubating normal human serum with mannan-agarose, artificially activating the lectin

pathway (LP) and releasing MASP-2/C1-INH complex into the sample. Likewise, a positive control for C1s/C1-INH complexes can be prepared by incubating normal human serum with immune complexes, artificially activating the CP and releasing C1s/C1-INH complex into the sample. Serial dilutions of the positive control may be used as calibrators.

As another alternative, standards and positive controls may be prepared by mixing recombinant C1-INH and either recombinant C1s or recombinant MASP-2 in stochiometric amounts, purifying the resulting C1s/C1-INH or MASP-2/C1-INH complexes by size exclusion chromatography and quantifying them by gel electrophoresis and /or Bradford assay or measurement of the optical absorbance at 280nm as further described in Example 27.

Bead-Based Assay Methods:

Antibody-coated magnetic beads: A panel of monoclonal antibodies known to bind to MASP-2 and C1s were tested as capture antibodies. Antibodies were diluted to 50µg/ml in phosphate buffered saline (PBS) and immobilized by carbodiimide coupling on MagPlex magnetic polystyrene microspheres (Luminex), using the xMAP antibody coupling kit, as described in the Luminex (xMAP) Cookbook (4th edition). After coupling, any remaining reactive sites on the beads were blocked by incubation with PBS containing 0.05% TWEEN 20, pH 7.4 (PBS TBN). BSA-coated beads were prepared as negative controls. MagPlex beads with different emission spectra were used for anti-C1s, anti-MASP-2 and BSA coated beads, to allow the assay to be multiplexed.

Assay procedure: Antibody and BSA-coupled MagPlex beads were diluted in PBS-TBN assay buffer to a final concentration of 50 microspheres/µL of each type of bead. Fifty µL of this mixture was aliquoted into each well of a 96-well plate. An equal volume of plasma or serum diluted in PBS-TBN was added to the wells, mixed and incubated for 30min at room temperature on a shaker. The beads were washed 3 times with assay buffer by retaining them with a magnetic separator, aspirating the supernatant and adding 100µL of fresh PBS-TBN assay buffer. After washing, the beads were resuspended in 50µL of assay buffer and bound ligand was detected by adding a biotinylated anti-C1-INH polyclonal antibody (R&D Systems, BAF2488) diluted 1:1000 in PBS-TBN assay buffer. The beads were incubated with the detection antibody for 30min at room temperature, before being washed 3x as described above. Streptavidin R-phycoerythrin (SAPE; Thermo Fisher Scientific) was diluted to 1µg/mL in assay buffer, 100µL added to each well, mixed and incubated for 30min at room temperature. After washing as above, 50–

75μL of each reaction were analyzed on the Luminex analyzer according to the system manual.

Antibody selection: In preliminary experiments designed to test pairs of capture and detection antibodies, we prepared control sera for the MASP-2/C1-INH assay by incubating normal human serum with mannan-agarose, artificially activating the LP and releasing MASP-2/C1-INH into the sample. Likewise, a positive control for C1s/C1-INH complexes was prepared by incubating normal human serum with sheep anti-HSA, generating immune complexes in situ to artificially activate the CP and release C1s/C1-INH into the sample. Serial dilutions of these sera, ranging from 1:10 to 1:1280 were assayed as described above. Controls were: Non-activated NHS, BSA-coated beads, no-serum (buffer only) reactions, and mixtures omitting the detection antibody. The following mAb were shown to work well as capture Ab.

- Anti-MASP-2 humanized mouse mAb #C8
- Anti-C1s affinity-purified polyclonal, Proteintech (14554-1-AP)

These capture antibodies gave a straightforward log/linear relationship between sample concentration and fluorescent intensity at sample dilutions from 1:10 to 1:640, with the signal falling to background levels at 1:1280. The anti-MASP-2 mAb clone 8B5 (Hycult Biotech), previously used successfully in sandwich ELISAs, performed poorly in the Luminex assay, with poor sensitivity and a low signal-to-noise ratio.

Exemplary Assay Protocol

(i) coat 250 μL of polystyrene, or magnetic polystyrene, microbeads (e.g., Magplex beads) with 12.5 μg of the capture antibody in 250 μL of phosphate buffered saline, using the xMAP antibody coupling kit, according to the manufacturer's description (see Luminex (xMAP) 4th Edition). The following monoclonal antibodies have been shown to work as capture antibodies.

- anti-MASP-2 capture Ab Clone #C8
- anti-C1s affinity-purified polyclonal, cat number 14554-1-AP, Proteintech

The capture antibodies should be coupled to distinct bead sets, one for each antibody.

(ii) to prepare activated control serum, dilute pooled normal human serum to 20% v/v Tris-buffered saline (TBS; 10mM Tris-Cl, 140 mM NaCl, pH 7.4) with 5mM Ca^{2+}. Wash

100µl of mannan-agarose (Sigma cat. M9917) twice with five volumes of TBS/Ca^{2+} and resuspend to 100µl in the same buffer. Add 500µL of 20% serum to the 100µL of mannan-agarose, and incubate at room temperature (RT) with gentle shaking or rotation for 30min. Add EDTA to an end concentration of 10mM and incubate for a further 2min then spin down the agarose and aspirate off the activated serum. Store at -20°C.

(iii) Select the appropriate antibody-coupled microsphere sets and carry out the assay as follows:

- Resuspend the microspheres by vortexing and sonication
- Prepare a working microsphere mixture by diluting the coupled microsphere stocks to a final concentration of 50 microspheres of each set/µL in assay buffer (TBS).
- Aliquot 50 µL of the working microsphere mixture into the appropriate wells of a 96-well plate.
- Add 50 µL of assay buffer (TBS) to each background well.
- Add 50 µL of standard or sample to the appropriate wells.
- Mix the reactions gently by pipetting up and down several times with a multi-channel pipettor.
- Cover the plate and incubate for 30 minutes at room temperature on a shaker set to approximately 800 rpm.
- Place the plate into the magnetic separator and allow separation to occur for 30-60 seconds.
- Use a multi-channel pipette to carefully aspirate the supernatant from each well.
- Leave the plate in the magnetic separator for the following wash steps:
  - Add 100 µL assay buffer to each well.
  - Use a multi-channel pipette to carefully aspirate the supernatant from each well
- Remove the plate from the magnetic separator and resuspend the microspheres in 50 µL of assay buffer by gently pipetting up and down several times using a multi-channel pipettor.

- Dilute the biotinylated detection antibody in assay buffer. A suitable detection antibody is R&D system anti-C1-INH affinity-purified polyclonal, cat. No BAF2488, diluted 1:1000.
- Add 50 µL of the diluted detection antibody to each well.
- Mix the reactions gently by pipetting up and down several times with a multi-channel pipettor.
- Cover the plate and incubate for 30 minutes at room temperature on a plate shaker set to approximately 800 rpm.
- Place the plate into the magnetic separator and allow separation to occur for 30-60 seconds.
- Use a multi-channel pipette to carefully aspirate the supernatant from each well.
- Leave the plate in the magnetic separator for the following wash steps:
  - Add 100 µL assay buffer to each well.
  - Use a multi-channel pipette to carefully aspirate the supernatant from each well.
- Remove the plate from the magnetic separator and resuspend the microspheres in 50 µL of assay buffer by gently pipetting up and down several times with a multi-channel pipettor.
- Dilute Streptavidin, R-Phycoerythrin conjugate (SAPE) reporter to 1 µg/mL in assay buffer.
- Add 50 µL of the diluted SAPE to each well.
- Mix the reactions gently by pipetting up and down several times with a multi-channel pipettor.
- Cover the plate and incubate for 30 minutes at room temperature on a plate shaker set to approximately 800 rpm.
- Place the plate into the magnetic separator and allow separation to occur for 30-60 seconds.
- Use a multi-channel pipette to carefully aspirate the supernatant from each well.
- Leave the plate in the magnetic separator for the following wash steps:
  - Add 100 µL assay buffer to each well.

- Use a multi-channel pipette to carefully aspirate the supernatant from each well.
- Remove the plate from the magnetic separator and resuspend the microspheres in 100 µL of assay buffer by gently pipetting up and down several times with a multi-channel pipettor.
- Analyze 50–75 µL on the Luminex analyzer according to the system manual.

anti-MASP-2 mAb #C8

mAb #C8 VH (SEQ ID NO:97)
QVTLKESGPVLVKPTETLTLTCTVSGFSLSATYWGVTWIRQPPGKALEWLAHIFS
SDEKSYRTSLKSRLTISKDTSKNQVVLTMTNMDPVDTATYYCARIRRGGIDYWG
QGTLVTVSS

mAb #C8 VL (SEQ ID NO:98)
QPVLTQPPSLSVSPGQTASITCSGEKLGDKYAYWYQQKPGQSPVLVMYQDKQRP
SGIPERFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTAVFGGGTKLTVL

Results:

FIGURE 63 graphically illustrates the detection of MASP-2/C1-INH complexes in pooled human serum from acute COVID-19 patients in a bead-based assay using anti-MASP-2 mAb #C8 as a capture antibody as compared to BSA coated control beads.

EXAMPLE 27

Method of Generating MASP-2/C1-INH complexes for Use as Reference Standards

Methods:

1. Mix human MASP-2 CCP1/CCP2/SP6His (MW 43,740) with C1 esterase inhibitor (Sigma E0518 (from human plasma) MW 105,000 at a molar ratio of MASP-2:C1 inhibitor 1:1.5 (300 µg total).
2. Shake 400 rpm in eppendorf tubes for 60 minutes, 37°C
3. Refrigerate overnight
4. Purify by size exclusion chromatography (SEC):

TABLE 16: SEC Analysis and Purification

Peak	Retention	Area%
1	8.478	56.09
2	10.588	40.6
3	13.242	3.31

Peak 1 and Peak 2 were collected from the SEC and run on a non-reducing gel along with control MASP-2 CCP1/CCP2/SP6HIS (MW 43,740) and C1 esterase inhibitor (MW 105,000).

Results:

FIGURE 64 is a photograph of a non-reducing gel loaded with 6 µg of samples obtained during SEC purification of recombinant MASP-2/C1-INH complexes in which: lane 1: Peak 1 flow through; lane 2: peak 1 concentrated; lane 3: peak 2 flow through; lane 4: peak 2 concentrated; lane 5: unpurified mixture; lane 6: MASP-2 CCP1/2/SP (43,740 KD); lane 7: C1-Inhibitor (100KD).

As shown in FIGURE 64, the purified MASP-2/C1-INH complex is present in concentrated Peak 1. This recombinant complex can be used as a reference standard in a bead-based assay as described in Example 26.

EXAMPLE 28

Acute COVID-19 Patients Tested for Levels of MASP-2/C1-INH and C1s/CI-INH complexes

Methods:

As described in Examples 24 and 25, a study is in progress to measure complement activation in longitudinal plasma and serum samples taken from various categories of COVID-19 patients and healthy volunteers. As described herein, acute COVID-19 infection leads to complement activation, de-complementation and the release of complement activation products, e.g., C5a.

As described in this Example, as a part of this ongoing longitudinal study, forty patients with severe acute COVID-19 were tested for production of MASP-2/C1-INH complex formation and C1s/C1-INH complex formation using the bead-based assay described in Example 26.

The forty (40) patients with severe acute COVID-19 analyzed in this Example were recruited at the Royal Papworth Hospital, UK. The WHO clinical scores for these patients ranged from 3-7, and 19 of them required extracorporeal membrane oxygenation (ECMO). Nineteen of the patients survived, and were recalled for a follow-up 3 months after discharge; 21 succumbed to the disease. Thirty normal health care workers (HCW) who had tested positive for COVID-19, but not been hospitalized, and 30 uninfected HCW served as controls.

Plasma samples taken from the various subjects at the times indicated (time shown is from hospital admission) were diluted 1:50 and analyzed in the bead-based duplexed Luminex assay for the level of MASP-2/C1-INH complex and C1s/C1-INH complex as described in Example 26.

Measurement of complement haemolysis (CH50)

Antibody-driven complement lysis of sheep erythrocytes (SE) was measured using rabbit anti-sheep IgG coated SE as follows. Sheep erythrocytes (Oxoid) were washed 3 times using GVB buffer (10mM barbital, 145mM NaCl, 0.1%w/v gelatine), containing 10mM EDTA. The final concentration of RBCs was adjusted to $1x10^9$/ml. RBCs were sensitized by incubation with anti-sheep RBCs (Sigma S1389, diluted 1:200) at 37°C with gentle shaking for 30 minutes. Finally, RBCs were washed with GVB buffer containing 2mM Ca^{2+} and 1mM Mg^{2+} (GVB^{++}). Serum samples were serially diluted in 100µl GVB^{++} buffer in 96 well plates and 10^7 RBCs in an equal volume of GVB^{++} were added to each well. Wells containing buffer only were used as a negative control. Wells containing water instead of buffer/plasma were used as a positive control (nominally 100% lysis). After 30 minutes of incubation at 37°C, plates were centrifuged, 100 µl of the supernatant aspirated and released haemoglobin determined by measurement of the OD at 405 nm. The percentage of haemolysis was calculated and plotted against the plasma dilution to determine the CH50.

Circulating C5a was measured using a proprietary sandwich ELISA supplied by R&D systems (Cat. No. DY2037).

Results:

FIGURE 65 graphically illustrates the levels of MASP-2/C1-INH complex in acute COVID-19 patients as determined in the duplexed bead-based assay described herein. As shown in FIGURE 65, MASP-2/C1-INH complex levels were elevated throughout the acute phase of the disease, as compared to healthy controls, which is indicative of lectin pathway activation in acute COVID-19. As further shown in FIGURE 65, reduced, but not normal, levels of MASP-2/C1-INH complex were observed in survivors three months after discharge from the hospital. In contrast, people with mild COVID-19 disease (seropositive health care workers (HCW)) showed no elevation of MASP-2/C1-INH levels. N=40 for hospitalized patients, 30 for non-hospitalized COVID-19 cases, 30 for healthy controls. Analysed by 1-way ANOVA with Dunnett's correction for multiple comparisons.

FIGURE 66 graphically illustrates the levels of C1s/C1-INH complex in acute COVID-19 patients as determined in the duplexed bead-based assay described herein. As shown in FIGURE 66, C1s/C1-INH complex levels were elevated throughout the acute phase of the disease, as compared to healthy controls, indicative of classical pathway activation in acute COVID-19. As further shown in FIGURE 66, reduced, but not normal, levels of C1s/C1-INH complex were observed in survivors three months after discharge from the hospital. In contrast, people with mild COVID-19 disease (seropositive health care workers (HCW)) showed no elevation of C1s/C1-INH levels. N=40 for hospitalized patients, 30 for non-hospitalized COVID-19 cases, 30 for healthy controls. Analysed by 1-way ANOVA with Dunnett's correction for multiple comparisons. It is noted that the C1s/C1inh complex levels correlates with anti-COVID-19 antibody titer and antibody-dependent complement deposition (ADCD) (data not shown).

FIGURE 67 graphically illustrates the CH_{50} values in the acute COVID-19 patients, convalescent patients, sero-positive staff and sero-negative staff in the longitudinal study described in this example. As shown in FIGURE 67, the CH_{50} values are lower in the acute phase of the disease, as compared to healthy controls, indicative of complement consumption and activation.

FIGURE 68 graphically illustrates the C5a values in the acute COVID-19 patients, convalescent patients, sero-positive staff and sero-negative staff in the longitudinal study

described in this example. As shown in FIGURE 68, the C5a values are higher in the acute phase of the disease, as compared to healthy controls, indicative of complement activation.

Taken together, the results demonstrate that complement consumption and activation occurs in the early acute phase of COVID-19 even in the absence of anti-COVID-19 antibodies.

As shown in FIGURES 65 and 66, the levels of MASP-2/C1-INH complexes and C1s/C1-INH complexes were significantly elevated in all of the hospitalized acute COVID-19 patients. In those patients that survived, the levels of serine protease/C1-INH complexes trended toward normal three months after discharge, although a subset of the patients still had elevated levels, pushing the values up above the control set, and indicating ongoing complement activation.

Assay performance: Summary

As described herein, acute COVID-19 infection leads to complement activation, de-complementation and the release of complement activation products, e.g., C3a and C5a. We tested the bead-based C1-INH complex assays using plasma from 40 patients with severe acute COVID-19, who were recruited at the Royal Papworth Hospital, UK. The WHO clinical scores for the patients ranged from 3-7, and 19 of them required extracorporeal membrane oxygenation (ECMO). Nineteen of the patients survived; 21 succumbed to the disease. Thirty uninfected health care workers (HCW) served as controls. Serial dilutions of pooled acute phase plasma were used for standards (range 1:10-1:1280). Individual samples were diluted 1:50 in assay buffer. A high and low standard (pooled acute and NHS) were included at 3 separate locations on each plate to determine intra- and inter-plate variation.

The standard curves for both assays were straight log/linear relationships, with usable plasma dilutions ranging from 1:20 to 1:640. The absolute fluorescence signal for the C1s/C1-INH complex is approximately 10-fold higher than that for the MASP-2/C1-INH complex, perhaps reflecting the difference in serum concentration between MASP-2 and C1s.

MASP-2/C1-INH complexes and C1s/C1-INH complexes were significantly elevated in all of the hospitalized acute COVID-19 patients compared to the healthy controls, indicating activation of both the LP and the CP.

EXAMPLE 29

Treatment with Narsoplimab reduces the levels of MASP-2/C1-INH complex in COVID-19 and leads to a better clinical outcome.

Background/Rationale:

As described in Examples 20, 21 and 22, it has been demonstrated that the lectin pathway contributes to the pulmonary injury associated with acute COVID-19 and that a representative MASP-2 inhibitory antibody, narsoplimab, is effective to alleviate the pulmonary symptoms in acute COVID-19 patients. In this example, acute COVID-19 patients treated with narsoplimab as described in Examples 20, 21 and 22 were analyzed to determine the effect of narsoplimab on the level of MASP-2/C1-INH complex.

Methods:

Eight patients suffering from acute COVID-19 were admitted to the ITU in Bergamo, Italy and were treated with narsoplimab at a dosage of 4 mg/kg twice weekly. Samples were taken at hospital admission (prior to treatment with narsoplimab), then, counting days after treatment with narsoplimab, samples were taken at day 3-4, day 7-8, and day 9 to discharge. 16 healthy control subjects were recruited at the same time.

The samples were analyzed for CH50, C5a and MASP-2/C1-INH complex using the bead-based assay described in Example 26.

Results:

FIGURE 69 graphically illustrates the levels MASP-2/C1-INH complex in samples from 8 acute COVID-19 patients at admission (prior to narsoplimab treatment) and after narsoplimab treatment (day 3-4 after starting treatment; day 7-8, day 9 to discharge) as compared to 16 healthy controls. As shown in FIGURE 69, the MASP-2/C1-INH complex levels were elevated in the acute COVID patients upon hospital admission (prior to narsoplimab treatment) as compared to healthy subjects. As further shown in FIGURE 69, at day 3-4 after narsoplimab treatment there was a dramatic reduction in MASP-2/C1-INH complex levels comparable to that observed in healthy controls, which persisted to

discharge. In contrast, in acute COVID-19 patients from the longitudinal study described in Example 28 who were not treated with narsoplimab, as shown in FIGURE 65, an elevated level of MASP-2/C1-INH complex was observed at 0-4 days, 5-10 days and day 11-discharge, with a reduced level of MASP-2/C1-INH complex level occurring at the 3 month follow up which was still higher than normal healthy controls.

FIGURE 70A graphically illustrates the CH_{50} values in samples from 8 acute COVID-19 patients at admission (prior to narsoplimab treatment) and after narsoplimab treatment (day 3-4 after starting treatment; day 7-8, day 9 to discharge) as compared to 16 healthy controls.

As shown in FIGURE 70A, the CH_{50} values in acute COVID-19 patients at admission (prior to narsoplimab treatment) were lower than healthy controls. As further shown in FIGURE 70A, at day 3-4 after narsoplimab treatment there was an increase in CH_{50} into the normal range which continued to increase by day 7-8 and remained in the normal range at day 9 to discharge. In contrast, in acute COVID-19 patients from the longitudinal study described in Example 28 who were not treated with narsoplimab, as shown in FIGURE 67, a lower CH_{50} value was observed as compared to sero-negative staff at day 0-4, and this lower level persisted through 5-10 day and 11-15 day and eventually increased in convalescent patients at the 3 month follow up to normal levels.

FIGURE 70B graphically illustrates the C5a values in samples from 8 acute COVID-19 patients at admission (prior to narsoplimab treatment) and after narsoplimab treatment (day 3-4 after starting treatment; day 7-8, day 9 to discharge) as compared to 16 healthy controls.

As shown in FIGURE 70B, the C5a values in acute COVID-19 patients at admission (prior to narsoplimab treatment) were significantly higher than the healthy control subjects. As further shown in FIGURE 70B, the C5a values dramatically dropped by day 3-4 after narsoplimab treatment and continued to decrease at day 7-8 and by day 9-discharge to nearly normal levels. In contrast, in acute COVID-19 patients from the longitudinal study described in Example 28 who were not treated with narsoplimab, as shown in FIGURE 68, significantly elevated C5a values were observed at day 0-4 as compared to sero-negative staff, which decreased over time, but remained higher than

normal at days 5-10 and days 11-15, then eventually reduced to normal levels by the 3 month follow up (convalescent patients).

Taken together, these results demonstrate that patients with acute COVID-19 have complement activation and consumption upon hospital admission and that treatment with narsoplimab rapidly reduces complement activation and consumption. It is further demonstrated that the MASP-2/C1-INH complex level is indicative of the status of complement activation in COVID-19 patients, which is found to be high at hospital admission and rapidly decreases upon treatment with narsoplimab. Therefore, the level of MASP-2/C1-INH complex may be used as a way to determine the need for treatment with narsoplimab and may also be used to monitor the efficacy of narsoplimab treatment.

Discussion:

As described in this Example, we explored the effect of Narsoplimab treatment on complement activation during acute COVID-19. Markers of complement activation and depletion were analysed in longitudinal plasma samples taken from eight patients hospitalized with acute Covid-19 (WHO scores 3-7). Samples taken from healthy health care workers served as controls.

Immediately prior to treatment, all patients were decomplemented (low CH_{50}), and showed evidence of alternative pathway activation and anaphylatoxin production (Bb and C5a production). Using the novel bead-based fluorescent immunoassay described herein to measure C1s/C1-INH and MASP-2/C1-INH complexes, specific markers of classical and lectin pathway activation respectively, we found significantly elevated levels of both complexes in all patients prior to treatment. Narsoplimab treatment resulted in a rapid and sustained reduction in MASP-2/C1Inh complexes, and a corresponding reduction in C5a production. C1s/C1Inh levels remained high throughout the acute phase.

Taken together with the previous clinical results, these findings suggest that targeting the lectin pathway with Narsoplimab may suffice to reduce complement activation and anaphylatoxin reduction to below the threshold for maintaining ARDS, even in the presence of ongoing classical pathway activation.

These data demonstrate that, very early in severe COVID-19, lectin pathway activation is exceedingly high. This excessive lectin pathway activation causes consumption of the complement components shared between the lectin and classical pathways, impairing classical pathway function. Evaluation of blood samples from the Bergamo trial show that lectin pathway inhibition through narsoplimab can restore the loss of classical pathway functional activity caused by uncontrolled consumption through hyperactivation of the lectin pathway. These results demonstrate that the MASP-2/C1-INH complex is useful as an early indicator of severe COVID-19 and as a means to assess therapeutic response in COVID-19 patients undergoing treatment.

EXAMPLE 30

Further Evidence that Treatment with Narsoplimab reduces the levels of MASP-2/C1-INH complex in COVID-19 and leads to a better clinical outcome.

Background/Rationale:

As described in Examples 20, 21, 22 and 23, it has been demonstrated that the lectin pathway contributes to the pulmonary injury associated with acute COVID-19 and that a representative MASP-2 inhibitory antibody, narsoplimab, is effective to alleviate the pulmonary symptoms in acute COVID-19 patients. In this Example, patients suffering from acute COVID-19 patients treated with narsoplimab as described in Examples 20, 21 and 22 were analyzed to determine the effect of narsoplimab on the level of MASP-2/C1-INH complex. As described in Example 29, it was determined that patients with acute COVID-19 have complement activation and consumption upon hospital admission and that treatment with narsoplimab rapidly reduces complement activation and consumption. It was further demonstrated that the MASP-2/C1-INH complex level is indicative of the status of complement activation in COVID-19 patients, which is found to be high at hospital admission and rapidly decreases upon treatment with narsoplimab. This example describes further analysis of longitudinal samples obtained from acute subjects suffering from acute COVID-19 patients treated with narsoplimab (treated group) as compared to longitudinal samples obtained from subjects suffering from acute COVID-19 that were admitted in Bergamo during the same time period that were not treated with narsoplimab (untreated group), wherein the samples were analyzed for CH50, C5a and MASP-2/C1-INH complex using the bead-based immunoassay described in Example 26.

Methods:

Patients and controls included in this study

We analysed longitudinal plasma samples from 16 moderately and severely ill COVID-19 patients; 7 treated with narsoplimab (all of which recovered and were discharged after treatment) and 9 untreated controls, all admitted to the ICU of the Papa Giovanni XXIII Hospital in Bergamo, Italy during the fourth quarter of 2020. All patients were PCR positive for SARS-CoV-2, with ARDS (according to the Berlin criteria (Ferguson N.D et al., *Intensive Care Med* 38(10): 1573-82, 2012), requiring a minimum of CPAP (continuous passive airway pressure). The most severely ill patients were chosen for treatment with narsoplimab. All patients received Enoxaparin, dexamethasone and 500mg azithromycin daily, but patients with active systemic bacterial or fungal infections requiring further antimicrobial therapy were not eligible for narsoplimab treatment.

In the treated cohort, narsoplimab (4 mg/kg) was administered intravenously twice weekly for 2–4 weeks. Blood was collected prior to each narsoplimab dose and then twice weekly. Citrate plasma was prepared and frozen at -80°C until analysis. In parallel, samples were collected from patients with COVID-19 that did not receive narsoplimab. Likewise, normal control plasma were collected from seventeen seronegative volunteers (healthcare workers). The samples were analyzed for CH50, C5a and MASP-2/C1-INH complex using the bead-based assay described in Example 26.

Measurement of complement haemolysis (CH_{50})

Antibody-driven complement lysis of sheep erythrocytes (SE) was measured using rabbit anti-sheep IgG coated SE as follows. Sheep erythrocytes (Oxoid) were washed 3 times using GVB buffer (10mM barbital, 145mM NaCl, 0.1%w/v gelatine), containing 10mM EDTA. The final concentration of RBCs was adjusted to $1x10^9$/ml. RBCs were sensitized by incubation with anti-sheep RBCs (Sigma S1389, diluted 1:200) at 37°C with gentle shaking for 30 minutes. Finally, RBCs were washed with GVB buffer containing 2mM Ca^{2+} and 1mM Mg^{2+} (GVB^{++}). Serum samples were serially diluted in 100µl GVB^{++} buffer in 96 well plates and 10^7 RBCs in an equal volume of GVB^{++} were added to each well. Wells containing buffer only were used as a negative control. Wells

containing water instead of buffer/plasma were used as a positive control (nominally 100% lysis). After 30 minutes of incubation at 37°C, plates were centrifuged, 100 µl of the supernatant aspirated and released haemoglobin determined by measurement of the OD at 405 nm. The percentage of haemolysis was calculated and plotted against the plasma dilution to determine the CH50.

C5a ELISAs

Circulating C5a was measured using a proprietary sandwich ELISA supplied by R&D systems (Cat. No. DY2037).

Serum bactericidal assay (SBA)

K. pneumoniae isolates were grown in nutrient broth at 37°C overnight with gentle shaking. The next day, 10 mL of fresh nutrient broth were seeded with 100 µL of overnight bacterial culture and incubated at 37°C with gentle shaking until mid-logarithmic phase. Bacterial cultures were collected, washed twice using BBS (4mM barbital, 145mM NaCl, 2mM CaCl2, 1mM $MgCl_2$, pH 7.4) and then adjusted to a final concentration of 1×10^7 CFU mL−1. 1×10^5 CFU were incubated with 50% serum from HCW or sera from acute COVID-19 patients prior to and after narsoplimab treatment in BBS at 37°C with gentle shaking. After 2 hours, samples were taken and plated out on a nutrient agar plate for overnight at 37°C. Sera from patients were incubated with mucoid K. pneumoniae (ATCC 43816) for 120 minutes and recoverable viable bacterial colonies were calculated by measuring the decrease in the viable bacterial count recovered after the 2-hour incubation with each serum compared to heat-inactivated normal human serum (HI-NHS).

Luminex assay for MASP-2/C1Inh and C1s/C1Inh complexes

MASP-2/C1-INH and C1s/C1-INH complexes are specific markers of activation of the lectin and classical pathways, respectively. To measure these markers, we used the multiplexed bead-based fluorescent sandwich assay as described in Example 26.

RESULTS:

In this study seven severely ill COVID-19 patients were treated with narsoplimab (final concentration 4mg/kg body weight administered through i.v. infusions twice weekly) all of which recovered after treatment. For comparison, longitudinal blood samples were collected from a control group of nine COVID-19 patients that were in the ICU during the same time period who did not receive narsoplimab (untreated group).

Narsoplimab treatment reduces highly elevated levels of lectin pathway activation in COVID-19 patients to levels seen in healthy controls.

We used the bead-based fluorescent assay described in Example 26, which we developed to monitor the activation state of the lectin pathway (LP) and the classical pathway (CP) through detection of MASP-2/C1-INH and C1s/C1-INH complexes in serum/plasma in COVID-19 patients.

FIGURE 71 graphically illustrates the levels MASP-2C1-INH complex in samples from 7 COVID-19 patients at admission (day 0, prior to narsoplimab treatment) and after narsoplimab treatment (day 2-4 after starting treatment; day 6-8 and day 9 to discharge) as compared to samples obtained from 9 COVID-19 patients that were not treated with narsoplimab (untreated controls) during the same time period and a pool of healthy control subjects (healthy controls). As shown in FIGURE 71, at the start of the study, all patients had high levels of MASP-2/C1-INH complex indicative of lectin pathway activation. Narsoplimab treatment reduced MASP-2/C1-INH to normal levels directly after the first dose. Levels of MASP-2/C1-INH complex in the untreated group were significantly ($p<0.001$) higher than in the treated group for the rest of the study. Results were analyzed using 1-way ANOVA. In contrast, narsoplimab had no effect on the classical pathway driven production of C1s/C1-INH complex, which remained high in both patient groups throughout the study (data not shown).

Narsoplimab treatment ameliorates hypocomplementemia in acute COVID 19

FIGURE 72A graphically illustrates the CH_{50} values in samples from 7 acute COVID-19 patients at admission (day 0, prior to narsoplimab treatment) and after narsoplimab treatment (day 2-4 after starting treatment; day 6-8 and day 9 to discharge) as compared to samples obtained from 9 acute COVID-19 patients that were not treated with narsoplimab (untreated controls) during the same time period and a pool of healthy control subjects (healthy controls).

FIGURE 72B graphically illustrates the C5a values in samples from 7 acute COVID-19 patients at admission (day 0, prior to narsoplimab treatment) and after narsoplimab treatment (day 2-4 after starting treatment; day 6-8 and day 9 to discharge) as compared to samples obtained from 9 acute COVID-19 patients that were not treated

with narsoplimab (untreated controls) during the same time period and a pool of healthy control subjects (healthy controls).

As shown in FIGURE 72A, at the beginning of the study, plasma from all sixteen COVID-19 patients had severe hypocomplementemia determined as by low CH_{50} values. As further shown in FIGURE 72A, narsoplimab treatment resulted in an immediate improvement of the CH_{50} values, indicating a restoration of complement activation. In contrast, untreated control COVID-19 patients had significantly lower CH_{50} values ($p=0.0093$) during the first nine days of the study, and only began to recover normal complement activation shortly before discharge. As shown in FIGURE 72B, on admission to the ICU, all of the COVID-19 patients had high levels of the anaphylatoxin C5a in their sera reflecting that complement activation had taken place. As further shown in FIGURE 72B, narsoplimab treatment reduced C5a plasma levels to normal levels by 3 days after the first dose, and remained normal for the duration of the study.

In summary, in the narsoplimab-treated group, both CH_{50} and C5a had returned to normal by 3 days after the first dose, and remained normal for the duration of the study. In the untreated group, CH_{50} values were significantly lower ($p=0.0093$), and C5a levels significantly higher ($p=0.023$), than in the treated group throughout the remainder of the study. Results were analyzed using 1-way ANOVA.

Narsoplimab treatment inhibits lectin pathway-mediated complement activation, which allows recovery of classical pathway activity and antibody-mediated bactericidal activity

Complement-mediated bacterial lysis plays a major role in the defence against microbial infection, especially against Gram-negative bacteria. Hypocomplementemia induced by severe COVID-19 seriously impairs the ability of serum to opsonise or kill *Klebsiella pneumoniae*, a major secondary comorbidity in COVID-19. To determine whether narsoplimab could reverse this loss of function, we measured the serum bactericidal activity (SBA) of sera from treated and untreated patients against *K. pneumoniae*.

FIGURE 73 graphically illustrates the viable bacterial count of *K. pneumoniae* after incubation of sera from COVID-19 patients prior to treatment with narsoplimab (pre-treatment) and in COVID-19 patients after treatment with narsoplimab as compared to sera from COVID-19 patients not treated with narsoplimab as compared to normal

healthy serum (NHS) and heat-inactivated normal healthy serum (HI-NHS). As shown in FIGURE 73, a significantly lower bacterial count was observed when using sera from patients after treatment with narsoplimab compared to sera taken before treatment. Opsonization of *K. pneumoniae* with C3b was also impaired when incubated in pooled (n=6) sera from acute COVID-19 patients. Following narsoplimab administration, pooled sera of the same patients (n=6) opsonized *K. pneumoniae* with C3b to a similar extent as pooled normal healthy controls. Heat-inactivated normal healthy controls were used as a negative control. Results in were analyzed using 1-way ANOVA, with Dunnett's correction for multiple comparisons.

Overall Summary of Results:

As described herein and reported in Rambaldi A. et al., *Immunobiology* 225(6):152001, 2020, treatment of severely ill COVID-19 patients with narsoplimab, a MASP-2 inhibitory antibody that inhibits the lectin pathway (LP), achieved a therapeutic breakthrough with rapid improvements of disease manifestations following infusions.

In this Example we explored the effect of narsoplimab treatment on complement activation during acute COVID-19. Markers of complement activation and depletion were analyzed in longitudinal plasma samples taken from seven patients hospitalized with acute COVID-19 (WHO scores 3-7) and treated with narsoplimab, all of which recovered and were discharged. Samples taken from healthy health care workers and untreated COVID-19 served as controls.

Prior to treatment, all patient plasma presented with low CH_{50} and high C5a anaphylatoxin levels, markers indicative of complement activation through all three complement activation pathways. Using a novel bead-based fluorescent immunoassay to measure C1s/C1-INH and MASP-2/C1-INH complexes, specific markers of classical and lectin pathway activation respectively, we found significantly elevated levels of both complexes in all patients prior to treatment. Narsoplimab treatment resulted in a rapid and sustained reduction in MASP-2/C1-INH complexes, and a corresponding reduction in C5a production. C1s/C1-INH levels remained high throughout the acute phase. Bactericidal activity against Gram-negative bacteria was also restored.

Taken together with the previous clinical results, these findings provide further evidence that targeting the lectin pathway with narsoplimab may suffice to reduce complement activation and anaphylatoxin reduction to below the threshold for

maintaining ARDS, even in the presence of ongoing classical pathway activation, and restore the SBA required for a successful defense against opportunistic secondary infection.

Other embodiments

All publications, patent applications, and patents mentioned in this specification are herein incorporated by reference.

Various modifications and variations of the described methods and compositions of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific desired embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments.

While illustrative embodiments have 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.

SEQUENCE LISTING

<110> Omeros Corporation
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Lynch, Nicholas James
Schwaeble, Hans-Wilhelm
Shaffer, Kathleen A.
Yabuki, Munehisa

<120> Biomarker for Assessing the Risk of Developing Acute Covid-19 and Post-Acute Covid-19

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            430                 435                 440

ggg cgt ata tat gga ggg caa aag gca aaa cct ggt gat ttt cct tgg       1395
Gly Arg Ile Tyr Gly Gly Gln Lys Ala Lys Pro Gly Asp Phe Pro Trp
        445                 450                 455

caa gtc ctg ata tta ggt gga acc aca gca gca ggt gca ctt tta tat       1443
Gln Val Leu Ile Leu Gly Gly Thr Thr Ala Ala Gly Ala Leu Leu Tyr
    460                 465                 470

gac aac tgg gtc cta aca gct gct cat gcc gtc tat gag caa aaa cat       1491
Asp Asn Trp Val Leu Thr Ala Ala His Ala Val Tyr Glu Gln Lys His
475                 480                 485                 490

gat gca tcc gcc ctg gac att cga atg ggc acc ctg aaa aga cta tca       1539
Asp Ala Ser Ala Leu Asp Ile Arg Met Gly Thr Leu Lys Arg Leu Ser
                495                 500                 505

cct cat tat aca caa gcc tgg tct gaa gct gtt ttt ata cat gaa ggt       1587
Pro His Tyr Thr Gln Ala Trp Ser Glu Ala Val Phe Ile His Glu Gly
            510                 515                 520

tat act cat gat gct ggc ttt gac aat gac ata gca ctg att aaa ttg       1635
Tyr Thr His Asp Ala Gly Phe Asp Asn Asp Ile Ala Leu Ile Lys Leu
        525                 530                 535

aat aac aaa gtt gta atc aat agc aac atc acg cct att tgt ctg cca       1683
Asn Asn Lys Val Val Ile Asn Ser Asn Ile Thr Pro Ile Cys Leu Pro
    540                 545                 550

aga aaa gaa gct gaa tcc ttt atg agg aca gat gac att gga act gca       1731
Arg Lys Glu Ala Glu Ser Phe Met Arg Thr Asp Asp Ile Gly Thr Ala
```

```
555                 560                 565                 570

tct gga tgg gga tta acc caa agg ggt ttt ctt gct aga aat cta atg       1779
Ser Gly Trp Gly Leu Thr Gln Arg Gly Phe Leu Ala Arg Asn Leu Met
                575                 580                 585

tat gtc gac ata ccg att gtt gac cat caa aaa tgt act gct gca tat       1827
Tyr Val Asp Ile Pro Ile Val Asp His Gln Lys Cys Thr Ala Ala Tyr
            590                 595                 600

gaa aag cca ccc tat cca agg gga agt gta act gct aac atg ctt tgt       1875
Glu Lys Pro Pro Tyr Pro Arg Gly Ser Val Thr Ala Asn Met Leu Cys
        605                 610                 615

gct ggc tta gaa agt ggg ggc aag gac agc tgc aga ggt gac agc gga       1923
Ala Gly Leu Glu Ser Gly Gly Lys Asp Ser Cys Arg Gly Asp Ser Gly
    620                 625                 630

ggg gca ctg gtg ttt cta gat agt gaa aca gag agg tgg ttt gtg gga       1971
Gly Ala Leu Val Phe Leu Asp Ser Glu Thr Glu Arg Trp Phe Val Gly
635                 640                 645                 650

gga ata gtg tcc tgg ggt tcc atg aat tgt ggg gaa gca ggt cag tat       2019
Gly Ile Val Ser Trp Gly Ser Met Asn Cys Gly Glu Ala Gly Gln Tyr
                655                 660                 665

gga gtc tac aca aaa gtt att aac tat att ccc tgg atc gag aac ata       2067
Gly Val Tyr Thr Lys Val Ile Asn Tyr Ile Pro Trp Ile Glu Asn Ile
            670                 675                 680

att agt gat ttt taa cttgcgtgtc tgcagtcaag gattcttcat ttttagaaat       2122
Ile Ser Asp Phe
        685

gcctgtgaag accttggcag cgacgtggct cgagaagcat tcatcattac tgtggacatg     2182

gcagttgttg ctccacccaa aaaaacagac tccaggtgag gctgctgtca tttctccact     2242

tgccagttta attccagcct tacccattga ctcaagggga cataaaccac gagagtgaca     2302

gtcatctttg cccacccagt gtaatgtcac tgctcaaatt acatttcatt accttaaaaa     2362

gccagtctct tttcatactg gctgttggca tttctgtaaa ctgcctgtcc atgctctttg     2422

tttttaaact tgttcttatt gaaaaaaaaa aaaaaaaa                             2460


<210>  5
<211>  686
<212>  PRT
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<213> Homo sapiens

<400> 5

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

<210> 6
<211> 671
<212> PRT
<213> Homo sapiens

<400> 6

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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

Ser Ser Gly Ala Lys Val Leu Ala Thr Leu Cys Gly Gln Glu Ser Thr
```

```
65                  70                  75                  80

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

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

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

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

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

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

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

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

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
```

```
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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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
```

<210> 7
<211> 4900
<212> DNA
<213> Homo sapiens

<400> 7
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ccagcgtagg actgcagacc aggccaggcc agctggacgg gcacaccatg aggtaggtgg 180

gcgccacagc ctccctgcag ggtgtggggt gggagcacag gcctgggcct caccgcccct 240

gccctgccca taggctgctg accctcctgg gccttctgtg tggctcggtg gccacccccт 300

taggcccgaa gtggcctgaa cctgtgttcg ggcgcctggc atcccccggc tttccagggg 360

agtatgccaa tgaccaggag cggcgctgga ccctgactgc acccccggc taccgcctgc 420

gcctctactt cacccacttc gacctggagc tctcccacct ctgcgagtac gacttcgtca 480

aggtgccgtc agacgggagg gctggggttt ctcagggtcg gggggtcccc aaggagtagc 540

cagggttcag ggacacctgg gagcaggggc caggcttggc caggagggag atcaggcctg 600

ggtcttgcct tcactccctg tgacacctga ccccacagct gagctcgggg gccaaggtgc 660

tggccacgct gtgcgggcag gagagcacag acacggagcg ggcccctggc aaggacactt 720

tctactcgct gggctccagc ctggacatta ccttccgctc cgactactcc aacgagaagc 780

cgttcacggg gttcgaggcc ttctatgcag ccgagggtga gccaagaggg gtcctgcaac 840

atctcagtct gcgcagctgg ctgtgggggt aactctgtct taggccaggc agccctgcct 900

tcagtttccc caccтttccc agggcagggg agaggcctct ggcctgacat catccacaat 960

gcaaagacca aaacagccgt gacctccatt cacatgggct gagtgccaac tctgagccag 1020

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ccacccattc ttctacctac ccatcctatc catccatcct tctatcagca tccttctacc 4860

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<210> 8
<211> 136
<212> PRT
<213> Homo sapiens

<400> 8

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

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

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

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

Ser Gly Ala Lys Val Leu Ala Thr Leu Cys Gly Gln Glu Ser Thr Asp

```
                85                  90                  95

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

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
    130                 135
```

<210> 9
<211> 181
<212> PRT
<213> Homo sapiens

<400> 9

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

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

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

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

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

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

```
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

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

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

Lys Arg Thr Cys Ser
            180
```

<210> 10
<211> 293
<212> PRT
<213> Homo sapiens

<400> 10

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Trp Lys Ile His Tyr
```

290

<210> 11
<211> 41
<212> PRT
<213> Homo sapiens

<400> 11

Glu Asp Ile Asp Glu Cys Gln Val Ala Pro Gly Glu Ala Pro Thr Cys
1 5 10 15

Asp His His Cys His Asn His Leu Gly Gly Phe Tyr Cys Ser Cys Arg
20 25 30

Ala Gly Tyr Val Leu His Arg Asn Lys
35 40

<210> 12
<211> 242
<212> PRT
<213> Homo sapiens

<400> 12

Ile Tyr Gly Gly Gln Lys Ala Lys Pro Gly Asp Phe Pro Trp Gln Val
1 5 10 15

Leu Ile Leu Gly Gly Thr Thr Ala Ala Gly Ala Leu Leu Tyr Asp Asn
20 25 30

Trp Val Leu Thr Ala Ala His Ala Val Tyr Glu Gln Lys His Asp Ala
35 40 45

Ser Ala Leu Asp Ile Arg Met Gly Thr Leu Lys Arg Leu Ser Pro His
50 55 60

Tyr Thr Gln Ala Trp Ser Glu Ala Val Phe Ile His Glu Gly Tyr Thr
65 70 75 80

His Asp Ala Gly Phe Asp Asn Asp Ile Ala Leu Ile Lys Leu Asn Asn

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                85                  90                  95

Lys Val Val Ile Asn Ser Asn Ile Thr Pro Ile Cys Leu Pro Arg Lys
            100                 105                 110

Glu Ala Glu Ser Phe Met Arg Thr Asp Asp Ile Gly Thr Ala Ser Gly
        115                 120                 125

Trp Gly Leu Thr Gln Arg Gly Phe Leu Ala Arg Asn Leu Met Tyr Val
    130                 135                 140

Asp Ile Pro Ile Val Asp His Gln Lys Cys Thr Ala Ala Tyr Glu Lys
145                 150                 155                 160

Pro Pro Tyr Pro Arg Gly Ser Val Thr Ala Asn Met Leu Cys Ala Gly
                165                 170                 175

Leu Glu Ser Gly Gly Lys Asp Ser Cys Arg Gly Asp Ser Gly Gly Ala
            180                 185                 190

Leu Val Phe Leu Asp Ser Glu Thr Glu Arg Trp Phe Val Gly Gly Ile
        195                 200                 205

Val Ser Trp Gly Ser Met Asn Cys Gly Glu Ala Gly Gln Tyr Gly Val
    210                 215                 220

Tyr Thr Lys Val Ile Asn Tyr Ile Pro Trp Ile Glu Asn Ile Ile Ser
225                 230                 235                 240

Asp Phe
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<210> 13
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 13

Gly Lys Asp Ser Cys Arg Gly Asp Ala Gly Gly Ala Leu Val Phe Leu
1 5 10 15

<210> 14
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 14

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

<210> 15
<211> 43
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 15

Thr Ala Pro Pro Gly Tyr Arg Leu Arg Leu Tyr Phe Thr His Phe Asp
1 5 10 15

Leu Glu Leu Ser His Leu Cys Glu Tyr Asp Phe Val Lys Leu Ser Ser
20 25 30

Gly Ala Lys Val Leu Ala Thr Leu Cys Gly Gln
35 40

<210> 16
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 16

Thr Phe Arg Ser Asp Tyr Ser Asn
1 5

<210> 17
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 17

Phe Tyr Ser Leu Gly Ser Ser Leu Asp Ile Thr Phe Arg Ser Asp Tyr
1 5 10 15

Ser Asn Glu Lys Pro Phe Thr Gly Phe
20 25

<210> 18
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 18

Ile Asp Glu Cys Gln Val Ala Pro Gly
1 5

<210> 19
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 19

Ala Asn Met Leu Cys Ala Gly Leu Glu Ser Gly Gly Lys Asp Ser Cys

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1               5                   10                  15


Arg Gly Asp Ser Gly Gly Ala Leu Val
            20                  25


<210>  20
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<212>  DNA
<213>  Homo sapiens


<220>
<221>  CDS
<222>  (51)..(797)

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ctg ttt cca tca ctc cct ctc ctt ctc ctg agt atg gtg gca gcg tct        104
Leu Phe Pro Ser Leu Pro Leu Leu Leu Leu Ser Met Val Ala Ala Ser
        5                   10                  15

tac tca gaa act gtg acc tgt gag gat gcc caa aag acc tgc cct gca        152
Tyr Ser Glu Thr Val Thr Cys Glu Asp Ala Gln Lys Thr Cys Pro Ala
    20                  25                  30

gtg att gcc tgt agc tct cca ggc atc aac ggc ttc cca ggc aaa gat        200
Val Ile Ala Cys Ser Ser Pro Gly Ile Asn Gly Phe Pro Gly Lys Asp
35                  40                  45                  50

ggg cgt gat ggc acc aag gga gaa aag ggg gaa cca ggc caa ggg ctc        248
Gly Arg Asp Gly Thr Lys Gly Glu Lys Gly Glu Pro Gly Gln Gly Leu
                55                  60                  65

aga ggc tta cag ggc ccc cct gga aag ttg ggg cct cca gga aat cca        296
Arg Gly Leu Gln Gly Pro Pro Gly Lys Leu Gly Pro Pro Gly Asn Pro
            70                  75                  80

ggg cct tct ggg tca cca gga cca aag ggc caa aaa gga gac cct gga        344
Gly Pro Ser Gly Ser Pro Gly Pro Lys Gly Gln Lys Gly Asp Pro Gly
        85                  90                  95

aaa agt ccg gat ggt gat agt agc ctg gct gcc tca gaa aga aaa gct        392
Lys Ser Pro Asp Gly Asp Ser Ser Leu Ala Ala Ser Glu Arg Lys Ala
    100                 105                 110
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ctg caa aca gaa atg gca cgt atc aaa aag tgg ctc acc ttc tct ctg        440
Leu Gln Thr Glu Met Ala Arg Ile Lys Lys Trp Leu Thr Phe Ser Leu
115                 120                 125                 130

ggc aaa caa gtt ggg aac aag ttc ttc ctg acc aat ggt gaa ata atg        488
Gly Lys Gln Val Gly Asn Lys Phe Phe Leu Thr Asn Gly Glu Ile Met
                135                 140                 145

acc ttt gaa aaa gtg aag gcc ttg tgt gtc aag ttc cag gcc tct gtg        536
Thr Phe Glu Lys Val Lys Ala Leu Cys Val Lys Phe Gln Ala Ser Val
            150                 155                 160

gcc acc ccc agg aat gct gca gag aat gga gcc att cag aat ctc atc        584
Ala Thr Pro Arg Asn Ala Ala Glu Asn Gly Ala Ile Gln Asn Leu Ile
        165                 170                 175

aag gag gaa gcc ttc ctg ggc atc act gat gag aag aca gaa ggg cag        632
Lys Glu Glu Ala Phe Leu Gly Ile Thr Asp Glu Lys Thr Glu Gly Gln
    180                 185                 190

ttt gtg gat ctg aca gga aat aga ctg acc tac aca aac tgg aac gag        680
Phe Val Asp Leu Thr Gly Asn Arg Leu Thr Tyr Thr Asn Trp Asn Glu
195                 200                 205                 210

ggt gaa ccc aac aat gct ggt tct gat gaa gat tgt gta ttg cta ctg        728
Gly Glu Pro Asn Asn Ala Gly Ser Asp Glu Asp Cys Val Leu Leu Leu
                215                 220                 225

aaa aat ggc cag tgg aat gac gtc ccc tgc tcc acc tcc cat ctg gcc        776
Lys Asn Gly Gln Trp Asn Asp Val Pro Cys Ser Thr Ser His Leu Ala
            230                 235                 240

gtc tgt gag ttc cct atc tga agggtcatat cactcaggcc ctccttgtct          827
Val Cys Glu Phe Pro Ile
        245

ttttactgca acccacaggc ccacagtatg cttgaaaaga taaattatat caatttcctc      887

atatccagta ttgttccttt tgtgggcaat cactaaaaat gatcactaac agcaccaaca      947

aagcaataat agt                                                        960
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<210> 21
<211> 248
<212> PRT
<213> Homo sapiens

<400> 21

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Met Ser Leu Phe Pro Ser Leu Pro Leu Leu Leu Leu Ser Met Val Ala
1               5                  10                  15

Ala Ser Tyr Ser Glu Thr Val Thr Cys Glu Asp Ala Gln Lys Thr Cys
            20                  25                  30

Pro Ala Val Ile Ala Cys Ser Ser Pro Gly Ile Asn Gly Phe Pro Gly
        35                  40                  45

Lys Asp Gly Arg Asp Gly Thr Lys Gly Glu Lys Gly Glu Pro Gly Gln
    50                  55                  60

Gly Leu Arg Gly Leu Gln Gly Pro Pro Gly Lys Leu Gly Pro Pro Gly
65                  70                  75                  80

Asn Pro Gly Pro Ser Gly Ser Pro Gly Pro Lys Gly Gln Lys Gly Asp
                85                  90                  95

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

Lys Ala Leu Gln Thr Glu Met Ala Arg Ile Lys Lys Trp Leu Thr Phe
        115                 120                 125

Ser Leu Gly Lys Gln Val Gly Asn Lys Phe Phe Leu Thr Asn Gly Glu
    130                 135                 140

Ile Met Thr Phe Glu Lys Val Lys Ala Leu Cys Val Lys Phe Gln Ala
145                 150                 155                 160

Ser Val Ala Thr Pro Arg Asn Ala Ala Glu Asn Gly Ala Ile Gln Asn
                165                 170                 175

Leu Ile Lys Glu Glu Ala Phe Leu Gly Ile Thr Asp Glu Lys Thr Glu
            180                 185                 190

Gly Gln Phe Val Asp Leu Thr Gly Asn Arg Leu Thr Tyr Thr Asn Trp
        195                 200                 205
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Asn Glu Gly Glu Pro Asn Asn Ala Gly Ser Asp Glu Asp Cys Val Leu
    210   215   220

Leu Leu Lys Asn Gly Gln Trp Asn Asp Val Pro Cys Ser Thr Ser His
225   230   235   240

Leu Ala Val Cys Glu Phe Pro Ile
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<210> 22
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> Wherein X at position 1 represents hydroxyproline

<220>
<221> MISC_FEATURE
<222> (4)..(4)
<223> Wherein X at position 4 represents hydrophobic residue

<400> 22

Xaa Gly Lys Xaa Gly Pro
1   5

<210> 23
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> MISC_FEATURE

<222> (1)..(1)
<223> Wherein X represents hydroxyproline

<400> 23

Xaa Gly Lys Leu Gly
1 5

<210> 24
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> MISC_FEATURE
<222> (9)..(15)
<223> Wherein X at positions 9 and 15 represents hydroxyproline

<400> 24

Gly Leu Arg Gly Leu Gln Gly Pro Xaa Gly Lys Leu Gly Pro Xaa Gly
1 5 10 15

<210> 25
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> MISC_FEATURE
<222> (3)..(27)
<223> Wherein X at positions 3, 6, 15, 21, 24, 27 represents
hydroxyproline

<400> 25

Gly Pro Xaa Gly Pro Xaa Gly Leu Arg Gly Leu Gln Gly Pro Xaa Gly
1 5 10 15

Lys Leu Gly Pro Xaa Gly Pro Xaa Gly Pro Xaa
            20   25

<210> 26
<211> 53
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> misc_feature
<222> (26)..(26)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (32)..(32)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (35)..(35)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (41)..(41)
<223> Xaa can be any naturally occurring amino acid

<220>
<221> misc_feature
<222> (50)..(50)
<223> Xaa can be any naturally occurring amino acid

<400> 26

Gly Lys Asp Gly Arg Asp Gly Thr Lys Gly Glu Lys Gly Glu Pro Gly
1   5   10   15

Gln Gly Leu Arg Gly Leu Gln Gly Pro Xaa Gly Lys Leu Gly Pro Xaa
            20   25   30

Gly Asn Xaa Gly Pro Ser Gly Ser Xaa Gly Pro Lys Gly Gln Lys Gly
        35   40   45

Asp Xaa Gly Lys Ser
    50

<210> 27
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> MISC_FEATURE
<222> (3)..(33)
<223> Wherein X at positions 3, 6, 12, 18, 21, 30, 33 represents
       hydroxyproline

<400> 27

Gly Ala Xaa Gly Ser Xaa Gly Glu Lys Gly Ala Xaa Gly Pro Gln Gly
1   5   10   15

Pro Xaa Gly Pro Xaa Gly Lys Met Gly Pro Lys Gly Glu Xaa Gly Asp
            20   25   30

Xaa

<210> 28
<211> 45
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> MISC_FEATURE
<222> (3)..(45)
<223> Wherein X at positions 3, 6, 9, 27, 30, 36, 42, 45 represents
       hydroxyproline

<400> 28

Gly Cys Xaa Gly Leu Xaa Gly Ala Xaa Gly Asp Lys Gly Glu Ala Gly
1 5 10 15

Thr Asn Gly Lys Arg Gly Glu Arg Gly Pro Xaa Gly Pro Xaa Gly Lys
20 25 30

Ala Gly Pro Xaa Gly Pro Asn Gly Ala Xaa Gly Glu Xaa
35 40 45

<210> 29
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 29

Leu Gln Arg Ala Leu Glu Ile Leu Pro Asn Arg Val Thr Ile Lys Ala
1 5 10 15

Asn Arg Pro Phe Leu Val Phe Ile
20

<210> 30
<211> 559
<212> DNA
<213> Homo sapiens

<400> 30
atgaggctgc tgaccctcct gggccttctg tgtggctcgg tggccacccc cttgggcccg 60

aagtggcctg aacctgtgtt cgggcgcctg gcatcccccg gctttccagg ggagtatgcc 120

aatgaccagg agcggcgctg gaccctgact gcacccccg gctaccgcct gcgcctctac 180

ttcacccact tcgacctgga gctctcccac ctctgcgagt acgacttcgt caagctgagc 240

tcgggggcca aggtgctggc cacgctgtgc gggcaggaga gcacagacac ggagcgggcc 300

cctggcaagg acactttcta ctcgctgggc tccagcctgg acattacctt ccgctccgac 360

tactccaacg agaagccgtt cacggggttc gaggccttct atgcagccga ggacattgac 420

gagtgccagg tggccccggg agaggcgccc acctgcgacc accactgcca caaccacctg 480

ggcggtttct actgctcctg ccgcgcaggc tacgtcctgc accgtaacaa gcgcacctgc 540

tcagccctgt gctccggcc 559

<210> 31
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 31
cgggcacacc atgaggctgc tgaccctcct gggc 34

<210> 32
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 32
gacattacct tccgctccga ctccaacgag aag 33

<210> 33
<211> 33
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<220>
<223> Synthetic

<400> 33
agcagccctg aatacccacg gccgtatccc aaa 33

<210> 34
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 34
cgggatccat gaggctgctg accctc 26

<210> 35
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 35
ggaattccta ggctgcata 19

<210> 36
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 36
ggaattccta cagggcgct 19

<210> 37
<211> 19
<212> DNA
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<220>
<223> Synthetic

<400> 37
ggaattccta gtagtggat 19

<210> 38
<211> 25
<212> DNA
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<220>
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<400> 38
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<210> 39
<211> 23
<212> DNA
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<220>
<223> Synthetic

<400> 39
ggaattcact cgttattctc gga 23

<210> 40
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 40
tccgagaata acgagtg 17

<210> 41
<211> 29
<212> DNA
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<220>
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<400> 41
cattgaaagc tttggggtag aagttgttc 29

<210> 42
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 42

cgcggccgca gctgctcaga gtgtaga 27

<210> 43
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 43
cggtaagctt cactggctca gggaaata 28

<210> 44
<211> 37
<212> DNA
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<220>
<223> Synthetic

<400> 44
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<210> 45
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<400> 45
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<210> 46
<211> 36
<212> DNA
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<220>
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<400> 46
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<210> 47
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 47
cgggatcctt ctccctctaa cactct 26

<210> 48
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 48

Glu Pro Lys Ser Cys Asp Lys Thr His
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<210> 49
<211> 4960
<212> DNA
<213> Homo Sapiens

<400> 49
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gctcgaaccc cggaggcaga ggtttggtgg ctcacacctg taatcccagc actttgcgag 120

gctgaggcag gtgcatcgct ttggctcagg agttcaagac cagcctgggc aacacaggga 180

gacccccatc tctacaaaaa acaaaaacaa atataaaggg gataaaaaaa aaaaaaagac 240

aagacatgaa tccatgagga cagagtgtgg aagaggaagc agcagcctca aagttctgga 300

agctggaaga acagataaac aggtgtgaaa taactgcctg gaaagcaact tctttttttt 360

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tctcggatca ctgcaacctc cgcctcccag gctcaagcaa ttctcctgcc tcagcctccc 480

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caaagttgta atcaatagca acatcacgcc tatttgtctg ccaagaaaag aagctgaatc 4200

ctttatgagg acagatgaca ttggaactgc atctggatgg ggattaaccc aaaggggttt 4260

tcttgctaga aatctaatgt atgtcgacat accgattgtt gaccatcaaa aatgtactgc 4320

tgcatatgaa aagccaccct atccaagggg aagtgtaact gctaacatgc tttgtgctgg 4380

cttagaaagt gggggcaagg acagctgcag aggtgacagc ggaggggcac tggtgtttct 4440

agatagtgaa acagagaggt ggtttgtggg aggaatagtg tcctggggtt ccatgaattg 4500

tggggaagca ggtcagtatg gagtctacac aaaagttatt aactatattc cctggatcga 4560

gaacataatt agtgattttt aacttgcgtg tctgcagtca aggattcttc atttttagaa 4620

atgcctgtga agaccttggc agcgacgtgg ctcgagaagc attcatcatt actgtggaca 4680

tggcagttgt tgctccaccc aaaaaaacag actccaggtg aggctgctgt catttctcca 4740

cttgccagtt taattccagc cttacccatt gactcaaggg gacataaacc acgagagtga 4800

cagtcatctt tgcccaccca gtgtaatgtc actgctcaaa ttacatttca ttaccttaaa 4860

aagccagtct cttttcatac tggctgttgg catttctgta aactgcctgt ccatgctctt 4920

tgtttttaaa cttgttctta ttgaaaaaaa aaaaaaaaaa 4960

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<211> 2090
<212> DNA
<213> Murine

<220>
<221> CDS
<222> (33)..(2090)

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Met Arg Leu Leu Ile Phe Leu
1 5

ggt ctg ctg tgg agt ttg gtg gcc aca ctt ctg ggt tca aag tgg cct 101
Gly Leu Leu Trp Ser Leu Val Ala Thr Leu Leu Gly Ser Lys Trp Pro
10 15 20

gaa cct gta ttc ggg cgc ctg gtg tcc cct ggc ttc cca gag aag tat 149
Glu Pro Val Phe Gly Arg Leu Val Ser Pro Gly Phe Pro Glu Lys Tyr
25 30 35

gct gac cat caa gat cga tcc tgg aca ctg act gca ccc cct ggc tac 197
Ala Asp His Gln Asp Arg Ser Trp Thr Leu Thr Ala Pro Pro Gly Tyr
40 45 50 55

cgc ctg cgc ctc tac ttc acc cac ttt gac ctg gaa ctc tct tac cgc 245
Arg Leu Arg Leu Tyr Phe Thr His Phe Asp Leu Glu Leu Ser Tyr Arg
60 65 70

tgc gag tat gac ttt gtc aag ttg agc tca ggg acc aag gtg ctg gcc 293
Cys Glu Tyr Asp Phe Val Lys Leu Ser Ser Gly Thr Lys Val Leu Ala
75 80 85

aca ctg tgt ggg cag gag agt aca gac act gag cag gca cct ggc aat 341
Thr Leu Cys Gly Gln Glu Ser Thr Asp Thr Glu Gln Ala Pro Gly Asn
90 95 100

gac acc ttc tac tca ctg ggt ccc agc cta aag gtc acc ttc cac tcc 389
Asp Thr Phe Tyr Ser Leu Gly Pro Ser Leu Lys Val Thr Phe His Ser
105 110 115

gac tac tcc aat gag aag ccg ttc aca ggg ttt gag gcc ttc tat gca 437
Asp Tyr Ser Asn Glu Lys Pro Phe Thr Gly Phe Glu Ala Phe Tyr Ala
120 125 130 135

gcg gag gat gtg gat gaa tgc aga gtg tct ctg gga gac tca gtc cct 485
Ala Glu Asp Val Asp Glu Cys Arg Val Ser Leu Gly Asp Ser Val Pro
140 145 150

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tgt gac cat tat tgc cac aac tac ttg ggc ggc tac tat tgc tcc tgc        533
Cys Asp His Tyr Cys His Asn Tyr Leu Gly Gly Tyr Tyr Cys Ser Cys
            155                 160                 165

aga gcg ggc tac att ctc cac cag aac aag cac acg tgc tca gcc ctt        581
Arg Ala Gly Tyr Ile Leu His Gln Asn Lys His Thr Cys Ser Ala Leu
        170                 175                 180

tgt tca ggc cag gtg ttc aca gga aga tct ggg tat ctc agt agc cct        629
Cys Ser Gly Gln Val Phe Thr Gly Arg Ser Gly Tyr Leu Ser Ser Pro
    185                 190                 195

gag tac ccg cag cca tac ccc aag ctc tcc agc tgc acc tac agc atc        677
Glu Tyr Pro Gln Pro Tyr Pro Lys Leu Ser Ser Cys Thr Tyr Ser Ile
200                 205                 210                 215

cgc ctg gag gac ggc ttc agt gtc atc ctg gac ttc gtg gag tcc ttc        725
Arg Leu Glu Asp Gly Phe Ser Val Ile Leu Asp Phe Val Glu Ser Phe
                220                 225                 230

gat gtg gag acg cac cct gaa gcc cag tgc ccc tat gac tcc ctc aag        773
Asp Val Glu Thr His Pro Glu Ala Gln Cys Pro Tyr Asp Ser Leu Lys
            235                 240                 245

att caa aca gac aag ggg gaa cac ggc cca ttt tgt ggg aag acg ctg        821
Ile Gln Thr Asp Lys Gly Glu His Gly Pro Phe Cys Gly Lys Thr Leu
        250                 255                 260

cct ccc agg att gaa act gac agc cac aag gtg acc atc acc ttt gcc        869
Pro Pro Arg Ile Glu Thr Asp Ser His Lys Val Thr Ile Thr Phe Ala
    265                 270                 275

act gac gag tcg ggg aac cac aca ggc tgg aag ata cac tac aca agc        917
Thr Asp Glu Ser Gly Asn His Thr Gly Trp Lys Ile His Tyr Thr Ser
280                 285                 290                 295

aca gca cgg ccc tgc cct gat cca acg gcg cca cct aat ggc agc att        965
Thr Ala Arg Pro Cys Pro Asp Pro Thr Ala Pro Pro Asn Gly Ser Ile
                300                 305                 310

tca cct gtg caa gcc acg tat gtc ctg aag gac agg ttt tct gtc ttc       1013
Ser Pro Val Gln Ala Thr Tyr Val Leu Lys Asp Arg Phe Ser Val Phe
            315                 320                 325

tgc aag aca ggc ttc gag ctt ctg caa ggt tct gtc ccc ctg aaa tca       1061
Cys Lys Thr Gly Phe Glu Leu Leu Gln Gly Ser Val Pro Leu Lys Ser
        330                 335                 340

ttc act gct gtc tgt cag aaa gat gga tct tgg gac cgg ccg atg cca       1109
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Phe Thr Ala Val Cys Gln Lys Asp Gly Ser Trp Asp Arg Pro Met Pro
    345                 350                 355

gag tgc agc att att gat tgt ggc cct ccc gat gac cta ccc aat ggc       1157
Glu Cys Ser Ile Ile Asp Cys Gly Pro Pro Asp Asp Leu Pro Asn Gly
360                 365                 370                 375

cat gtg gac tat atc aca ggc cct caa gtg act acc tac aaa gct gtg       1205
His Val Asp Tyr Ile Thr Gly Pro Gln Val Thr Thr Tyr Lys Ala Val
                380                 385                 390

att cag tac agc tgt gaa gag act ttc tac aca atg agc agc aat ggt       1253
Ile Gln Tyr Ser Cys Glu Glu Thr Phe Tyr Thr Met Ser Ser Asn Gly
            395                 400                 405

aaa tat gtg tgt gag gct gat gga ttc tgg acg agc tcc aaa gga gaa       1301
Lys Tyr Val Cys Glu Ala Asp Gly Phe Trp Thr Ser Ser Lys Gly Glu
        410                 415                 420

aaa ctc ccc ccg gtt tgt gag cct gtt tgt ggg ctg tcc aca cac act       1349
Lys Leu Pro Pro Val Cys Glu Pro Val Cys Gly Leu Ser Thr His Thr
    425                 430                 435

ata gga gga cgc ata gtt gga ggg cag cct gca aag cct ggt gac ttt       1397
Ile Gly Gly Arg Ile Val Gly Gly Gln Pro Ala Lys Pro Gly Asp Phe
440                 445                 450                 455

cct tgg caa gtc ttg ttg ctg ggt caa act aca gca gca gca ggt gca       1445
Pro Trp Gln Val Leu Leu Leu Gly Gln Thr Thr Ala Ala Ala Gly Ala
                460                 465                 470

ctt ata cat gac aat tgg gtc cta aca gcc gct cat gct gta tat gag       1493
Leu Ile His Asp Asn Trp Val Leu Thr Ala Ala His Ala Val Tyr Glu
            475                 480                 485

aaa aga atg gca gcg tcc tcc ctg aac atc cga atg ggc atc ctc aaa       1541
Lys Arg Met Ala Ala Ser Ser Leu Asn Ile Arg Met Gly Ile Leu Lys
        490                 495                 500

agg ctc tca cct cat tac act caa gcc tgg ccc gag gaa atc ttt ata       1589
Arg Leu Ser Pro His Tyr Thr Gln Ala Trp Pro Glu Glu Ile Phe Ile
    505                 510                 515

cat gaa ggc tac act cac ggt gct ggt ttt gac aat gat ata gca ttg       1637
His Glu Gly Tyr Thr His Gly Ala Gly Phe Asp Asn Asp Ile Ala Leu
520                 525                 530                 535

att aaa ctc aag aac aaa gtc aca atc aac gga agc atc atg cct gtt       1685
Ile Lys Leu Lys Asn Lys Val Thr Ile Asn Gly Ser Ile Met Pro Val
                540                 545                 550
```

```
tgc cta ccg cga aaa gaa gct gca tcc tta atg aga aca gac ttc act       1733
Cys Leu Pro Arg Lys Glu Ala Ala Ser Leu Met Arg Thr Asp Phe Thr
            555                 560                 565

gga act gtg gct ggc tgg ggg tta acc cag aag ggg ctt ctt gct aga       1781
Gly Thr Val Ala Gly Trp Gly Leu Thr Gln Lys Gly Leu Leu Ala Arg
        570                 575                 580

aac cta atg ttt gtg gac ata cca att gct gac cac caa aaa tgt acc       1829
Asn Leu Met Phe Val Asp Ile Pro Ile Ala Asp His Gln Lys Cys Thr
    585                 590                 595

acc gtg tat gaa aag ctc tat cca gga gta aga gta agc gct aac atg       1877
Thr Val Tyr Glu Lys Leu Tyr Pro Gly Val Arg Val Ser Ala Asn Met
600                 605                 610                 615

ctc tgt gct ggc tta gag act ggt ggc aag gac agc tgc aga ggt gac       1925
Leu Cys Ala Gly Leu Glu Thr Gly Gly Lys Asp Ser Cys Arg Gly Asp
                620                 625                 630

agt ggg ggg gca tta gtg ttt cta gat aat gag aca cag cga tgg ttt       1973
Ser Gly Gly Ala Leu Val Phe Leu Asp Asn Glu Thr Gln Arg Trp Phe
            635                 640                 645

gtg gga gga ata gtt tcc tgg ggt tcc att aat tgt ggg gcg gca ggc       2021
Val Gly Gly Ile Val Ser Trp Gly Ser Ile Asn Cys Gly Ala Ala Gly
        650                 655                 660

cag tat ggg gtc tac aca aaa gtc atc aac tat att ccc tgg aat gag       2069
Gln Tyr Gly Val Tyr Thr Lys Val Ile Asn Tyr Ile Pro Trp Asn Glu
    665                 670                 675

aac ata ata agt aat ttc taa                                          2090
Asn Ile Ile Ser Asn Phe
680                 685
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<210> 51
<211> 685
<212> PRT
<213> Murine

<400> 51

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Met Arg Leu Leu Ile Phe 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
```

```
            20                  25                  30

Pro Gly Phe Pro Glu Lys Tyr Ala Asp His Gln Asp Arg Ser Trp Thr
        35                  40                  45

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

Asp Leu Glu Leu Ser Tyr Arg Cys Glu Tyr Asp Phe Val Lys Leu Ser
65                  70                  75                  80

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

Thr Glu Gln Ala Pro Gly Asn Asp Thr Phe Tyr Ser Leu Gly Pro Ser
            100                 105                 110

Leu Lys Val Thr Phe His Ser Asp Tyr Ser Asn Glu Lys Pro Phe Thr
        115                 120                 125

Gly Phe Glu Ala Phe Tyr Ala Ala Glu Asp Val Asp Glu Cys Arg Val
    130                 135                 140

Ser Leu Gly Asp Ser Val Pro Cys Asp His Tyr Cys His Asn Tyr Leu
145                 150                 155                 160

Gly Gly Tyr Tyr Cys Ser Cys Arg Ala Gly Tyr Ile Leu His Gln Asn
                165                 170                 175

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Trp Thr Ser Ser Lys Gly Glu Lys Leu Pro Pro Val Cys Glu Pro Val
```

```
            420                 425                 430

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

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

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

Ala Ala His Ala Val Tyr Glu Lys Arg Met Ala Ala Ser Ser Leu Asn
                485                 490                 495

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

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

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

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

Leu Met Arg Thr Asp Phe Thr Gly Thr Val Ala Gly Trp Gly Leu Thr
                565                 570                 575

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

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

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

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


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


Ile Asn Cys Gly Ala Ala Gly Gln Tyr Gly Val Tyr Thr Lys Val Ile
            660                 665                 670


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


<210>  52
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<212>  PRT
<213>  Murine

<400>  52

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


Ser Pro Gly Phe Pro Glu Lys Tyr Ala Asp His Gln Asp Arg Ser 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 Tyr Arg Cys Glu Tyr Asp Phe Val Lys Leu
    50                  55                  60


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


Asp Thr Glu Gln Ala Pro Gly Asn Asp Thr Phe Tyr Ser Leu Gly Pro
                85                  90                  95


Ser Leu Lys Val Thr Phe His Ser Asp Tyr Ser Asn Glu Lys Pro Phe
            100                 105                 110
```

```
Thr Gly Phe Glu Ala Phe Tyr Ala Ala Glu Asp Val Asp Glu Cys Arg
        115                 120                 125

Val Ser Leu Gly Asp Ser Val Pro Cys Asp His Tyr Cys His Asn Tyr
    130                 135                 140

Leu Gly Gly Tyr Tyr Cys Ser Cys Arg Ala Gly Tyr Ile Leu His Gln
145                 150                 155                 160

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

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

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

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

Gln Cys Pro Tyr Asp Ser Leu Lys Ile Gln Thr Asp Lys Gly Glu His
225                 230                 235                 240

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

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

Gly Trp Lys Ile His Tyr Thr Ser Thr Ala Arg Pro Cys Pro Asp Pro
        275                 280                 285

Thr Ala Pro Pro Asn Gly Ser Ile Ser Pro Val Gln Ala Thr Tyr Val
    290                 295                 300
```

```
Leu Lys Asp Arg Phe Ser Val Phe Cys Lys Thr Gly Phe Glu Leu Leu
305                 310                 315                 320

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

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

Pro Pro Asp Asp Leu Pro Asn Gly His Val Asp Tyr Ile Thr Gly Pro
        355                 360                 365

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

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

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

Val Cys Gly Leu Ser Thr His Thr Ile Gly Gly Arg Ile Val Gly Gly
            420                 425                 430

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

Gln Thr Thr Ala Ala Ala Gly Ala Leu Ile His Asp Asn Trp Val Leu
    450                 455                 460

Thr Ala Ala His Ala Val Tyr Glu Lys Arg Met Ala Ala Ser Ser Leu
465                 470                 475                 480

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

Ala Trp Pro Glu Glu Ile Phe Ile His Glu Gly Tyr Thr His Gly Ala
            500                 505                 510
```

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

Ile Asn Gly Ser Ile Met Pro Val Cys Leu Pro Arg Lys Glu Ala Ala
    530                 535                 540

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

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

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

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

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

Asp Asn Glu Thr Gln Arg Trp Phe Val Gly Gly Ile Val Ser Trp Gly
625                 630                 635                 640

Ser Ile Asn Cys Gly Ala Ala Gly Gln Tyr Gly Val Tyr Thr Lys Val
                645                 650                 655

Ile Asn Tyr Ile Pro Trp Asn Glu Asn Ile Ile Ser Asn Phe
            660                 665                 670
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<210> 53
<211> 2091
<212> DNA
<213> Rat

<220>
<221> CDS

<222> (10)..(2067)

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          Met Arg Leu Leu Ile Val Leu Gly Leu Leu Trp Ser Leu Val
          1   5   10

gcc aca ctt ttg ggc tcc aag tgg cct gag cct gta ttc ggg cgc ctg 99
Ala Thr Leu Leu Gly Ser Lys Trp Pro Glu Pro Val Phe Gly Arg Leu
15   20   25   30

gtg tcc ctg gcc ttc cca gag aag tat ggc aac cat cag gat cga tcc 147
Val Ser Leu Ala Phe Pro Glu Lys Tyr Gly Asn His Gln Asp Arg Ser
                35   40   45

tgg acg ctg act gca ccc cct ggc ttc cgc ctg cgc ctc tac ttc acc 195
Trp Thr Leu Thr Ala Pro Pro Gly Phe Arg Leu Arg Leu Tyr Phe Thr
            50   55   60

cac ttc aac ctg gaa ctc tct tac cgc tgc gag tat gac ttt gtc aag 243
His Phe Asn Leu Glu Leu Ser Tyr Arg Cys Glu Tyr Asp Phe Val Lys
        65   70   75

ttg acc tca ggg acc aag gtg cta gcc acg ctg tgt ggg cag gag agt 291
Leu Thr Ser Gly Thr Lys Val Leu Ala Thr Leu Cys Gly Gln Glu Ser
    80   85   90

aca gat act gag cgg gca cct ggc aat gac acc ttc tac tca ctg ggt 339
Thr Asp Thr Glu Arg Ala Pro Gly Asn Asp Thr Phe Tyr Ser Leu Gly
95   100   105   110

ccc agc cta aag gtc acc ttc cac tcc gac tac tcc aat gag aag cca 387
Pro Ser Leu Lys Val Thr Phe His Ser Asp Tyr Ser Asn Glu Lys Pro
                115   120   125

ttc aca gga ttt gag gcc ttc tat gca gcg gag gat gtg gat gaa tgc 435
Phe Thr Gly Phe Glu Ala Phe Tyr Ala Ala Glu Asp Val Asp Glu Cys
            130   135   140

aga aca tcc ctg gga gac tca gtc cct tgt gac cat tat tgc cac aac 483
Arg Thr Ser Leu Gly Asp Ser Val Pro Cys Asp His Tyr Cys His Asn
        145   150   155

tac ctg ggc ggc tac tac tgc tcc tgc cga gtg ggc tac att ctg cac 531
Tyr Leu Gly Gly Tyr Tyr Cys Ser Cys Arg Val Gly Tyr Ile Leu His
    160   165   170

cag aac aag cat acc tgc tca gcc ctt tgt tca ggc cag gtg ttc act 579
Gln Asn Lys His Thr Cys Ser Ala Leu Cys Ser Gly Gln Val Phe Thr
175   180   185   190

```
ggg agg tct ggc ttt ctc agt agc cct gag tac cca cag cca tac ccc        627
Gly Arg Ser Gly Phe Leu Ser Ser Pro Glu Tyr Pro Gln Pro Tyr Pro
                195                 200                 205

aaa ctc tcc agc tgc gcc tac aac atc cgc ctg gag gaa ggc ttc agt        675
Lys Leu Ser Ser Cys Ala Tyr Asn Ile Arg Leu Glu Glu Gly Phe Ser
            210                 215                 220

atc acc ctg gac ttc gtg gag tcc ttt gat gtg gag atg cac cct gaa        723
Ile Thr Leu Asp Phe Val Glu Ser Phe Asp Val Glu Met His Pro Glu
        225                 230                 235

gcc cag tgc ccc tac gac tcc ctc aag att caa aca gac aag agg gaa        771
Ala Gln Cys Pro Tyr Asp Ser Leu Lys Ile Gln Thr Asp Lys Arg Glu
    240                 245                 250

tac ggc ccg ttt tgt ggg aag acg ctg ccc ccc agg att gaa act gac        819
Tyr Gly Pro Phe Cys Gly Lys Thr Leu Pro Pro Arg Ile Glu Thr Asp
255                 260                 265                 270

agc aac aag gtg acc att acc ttt acc acc gac gag tca ggg aac cac        867
Ser Asn Lys Val Thr Ile Thr Phe Thr Thr Asp Glu Ser Gly Asn His
                275                 280                 285

aca ggc tgg aag ata cac tac aca agc aca gca cag ccc tgc cct gat        915
Thr Gly Trp Lys Ile His Tyr Thr Ser Thr Ala Gln Pro Cys Pro Asp
            290                 295                 300

cca acg gcg cca cct aat ggt cac att tca cct gtg caa gcc acg tat        963
Pro Thr Ala Pro Pro Asn Gly His Ile Ser Pro Val Gln Ala Thr Tyr
        305                 310                 315

gtc ctg aag gac agc ttt tct gtc ttc tgc aag act ggc ttc gag ctt       1011
Val Leu Lys Asp Ser Phe Ser Val Phe Cys Lys Thr Gly Phe Glu Leu
    320                 325                 330

ctg caa ggt tct gtc ccc ctg aag tca ttc act gct gtc tgt cag aaa       1059
Leu Gln Gly Ser Val Pro Leu Lys Ser Phe Thr Ala Val Cys Gln Lys
335                 340                 345                 350

gat gga tct tgg gac cgg ccg ata cca gag tgc agc att att gac tgt       1107
Asp Gly Ser Trp Asp Arg Pro Ile Pro Glu Cys Ser Ile Ile Asp Cys
                355                 360                 365

ggc cct ccc gat gac cta ccc aat ggc cac gtg gac tat atc aca ggc       1155
Gly Pro Pro Asp Asp Leu Pro Asn Gly His Val Asp Tyr Ile Thr Gly
            370                 375                 380

cct gaa gtg acc acc tac aaa gct gtg att cag tac agc tgt gaa gag       1203
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```
Pro Glu Val Thr Thr Tyr Lys Ala Val Ile Gln Tyr Ser Cys Glu Glu
        385                 390                 395

act ttc tac aca atg agc agc aat ggt aaa tat gtg tgt gag gct gat       1251
Thr Phe Tyr Thr Met Ser Ser Asn Gly Lys Tyr Val Cys Glu Ala Asp
    400                 405                 410

gga ttc tgg acg agc tcc aaa gga gaa aaa tcc ctc ccg gtt tgc aag       1299
Gly Phe Trp Thr Ser Ser Lys Gly Glu Lys Ser Leu Pro Val Cys Lys
415                 420                 425                 430

cct gtc tgt gga ctg tcc aca cac act tca gga ggc cgt ata att gga       1347
Pro Val Cys Gly Leu Ser Thr His Thr Ser Gly Gly Arg Ile Ile Gly
                435                 440                 445

gga cag cct gca aag cct ggt gac ttt cct tgg caa gtc ttg tta ctg       1395
Gly Gln Pro Ala Lys Pro Gly Asp Phe Pro Trp Gln Val Leu Leu Leu
            450                 455                 460

ggt gaa act aca gca gca ggt gct ctt ata cat gac gac tgg gtc cta       1443
Gly Glu Thr Thr Ala Ala Gly Ala Leu Ile His Asp Asp Trp Val Leu
        465                 470                 475

aca gcg gct cat gct gta tat ggg aaa aca gag gcg atg tcc tcc ctg       1491
Thr Ala Ala His Ala Val Tyr Gly Lys Thr Glu Ala Met Ser Ser Leu
    480                 485                 490

gac atc cgc atg ggc atc ctc aaa agg ctc tcc ctc att tac act caa       1539
Asp Ile Arg Met Gly Ile Leu Lys Arg Leu Ser Leu Ile Tyr Thr Gln
495                 500                 505                 510

gcc tgg cca gag gct gtc ttt atc cat gaa ggc tac act cac gga gct       1587
Ala Trp Pro Glu Ala Val Phe Ile His Glu Gly Tyr Thr His Gly Ala
                515                 520                 525

ggt ttt gac aat gat ata gca ctg att aaa ctc aag aac aaa gtc aca       1635
Gly Phe Asp Asn Asp Ile Ala Leu Ile Lys Leu Lys Asn Lys Val Thr
            530                 535                 540

atc aac aga aac atc atg ccg att tgt cta cca aga aaa gaa gct gca       1683
Ile Asn Arg Asn Ile Met Pro Ile Cys Leu Pro Arg Lys Glu Ala Ala
        545                 550                 555

tcc tta atg aaa aca gac ttc gtt gga act gtg gct ggc tgg ggg tta       1731
Ser Leu Met Lys Thr Asp Phe Val Gly Thr Val Ala Gly Trp Gly Leu
    560                 565                 570

acc cag aag ggg ttt ctt gct aga aac cta atg ttt gtg gac ata cca       1779
Thr Gln Lys Gly Phe Leu Ala Arg Asn Leu Met Phe Val Asp Ile Pro
575                 580                 585                 590
```

```
att gtt gac cac caa aaa tgt gct act gcg tat aca aag cag ccc tac       1827
Ile Val Asp His Gln Lys Cys Ala Thr Ala Tyr Thr Lys Gln Pro Tyr
                595                 600                 605

cca gga gca aaa gtg act gtt aac atg ctc tgt gct ggc cta gac cgc       1875
Pro Gly Ala Lys Val Thr Val Asn Met Leu Cys Ala Gly Leu Asp Arg
            610                 615                 620

ggt ggc aag gac agc tgc aga ggt gac agc gga ggg gca tta gtg ttt       1923
Gly Gly Lys Asp Ser Cys Arg Gly Asp Ser Gly Gly Ala Leu Val Phe
        625                 630                 635

cta gac aat gaa aca cag aga tgg ttt gtg gga gga ata gtt tcc tgg       1971
Leu Asp Asn Glu Thr Gln Arg Trp Phe Val Gly Gly Ile Val Ser Trp
    640                 645                 650

ggt tct att aac tgt ggg ggg tca gaa cag tat ggg gtc tac acg aaa       2019
Gly Ser Ile Asn Cys Gly Gly Ser Glu Gln Tyr Gly Val Tyr Thr Lys
655                 660                 665                 670

gtc acg aac tat att ccc tgg att gag aac ata ata aat aat ttc taa       2067
Val Thr Asn Tyr Ile Pro Trp Ile Glu Asn Ile Ile Asn Asn Phe
                675                 680                 685

tttgcaaaaa aaaaaaaaaa aaaa                                           2091
```

<210> 54
<211> 685
<212> PRT
<213> Rat

<400> 54

```
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
            20                  25                  30

Leu Ala Phe Pro Glu Lys Tyr Gly Asn His Gln Asp Arg Ser Trp Thr
        35                  40                  45

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

```
Asn Leu Glu Leu Ser Tyr Arg Cys Glu Tyr Asp Phe Val Lys Leu Thr
65                  70                  75                  80

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

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

Leu Lys Val Thr Phe His Ser Asp Tyr Ser Asn Glu Lys Pro Phe Thr
        115                 120                 125

Gly Phe Glu Ala Phe Tyr Ala Ala Glu Asp Val Asp Glu Cys Arg Thr
    130                 135                 140

Ser Leu Gly Asp Ser Val Pro Cys Asp His Tyr Cys His Asn Tyr Leu
145                 150                 155                 160

Gly Gly Tyr Tyr Cys Ser Cys Arg Val Gly Tyr Ile Leu His Gln Asn
                165                 170                 175

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

Ser Gly Phe Leu Ser Ser Pro Glu Tyr Pro Gln Pro Tyr Pro Lys Leu
        195                 200                 205

Ser Ser Cys Ala Tyr Asn Ile Arg Leu Glu Glu Gly Phe Ser Ile Thr
    210                 215                 220

Leu Asp Phe Val Glu Ser Phe Asp Val Glu Met His Pro Glu Ala Gln
225                 230                 235                 240

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

Pro Phe Cys Gly Lys Thr Leu Pro Pro Arg Ile Glu Thr Asp Ser Asn
```

```
            260                 265                 270

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Ile Asn Cys Gly Gly Ser Glu Gln Tyr Gly Val Tyr Thr Lys Val Thr
```

660 665 670

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

<210> 55
<211> 670
<212> PRT
<213> Rat

<400> 55

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

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

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

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

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

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

Ser Leu Lys Val Thr Phe His Ser Asp Tyr Ser Asn Glu Lys Pro Phe
100 105 110

Thr Gly Phe Glu Ala Phe Tyr Ala Ala Glu Asp Val Asp Glu Cys Arg
115 120 125

Thr Ser Leu Gly Asp Ser Val Pro Cys Asp His Tyr Cys His Asn Tyr
130 135 140

```
Leu Gly Gly Tyr Tyr Cys Ser Cys Arg Val Gly Tyr Ile Leu His Gln
145                 150                 155                 160

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

Arg Ser Gly Phe Leu Ser Ser Pro Glu Tyr Pro Gln Pro Tyr Pro Lys
            180                 185                 190

Leu Ser Ser Cys Ala Tyr Asn Ile Arg Leu Glu Glu Gly Phe Ser Ile
        195                 200                 205

Thr Leu Asp Phe Val Glu Ser Phe Asp Val Glu Met His Pro Glu Ala
    210                 215                 220

Gln Cys Pro Tyr Asp Ser Leu Lys Ile Gln Thr Asp Lys Arg Glu Tyr
225                 230                 235                 240

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

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

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

Thr Ala Pro Pro Asn Gly His Ile Ser Pro Val Gln Ala Thr Tyr Val
    290                 295                 300

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

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

Gly Ser Trp Asp Arg Pro Ile Pro Glu Cys Ser Ile Ile Asp Cys Gly
            340                 345                 350
```

```
Pro Pro Asp Asp Leu Pro Asn Gly His Val Asp Tyr Ile Thr Gly Pro
        355                 360                 365

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

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

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

Val Cys Gly Leu Ser Thr His Thr Ser Gly Gly Arg Ile Ile Gly Gly
            420                 425                 430

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

Glu Thr Thr Ala Ala Gly Ala Leu Ile His Asp Asp Trp Val Leu Thr
    450                 455                 460

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

Ile Arg Met Gly Ile Leu Lys Arg Leu Ser Leu Ile Tyr Thr Gln Ala
                485                 490                 495

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

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

Asn Arg Asn Ile Met Pro Ile Cys Leu Pro Arg Lys Glu Ala Ala Ser
    530                 535                 540
```

```
Leu Met Lys Thr Asp Phe Val Gly Thr Val Ala Gly Trp Gly Leu Thr
545                 550                 555                 560


Gln Lys Gly Phe Leu Ala Arg Asn Leu Met Phe Val Asp Ile Pro Ile
                565                 570                 575


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


Gly Ala Lys Val Thr Val Asn Met Leu Cys Ala Gly Leu Asp Arg Gly
        595                 600                 605


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


Asp Asn Glu Thr Gln Arg Trp Phe Val Gly Gly Ile Val Ser Trp Gly
625                 630                 635                 640


Ser Ile Asn Cys Gly Gly Ser Glu Gln Tyr Gly Val Tyr Thr Lys Val
                645                 650                 655


Thr Asn Tyr Ile Pro Trp Ile Glu Asn Ile Ile Asn Asn Phe
            660                 665                 670


<210>  56
<211>  28
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Homo Sapiens

<400>  56
atgaggctgc tgaccctcct gggccttc                                          28


<210>  57
<211>  23
<212>  DNA
<213>  Artificial Sequence

<220>
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<223> Homo Sapiens

<400> 57
gtgcccctcc tgcgtcacct ctg 23

<210> 58
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Homo Sapiens

<400> 58
cagaggtgac gcaggagggg cac 23

<210> 59
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Homo Sapiens

<400> 59
ttaaaatcac taattatgtt ctcgatc 27

<210> 60
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Murine

<400> 60
atgaggctac tcatcttcct gg 22

<210> 61
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Murine

<400> 61
ctgcagaggt gacgcagggg ggg 23

<210> 62
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Murine

<400> 62
ccccccctgc gtcacctctg cag 23

<210> 63
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Murine

<400> 63
ttagaaatta cttattatgt tctcaatcc 29

<210> 64
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Rat

<400> 64
gaggtgacgc aggaggggca ttagtgttt 29

<210> 65
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Rat

<400> 65
ctagaaacac taatgcccct cctgcgtcac ctctgca 37

<210> 66
<211> 354
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 66
caggtcacct tgaaggagtc tggtcctgtg ctggtgaaac ccacagagac cctcacgctg 60

acctgcaccg tctctgggtt ctcactcagc aggggtaaaa tgggtgtgag ctggatccgt 120

cagcccccag ggaaggccct ggagtggctt gcacacattt tttcgagtga cgaaaaatcc 180

tacaggacat cgctgaagag caggctcacc atctccaagg acacctccaa aaaccaggtg 240

gtccttacaa tgaccaacat ggaccctgtg gacacagcca cgtattactg tgcacggata 300

cgacgtggag gaattgacta ctggggccag ggaaccctgg tcactgtctc ctca 354

<210> 67
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 67

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

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

```
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80


Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
                85                  90                  95


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
```

<210> 68
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 68

```
Gln Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1               5                   10                  15


Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp Ser Val Ser Ser Thr
            20                  25                  30


Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro Ser Arg Gly Leu Glu
        35                  40                  45


Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr Asn Asp Tyr Ala
    50                  55                  60


Val Ser Val Lys Ser Arg Ile Thr Ile Asn Pro Asp Thr Ser Lys Asn
65                  70                  75                  80


Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro Glu Asp Thr Ala Val
                85                  90                  95
```

Tyr Tyr Cys Ala Arg Asp Pro Phe Gly Val Pro Phe Asp Ile Trp Gly
100 105 110

Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 69
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 69

Gln Pro Val Leu Thr Gln Pro Pro Ser Leu Ser Val Ser Pro Gly Gln
1 5 10 15

Thr Ala Ser Ile Thr Cys Ser Gly Glu Lys Leu Gly Asp Lys Tyr Ala
20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Met Tyr
35 40 45

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

Asp Glu Ala Asp Tyr Tyr Cys Gln Ala Trp Asp Ser Ser Thr Ala Val
85 90 95

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

<210> 70
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 70
tcctatgagc tgatacagcc accctcggtg tcagtggccc caggacagac ggccaccatt 60

acctgtgcgg gagacaacct tgggaagaaa cgtgtgcact ggtaccagca gaggccaggc 120

caggcccctg tgttggtcat ctatgatgat agcgaccggc cctcagggat ccctgaccga 180

ttctctgcct ccaactctgg gaacacggcc accctgacca tcactagggg cgaagccggg 240

gatgaggccg actattattg tcaggtgtgg gacattgcta ctgatcatgt ggtcttcggc 300

ggagggacca agctcaccgt ccta 324

<210> 71
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 71

Ser Tyr Glu Leu Ile Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
1   5   10   15

Thr Ala Thr Ile Thr Cys Ala Gly Asp Asn Leu Gly Lys Lys Arg Val
            20   25   30

His Trp Tyr Gln Gln Arg Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35   40   45

Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Ala Ser
    50   55   60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Thr Arg Gly Glu Ala Gly
65   70   75   80

Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ile Ala Thr Asp His
                85   90   95

Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ala Ala Ala Gly
100 105 110

Ser Glu Gln Lys Leu Ile Ser Glu
115 120

<210> 72
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 72

Leu Glu Val Thr Cys Glu Pro Gly Thr Thr Phe Lys Asp Lys Cys Asn
1 5 10 15

Thr Cys Arg Cys Gly Ser Asp Gly Lys Ser Ala Val Cys Thr Lys Leu
20 25 30

Trp Cys Asn Gln
35

<210> 73
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 73

Thr Cys Glu Pro Gly Thr Thr Phe Lys Asp Lys Cys Asn Thr Cys Arg
1 5 10 15

Cys Gly Ser Asp Gly Lys Ser Ala Val Cys Thr Lys Leu Trp Cys Asn
20 25 30

Gln

<210> 74
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 74

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

Trp Cys Asn Gln
20

<210> 75
<211> 491
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 75

Leu Glu Val Thr Cys Glu Pro Gly Thr Thr Phe Lys Asp Lys Cys Asn
1 5 10 15

Thr Cys Arg Cys Gly Ser Asp Gly Lys Ser Ala Val Cys Thr Lys Leu
20 25 30

Trp Cys Asn Gln Gly Thr Gly Gly Gly Ser Gly Ser Ser Ser Gln Val
35 40 45

Thr Leu Lys Glu Ser Gly Pro Val Leu Val Lys Pro Thr Glu Thr Leu
50 55 60

Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Arg Gly Lys Met
65 70 75 80

```
Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
                85                  90                  95

Ala His Ile Phe Ser Ser Asp Glu Lys Ser Tyr Arg Thr Ser Leu Lys
            100                 105                 110

Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu
        115                 120                 125

Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
    130                 135                 140

Arg Ile Arg Arg Gly Gly Ile Asp Tyr Trp Gly Gln Gly Thr Leu Val
145                 150                 155                 160

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
                165                 170                 175

Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
            180                 185                 190

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
        195                 200                 205

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
    210                 215                 220

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
225                 230                 235                 240

Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
                245                 250                 255

Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
            260                 265                 270
```

```
Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
        275                 280                 285

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
    290                 295                 300

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
305                 310                 315                 320

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
                325                 330                 335

Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
            340                 345                 350

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
        355                 360                 365

Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
    370                 375                 380

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
385                 390                 395                 400

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
                405                 410                 415

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
            420                 425                 430

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
        435                 440                 445

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
    450                 455                 460

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
465                 470                 475                 480
```

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
485 490

<210> 76
<211> 491
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 76

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

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

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95

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 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
130 135 140

```
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190

Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
        195                 200                 205

Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
    210                 215                 220

Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
225                 230                 235                 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                245                 250                 255

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
            260                 265                 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        275                 280                 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    290                 295                 300

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305                 310                 315                 320

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
                325                 330                 335
```

```
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            340                 345                 350

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        355                 360                 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    370                 375                 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385                 390                 395                 400

Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
                405                 410                 415

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
            420                 425                 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Ala Ala Gly
        435                 440                 445

Gly Ser Gly Leu Glu Val Thr Cys Glu Pro Gly Thr Thr Phe Lys Asp
    450                 455                 460

Lys Cys Asn Thr Cys Arg Cys Gly Ser Asp Gly Lys Ser Ala Val Cys
465                 470                 475                 480

Thr Lys Leu Trp Cys Asn Gln Gly Ser Gly Ala
                485                 490
```

<210> 77
<211> 258
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 77

```
Leu Glu Val Thr Cys Glu Pro Gly Thr Thr Phe Lys Asp Lys Cys Asn
1               5                  10                  15

Thr Cys Arg Cys Gly Ser Asp Gly Lys Ser Ala Val Cys Thr Lys Leu
            20                  25                  30

Trp Cys Asn Gln Gly Thr Gly Gly Gly Ser Gly Ser Ser Ser Gln Pro
        35                  40                  45

Val Leu Thr Gln Pro Pro Ser Leu Ser Val Ser Pro Gly Gln Thr Ala
    50                  55                  60

Ser Ile Thr Cys Ser Gly Glu Lys Leu Gly Asp Lys Tyr Ala Tyr Trp
65                  70                  75                  80

Tyr Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Met Tyr Gln Asp
                85                  90                  95

Lys Gln Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser Asn Ser
            100                 105                 110

Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Met Asp Glu
        115                 120                 125

Ala Asp Tyr Tyr Cys Gln Ala Trp Asp Ser Ser Thr Ala Val Phe Gly
    130                 135                 140

Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro Lys Ala Ala Pro Ser
145                 150                 155                 160

Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala
                165                 170                 175

Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val
            180                 185                 190

Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala Gly Val Glu Thr Thr
        195                 200                 205
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35 40 45

Gln Asp Lys Gln Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
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Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Met
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Asp Glu Ala Asp Tyr Tyr Cys Gln Ala Trp Asp Ser Ser Thr Ala Val
85 90 95

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Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro Lys Ala Ala
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Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Gln Ala Asn
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Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val
    130                 135                 140

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

Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser
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Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg Ser Tyr Ser
            180                 185                 190

Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val Ala Pro
        195                 200                 205

Thr Glu Cys Ser Ala Ala Gly Gly Ser Gly Leu Glu Val Thr Cys Glu
    210                 215                 220

Pro Gly Thr Thr Phe Lys Asp Lys Cys Asn Thr Cys Arg Cys Gly Ser
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Gly Ala
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tcgggatcca gctcacaggt caccttgaag gagtctggtc ctgtgctggt gaaacccaca 240

gagaccctca cgctgacctg caccgtctct gggttctcac tcagcagggg taaaatgggt 300

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cgtggaggaa ttgactactg gggccaggga accctggtca ctgtctcctc agcctccacc 420

aagggcccat ccgtcttccc cctggcgccc tgctccagga gcacctccga gagcacagcc 480

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ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 600

tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacgaagac ctacacctgc 660

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gggacccagg ctatggatga ggctgactat tactgtcagg cgtgggacag cagcactgcg 480

gtattcggcg gagggaccaa gctgaccgtc ctaggccagc ctaaggcggc gccctcggtc 540

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ataagtgact tctacccggg agccgtgaca gtggcctgga aggcagatag cagccccgtc 660

aaggcgggag tggagaccac cacaccctcc aaacaaagca acaacaagta cgcggccagc 720

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        35                  40                  45

Val Ile Ser Lys Met Leu Phe Val Glu Pro Ile Leu Glu Val Ser Ser
    50                  55                  60

Leu Pro Thr Thr Asn Ser Thr Thr Asn Ser Ala Thr Lys Ile Thr Ala
65                  70                  75                  80

Asn Thr Thr Asp Glu Pro Thr Thr Gln Pro Thr Thr Glu Pro Thr Thr
                85                  90                  95

Gln Pro Thr Ile Gln Pro Thr Gln Pro Thr Thr Gln Leu Pro Thr Asp
            100                 105                 110

Ser Pro Thr Gln Pro Thr Thr Gly Ser Phe Cys Pro Gly Pro Val Thr
        115                 120                 125

Leu Cys Ser Asp Leu Glu Ser His Ser Thr Glu Ala Val Leu Gly Asp
    130                 135                 140

Ala Leu Val Asp Phe Ser Leu Lys Leu Tyr His Ala Phe Ser Ala Met
145                 150                 155                 160

Lys Lys Val Glu Thr Asn Met Ala Phe Ser Pro Phe Ser Ile Ala Ser
                165                 170                 175

Leu Leu Thr Gln Val Leu Leu Gly Ala Gly Glu Asn Thr Lys Thr Asn
            180                 185                 190

Leu Glu Ser Ile Leu Ser Tyr Pro Lys Asp Phe Thr Cys Val His Gln
        195                 200                 205

Ala Leu Lys Gly Phe Thr Thr Lys Gly Val Thr Ser Val Ser Gln Ile
    210                 215                 220
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Arg Thr Leu Tyr Ser Ser Ser Pro Arg Val Leu Ser Asn Asn Ser Asp
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Ala Asn Leu Glu Leu Ile Asn Thr Trp Val Ala Lys Asn Thr Asn Asn
            260                 265                 270

Lys Ile Ser Arg Leu Leu Asp Ser Leu Pro Ser Asp Thr Arg Leu Val
        275                 280                 285

Leu Leu Asn Ala Ile Tyr Leu Ser Ala Lys Trp Lys Thr Thr Phe Asp
    290                 295                 300

Pro Lys Lys Thr Arg Met Glu Pro Phe His Phe Lys Asn Ser Val Ile
305                 310                 315                 320

Lys Val Pro Met Met Asn Ser Lys Lys Tyr Pro Val Ala His Phe Ile
                325                 330                 335

Asp Gln Thr Leu Lys Ala Lys Val Gly Gln Leu Gln Leu Ser His Asn
            340                 345                 350

Leu Ser Leu Val Ile Leu Val Pro Gln Asn Leu Lys His Arg Leu Glu
        355                 360                 365

Asp Met Glu Gln Ala Leu Ser Pro Ser Val Phe Lys Ala Ile Met Glu
    370                 375                 380

Lys Leu Glu Met Ser Lys Phe Gln Pro Thr Leu Leu Thr Leu Pro Arg
385                 390                 395                 400

Ile Lys Val Thr Thr Ser Gln Asp Met Leu Ser Ile Met Glu Lys Leu
                405                 410                 415

Glu Phe Phe Asp Phe Ser Tyr Asp Leu Asn Leu Cys Gly Leu Thr Glu
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            420                 425                 430

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Leu Thr Glu Thr Gly Val Glu Ala Ala Ala Ala Ser Ala Ile Ser Val
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Ala Arg Thr Leu Leu Val Phe Glu Val Gln Gln Pro Phe Leu Phe Val
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Leu Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
        35                  40                  45

Ala Thr Ile Ser Gly Gly Gly Gly Asn Thr Tyr His Pro Asp Ser Met
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
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65 70 75 80

Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg His Gly Asp Phe Gly Asn Tyr Phe Asp Tyr Trp Gly Gln Gly
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35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

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85 90 95

Ser Tyr Asn Leu Phe Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys

100 105 110

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Gly

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<210> 92
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<212> PRT
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<210> 93
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<210> 95

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tggtaccagc agaaaccagg gcagtctcct aaactgctga tctactgggc atccactagg 180

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35 40 45

Trp Leu Ala His Ile Phe Ser Ser Asp Glu Lys Ser Tyr Arg Thr Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Ile Arg Arg Gly Gly Ile Asp Tyr Trp Gly Gln Gly Thr
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Leu Val Thr Val Ser Ser
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<210> 98
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 98

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Thr Ala Ser Ile Thr Cys Ser Gly Glu Lys Leu Gly Asp Lys Tyr Ala

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Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Met Tyr
        35                  40                  45

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

Asp Glu Ala Asp Tyr Tyr Cys Gln Ala Trp Asp Ser Ser Thr Ala Val
                85                  90                  95

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

CLAIMS

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

1. A method for treating, inhibiting, alleviating or preventing acute respiratory distress syndrome, pneumonia or some other pulmonary or other acute manifestation of COVID-19, such as thrombosis, in a mammalian subject infected with SARS-CoV-2, comprising

(i) determining the level of MASP-2/C1-INH complex in a biological sample obtained from the subject, wherein an increased level of MASP-2/C1-INH complex as compared to a healthy control sample is indicative of an increased risk of developing one or more acute manifestations of COVID-19; and

(ii) administering to the subject having an increased level of MASP-2/C1-INH complex an amount of a MASP-2 inhibitory agent effective to inhibit MASP-2-dependent complement activation, optionally, wherein the amount of said MASP-2 inhibitory agent is sufficient to reduce the level of MASP-2/C1-INH to a control level or reference standard.

2. The method of claim 1, wherein the MASP-2 inhibitory agent is a MASP-2 antibody or fragment thereof.

3. The method of claim 2, 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:6.

4. The method of claim 2, wherein the MASP-2 antibody or fragment thereof specifically binds to a polypeptide comprising SEQ ID NO:6 with an affinity of at least 10 times greater than it binds to a different antigen in the complement system.

5. The method of claim 2, wherein the antibody or fragment thereof is selected from the group consisting of a recombinant antibody, an antibody having reduced effector function, a chimeric antibody, a humanized antibody and a human antibody.

6. The method of claim 1, wherein the MASP-2 inhibitory agent selectively inhibits lectin pathway complement activation without substantially inhibiting C1q-dependent complement activation.

7. The method of claim 1, wherein the MASP-2 inhibitory agent is a small molecule MASP-2 inhibitory compound.

8. The method of claim 7, wherein the MASP-2 inhibitory compound is a synthetic or semi-synthetic small molecule.

9. The method of claim 1, wherein the MASP-2 inhibitory agent is an expression inhibitor of MASP-2.

10. The method of claim 1, wherein the MASP-2 inhibitory agent is administered subcutaneously, intraperitoneally, intra-muscularly, intra-arterially, intravenously, orally, or as an inhalant.

11. The method of claim 2, wherein the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising CDR-H1, CDR-H2 and CDR-H3 of the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising CDR-L1, CDR-L2 and CDR-L3 of the amino acid sequence set forth as SEQ ID NO:69.

12. The method of claim 2, wherein the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO:67 and a light chain variable region comprising SEQ ID NO:69.

13. A method for treating, ameliorating, preventing or reducing the risk of developing one or more COVID-19-related long-term sequelae in a mammalian subject that has been infected with SARS-CoV-2, comprising

(i) determining the level of MASP-2/C1-INH complex in a biological sample obtained from the subject, wherein an increased level of MASP-2/C1-INH complex as compared to a healthy control sample is indicative of an increased risk of developing one or more COVID-19-related long term sequelae; and

(ii) administering to the subject having an increased level of MASP-2/C1-INH complex an amount of a MASP-2 inhibitory agent effective to inhibit MASP-2-dependent complement activation.

14. The method of claim 13, wherein the MASP-2 inhibitory agent is a MASP-2 antibody or fragment thereof.

15. The method of claim 14, 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:6.

16. The method of claim 14, wherein the MASP-2 antibody or fragment thereof specifically binds to a polypeptide comprising SEQ ID NO:6 with an affinity of at least 10 times greater than it binds to a different antigen in the complement system.

17. The method of claim 14, wherein the antibody or fragment thereof is selected from the group consisting of a recombinant antibody, an antibody having reduced effector function, a chimeric antibody, a humanized antibody and a human antibody.

18. The method of claim 14, wherein the MASP-2 inhibitory agent selectively inhibits lectin pathway complement activation without substantially inhibiting C1q-dependent complement activation.

19. The method of claim 13, wherein the MASP-2 inhibitory agent is a small molecule MASP-2 inhibitory compound.

20. The method of claim 13, wherein the MASP-2 inhibitory compound is a synthetic or semi-synthetic small molecule.

21. The method of claim 13, wherein the MASP-2 inhibitory agent is an expression inhibitor of MASP-2.

22. The method of claim 14, wherein the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising CDR-H1, CDR-H2 and CDR-H3 of the amino acid sequence set forth as SEQ ID NO:67 and a light chain variable region comprising CDR-L1, CDR-L2 and CDR-L3 of the amino acid sequence set forth as SEQ ID NO:69.

23. The method of claim 14, wherein the MASP-2 inhibitory antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising SEQ ID NO:67 and a light chain variable region comprising SEQ ID NO:69.

24. The method of claim 13, wherein the one or more COVID-19 related long term sequelae is selected from the group consisting of a cardiovascular complication (including myocardial injury, cardiomyopathy, myocarditis, intravascular coagulation, stroke, venous and arterial complications and pulmonary thrombosis); a neurological complication (including cognitive difficulties, confusion, memory loss, also referred to as "brain fog," headache, stroke, dizziness, syncope, seizure, anorexia, insomnia, anosmia, ageusia, myoclonus, neuropathic pain, myalgias, development of neurological disease such as Alzheimer's disease, Guillian Barre Syndrome, Miller-Fisher Syndrome, Parkinson's disease) kidney injury (such as acute kidney injury (AKI); a pulmonary complication (including lung fibrosis, dyspnea, pulmonary embolism),an inflammatory condition such as Kawasaki disease, Kawasaki-like disease, multisystem inflammatory syndrome in children, multi-system organ failure, extreme fatigue, muscle weakness, low grade fever, inability to concentrate, memory lapses, changes in mood, sleep difficulties, needle pains in arms and legs, diarrhea and vomiting, loss of taste and smell, sore throat and difficulties in swallowing, new onset of diabetes and hypertension, skin rash, shortness of breath, chest pains and palpitations.

25. A monoclonal antibody, or antigen binding fragment thereof, that specifically binds to human MASP-2 in complex with C1-INH, wherein the antibody comprises a binding domain comprising (a) HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:88, or (b) HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:98,

wherein the CDRs are numbered according to the Kabat numbering system.

26. The monoclonal antibody of claim 25, wherein said antibody comprises a heavy chain variable region having at least 95% identify with the amino acid sequence set forth

as SEQ ID NO:87 and a light chain variable region having at least 95% identify with the amino acid sequence set forth as SEQ ID NO:88; or wherein said antibody comprises a heavy chain variable region having at least 95% identify with the amino acid sequence set forth as SEQ ID NO:97 and a light chain variable region having at least 95% identify with the amino acid sequence set forth as SEQ ID NO:98.

27. The monoclonal antibody of claim 25, wherein said antibody is a humanized, chimeric or fully human antibody.

28. A method of measuring the amount of MASP-2/C1-INH in a biological sample comprising:

(a) providing a test biological sample from a human subject;

(b) performing an immunoassay comprising capturing and detecting MASP-2/C1-INH complex in the test sample, wherein MASP-2/C1-INH is captured with a monoclonal antibody that specifically binds to human MASP-2; and the MASP-2/C1-INH complex is detected directly or indirectly with an antibody that specifically binds to C1-INH; and

(c) comparing the level of MASP-2/C1-INH complex detected in accordance with (b) with a predetermined level or control sample wherein the level of MASP-2/C1-INH complex detected in the test sample is indicative of the extent of Lectin Pathway Complement activation.

29. The method of claim 28, wherein the biological sample is a fluid sample selected from the group consisting of whole blood, serum, plasma, urine and cerebrospinal fluid.

30. The method of claim 28, wherein the antibody that specifically binds to MASP-2 comprises a binding domain comprising (a) HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:88, or (b) HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable

region set forth as SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

31. The method of claim 28, wherein the human subject is currently infected with SARS-CoV-2, or has previously been infected with SARS-CoV-2, or wherein the subject is suffering or at risk for developing another lectin-pathway disease or condition (e.g., HSCT-TMA, IgAN, GvHD or other lectin pathway disease or disorder). .

32. A method of determining the risk of a subject that is or has been infected with SARS-CoV-2 for developing COVID-19-related ARDS or other poor outcome, or long-term sequelae associated with COVID-19 comprising:

(a) obtaining a biological sample from the subject;

(b) measuring the level of MASP-2/C1-INH complex in the sample;

(c) comparing the measured level with a predetermined level of MASP-2/C1-INH complex or a reference standard to assess the risk of developing COVID-19-related ARDS and/or long-term sequelae associated with COVID-19; and

(d) determining the risk of the subject for developing COVID-19-related ARDS or other poor outcome and/or long-term sequelae associated with COVID-19 and reporting the results to the patient, physician or database;

(e) optionally, administering a treatment to the subject determined to be likely to develop acute disease and/or long-term sequelae associated with COVID-19 infection.

33. The method of claim 32, wherein the level of MASP-2/C1-INH complex is measured in an immunoassay.

34. The method of claim 32, wherein the method comprises performing an immunoassay to measure the level of MASP-2/C1-INH complex in the biological sample.

35. The method of claim 34, wherein the immunoassay is an ELISA assay.

36. The method of claim 35, wherein the immunoassay comprises the use of a capture antibody that specifically binds to MASP-2 comprises a binding domain comprising HC-

CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:87 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:88, wherein the CDRs are numbered according to the Kabat numbering system.

37. The method of claim 34, wherein the immunoassay is a bead-based immunofluorescence assay.

38. The method of claim 37, wherein the immunoassay comprises the use of a capture antibody that specifically binds to MASP-2 comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

39. The method of claim 37 or 38, wherein the biological sample is serum or plasma.

40. The method of claim 39, wherein the biological sample is from 1% to 5% serum or plasma.

41. A method for monitoring the efficacy of treatment with a MASP-2 inhibitory antibody, or antigen-binding fragment thereof, in a mammalian subject in need thereof, the method comprising:

(a) administering a dose of a MASP-2 inhibitory antibody, or antigen-binding fragment thereof, to a mammalian subject at a first point in time;

(b) assessing a first level of MASP-2/C1-INH complex in a biological sample obtained from the subject after step (a);

(c) treating the subject with the MASP-2 inhibitory antibody, or antigen-binding fragment thereof, at a second point in time;

(d) assessing a second level of MASP-2/C1-INH complex in a biological sample obtained from the subject after step (c); and

(e) comparing the level of MASP-2/C1-INH complex assessed in step (b) with the level of MASP-2/C1-INH complex assessed in step (d) to determine the efficacy of the MASP-2 inhibitory antibody or antigen-binding fragment thereof in the mammalian subject.

42. The method of claim 41, wherein the method further comprises adjusting the dose of the MASP-2 inhibitory antibody or antigen-binding fragment thereof.

43. The method of claim 42, wherein the dose of MASP-2 inhibitory antibody or antigen-binding fragment thereof administered to the subject is increased if the level of MASP-2/C1-INH complex is higher than the control or reference standard.

44. The method of claim 43, wherein if the subject is administered an increased dose of the MASP-2 inhibitory antibody or antigen-binding fragment thereof, steps (b) to (e) are repeated to determine whether the increased dose is sufficient to adjust the level of MASP-2/C1-INH complex to the desired level as compared to the respective control or reference standard.

45. The method of claim 41, wherein steps (b) and (d) comprise assessing the concentration of MASP-2/CI-INH complex in the biological samples in an immunoassay.

46. The method of claim 45, wherein the immunoassay is a bead-based immunofluorescence assay.

47. The method of claim 46, wherein the immunoassay comprises the use of a capture antibody that specifically binds to MASP-2 comprises a binding domain comprising HC-CDR1, HC-CDR2 and HC-CDR3 in a heavy chain variable region set forth as SEQ ID NO:97 and comprising LC-CDR1, LC-CDR2 and LC-CDR3 in a light chain variable region set forth as SEQ ID NO:98, wherein the CDRs are numbered according to the Kabat numbering system.

48. The method of claim 46 or 47, wherein the biological sample is serum or plasma.

49. The method of claim 48, wherein the biological sample is from 1% to 5% serum or plasma.

50. The method of any one of claims 41-50, wherein the mammalian subject is a human subject.

51. The method of claim 50, wherein the human subject is suffering from, or at risk of developing a lectin pathway disease or disorder selected from the group consisting of HSCT-TMA, IgAN, Lupus Nephritis and Graft-versus-Host Disease or some other lectin pathway disease or disorder.

52. The method of claim 41, wherein the human subject is suffering from, or at risk of developing COVID-19 or long-term sequelae associated with COVID-19.

53. The method of claim 41, wherein the second point in time is from 2 to 14 days after the first point in time.

54. The method of claim 41, wherein the second point in time is within 2 to 7 days from the first point in time.

55. The method of claim 41, wherein the second point in time is within 2 to 4 days from the first point in time.