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.

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 disclosure refers to use of a MASP-2 inhibitory monoclonal antibody or antigen-binding fragment thereof in an amount effective to reduce or prevent proteinuria in a subject in the manufacture of medicament for preventing or reducing renal damage in a subject suffering from steroid-dependent Immunoglobulin A Nephropathy (IgAN).

Disclosed is a method 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, comprising administering to the subject an amount of a MASP-2 inhibitory agent effective to inhibit fibrosis. In one example, the MASP-2 inhibitory agent is a MASP-2 antibody or

group consisting of a recombinant antibody, an antibody having reduced effector function, a chimeric antibody, a humanized antibody, and a human antibody. In one example, the MASP-2 inhibitory antibody does not substantially inhibit the classical pathway. In one example, the MASP-2 inhibitory antibody inhibits C3b deposition in 90% human serum with an IC_{50} of 30 nM or less. In one example, the method further comprises identifying a human subject having steroid-dependent IgAN prior to the step of administering to the subject a composition comprising an amount of a MASP-2 inhibitory antibody, or antigen-binding fragment thereof, effective to improve renal function. In one example, the MASP-2 inhibitory antibody or antigen-binding fragment thereof is administered in an amount effective to improve renal function. In one example, the MASP-2 inhibitory antibody or antigen-binding fragment thereof is administered in an amount effective and for a time sufficient to achieve at least a 20 percent reduction in 24-hour urine protein excretion as compared to baseline 24-hour urine protein excretion in the subject prior to treatment. In one example, the composition is administered in an amount sufficient to improve renal function and decrease the corticosteroid dosage in said subject. In one example, 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.

Also disclosed is a method of treating a human subject suffering from membranous nephropathy (MN) comprising administering to the subject a composition comprising an amount of a MASP-2 inhibitory antibody, or antigen-binding fragment thereof, effective to inhibit MASP-2-dependent complement activation. In one example, the subject is suffering from steroid-dependent MN. In one example, the MASP-2 inhibitory antibody is a monoclonal antibody, or fragment thereof that specifically binds to human MASP-2. In one example, the MASP-2 inhibitory antibody or antigen-binding fragment thereof is administered in an amount effective to improve renal function. In one example, the MASP-2 inhibitory antibody or antigen-binding fragment thereof is administered in an amount effective and for a time sufficient to achieve at least a 20

percent reduction in 24-hour urine protein excretion as compared to baseline 24-hour urine protein excretion in the subject prior to treatment. In one example, the composition is administered in an amount sufficient to improve renal function and decrease the corticosteroid dosage in said subject. In one example, 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.

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

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: 70 DNA encoding 17D20_dc35VH21N11VL (OMS646) light chain variable region (VL)

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

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

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.

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**K**MG**VSWIRQPPGKALEW **LAHIFSSDEKSYRTSL**KSRLTISKDTSKNQVVLTMTNMDPVDTAT**YYCARIRRG GIDY**WGQGTLVTVSS

d17N9 heavy chain variable region (VH) (SEQ ID NO:68)

QVQLQQSGPGLVKPSQTLSLTCAISGDSVS**ST**S**AA**WNWIRQSPSRGLEW**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

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.

CLAIMS

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

1. Use of a MASP-2 inhibitory monoclonal antibody or antigen-binding fragment thereof in an amount effective to reduce or prevent proteinuria in a subject in the manufacture of medicament for preventing or reducing renal damage in a subject suffering from steroid-dependent Immunoglobulin A Nephropathy (IgAN).

2. The use of claim 1, wherein the MASP-2 inhibitory antibody or antigen-binding fragment thereof is to be administered in an amount and for a time effective to achieve at least a 20 percent reduction in 24-hour urine protein excretion as compared to baseline 24-hour urine protein excretion prior to treatment.

3. The use of claim 1, 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.

4. The use of any of claims 1 to 3, wherein the MASP-2 inhibitory antibody does not substantially inhibit the classical pathway.

5. The use of any of claims 1 to 3, wherein the MASP-2 inhibitory antibody inhibits C3b deposition in 90% human serum with an IC_{50} of 30 nM or less.

6. The use of claim 1, wherein the human subject has been identified to have steroid-dependent IgAN.

7. The use of claim 1, wherein the MASP-2 inhibitory antibody or antigen-binding fragment thereof is to be administered in an amount effective to improve renal function.

8. The use of claim 1, wherein the composition is to be administered in an amount sufficient to improve renal function and decrease the corticosteroid dosage in said subject.

9. The use of any of claims 1 to 8, 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 use of any of claims 1 to 9, wherein the MASP-2 inhibitory antibody is to be administered subcutaneously, intraperitoneally, intra-muscularly, intra-arterially, intravenously, or as an inhalant.

11. The use of any of claims 1-10, wherein 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.