Title: TREATMENT OF HUMAN METAPNEUMO VIRUS

Abstract: The present disclosure provides compositions and methods for treating an infection by human metapneumovirus (hMPV). In particular, the present disclosure provides methods that entail administering agents having an anchoring domain that anchors the compound to the surface of a target cell, and a sialidase domain that can act extracellularly to inhibit infection of a target cell by hMPV.
TREATMENT OF HUMAN METAPNEUMOVIRUS

PRIORITY CLAIM

This application claims priority to U.S. Provisional Patent Application No. 62/172,725, entitled "TREATMENT OF HUMAN METAPNEUMO VIRUS," filed June 8, 2015, the entire contents of which are hereby incorporated by reference.

Incorporation by reference of Sequence Listing provided electronically

An electronic version of the Sequence Listing is filed herewith, the contents of which are incorporated by reference in their entirety. The electronic file, created June 6, 2016, is 31 kilobytes in size and titled 21865-0027WOI.txt.

BACKGROUND

Human metapneumovirus (hMPV) was first described in children in the Netherlands suffering from respiratory tract illness (Clinical Microbiology Reviews (2006) 19:546; Nature Medicine (2001) 7:719-724). Subsequent genetic characterization revealed that hMPV belongs to the Metapneumovirus genus, which is a branch of the family Paramyxoviridae and the complete genomic sequence is known (Virology (2002) 295:119-132). It is thought that hMPV is responsible for a significant fraction of the lower respiratory tract infections in young children and infants, and studies suggest that, after respiratory syncytial virus, hMPV is the second leading cause of bronchiolitis in young children (Journal of Infectious Diseases (2003) 188:1571-1577). In addition, hMPV can cause serious infections in immunocompromised patients (Journal of Infectious Diseases (2005) 192:1061-1065).

SUMMARY

The present disclosure provides compositions and methods for treating (including prophylactically treating) hMPV infection and disorders associated with hMPV infection (e.g., bronchitis caused by hMPV infection). Specifically, it provides compounds which can act extracellularly to reduce (e.g., reduce the risk of) or prevent infection of a cell by hMPV. Some preferred embodiments of the disclosure include therapeutic compounds having an anchoring
domain that facilitates association of the compound with the surface of a target cell and a sialidase domain that can act extracellularly to reduce or prevent infection of the target cell by hMPV. In some embodiments the compound comprises, consists of, or consists essentially of all or a catalytically active portion of a sialidase.

Thus, described herein are methods of treating an infection by hMPV or an hMPV-associated disorder in a patient, the method comprising administering to the patient a therapeutically effective amount of an agent having sialidase activity. In various embodiments: the patient is immunocompromised; the patient is undergoing immunosuppressive therapy; the patient is under age 10; the patient is an infant; the patient is suffering from bronchitis, pneumonia, asthma or chronic obstructive pulmonary disease (COPD); and the agent having sialidase activity is a polypeptide comprising a portion of a sialidase having sialidase activity. In some cases, the polypeptide comprises or consists of a fusion protein wherein the fusion protein comprises at least a first portion comprising a portion of a sialidase having sialidase activity and a second portion that binds to a glycosaminoglycan (GAG). In some cases, the polypeptide comprises or consists of a fusion protein comprising at least a first portion comprising a portion of a sialidase having sialidase activity and a second portion that has a net positive charge at physiological pH. In some cases, the portion that binds to a GAG is selected from the group comprising: human platelet factor 4 (SEQ ID NO: 2), human interleukin 8 (SEQ ID NO: 3), human antithrombin III (SEQ ID NO: 4), human apoprotein E (SEQ ID NO: 5), human angio-associated migratory protein (SEQ ID NO: 6), and human amphiregulin (SEQ ID NO: 7). In some cases, the agent having sialidase activity is abacterial sialidase (e.g., the bacterial sialidase is selected from a group comprising: Vibrio cholera, Arthrobacter ureafaciens, Clostridium perfringens, Actinomyces viscosus, and Micromonaspora viridifaciens). In some cases, the agent having sialidase activity is a human sialidase.

In one aspect, the disclosure provides a method for treating or prophylactically treating infection by hMPV. In preferred embodiments, the method comprises administering an agent having sialidase activity, such as a sialidase or a fragment thereof containing a sialidase catalytic domain, including a sialidase catalytic domain fusion protein, to a subject to treat an infection. For example, the infection can be by a pathogen. A pathogen can be, for example, a viral pathogen. The method includes administering a pharmaceutically effective amount of an agent of
the present disclosure to at least one target cell of a subject. Preferably, the pharmaceutical composition can be administered by the use of a topical formulation.

In some cases the agent includes a glycosaminoglycan (GAG) binding domain. The GAG binding domain can be all or a fragment of: human platelet factor 4, human interleukin 8, human antithrombin III, human apoprotein E, human angio-associated migratory protein, or human amphiregulin.

The source of the sialidase activity can be bacterial or human. In preferred embodiments, the bacterial source of the sialidase is selected from *Vibrio cholera, Arthrobacter ureafaciens, Clostridium perfringens, Actinomyces viscosus,* and *Micromonospora viridifaciens.*

In some embodiments, administration of the agent having sialidase activity leads to an improvement in one or more symptoms of the infection (*e.g.*, fever, cough, hypoxia, presence of infiltrate in the lungs) and reduces viral load.

In some cases the agent is administered to the lung, *e.g.*, by inhalation.

In some cases, the agent having sialidase activity is DAS181 (SEQ ID NO: 13; SEQ ID NO: 14 is DAS181 without an initial methionine, either can be used in the methods described herein). In some cases the method comprises administering a composition comprising microparticles comprising DAS181 (SEQ ID NOS: 13 and 14).

**BRIEF DESCRIPTION OF THE FIGURES**

**Figures 1A and 1B** show the effect of DAS181 treatment of HEp-2 cells on hMPV infectivity. **Figure 1A** depicts the optical densities of five different hMPV isolates comprising strains A1, A2, and B2 in HEp-2 cells pre-treated with either DAS181, DAS 185, or no treatment (control). **Figure 1B** illustrates the dose-dependent effect of inhibition of hMPV infectivity in HEp-2 cells by DAS181. Results are expressed as percent inhibition of infection relative to that of untreated cells.

**Figure 2** shows the effect of DAS181 pre-treatment of HEp-2 cells on hMPV G protein binding. Results are expressed as percentage binding relative to G protein binding of untreated cells.
In general, the present disclosure relates to methods for treating hMPV infection using agents having sialidase activity. Suitable agents are described in U.S. Patent Nos. 8,084,036 and 7,807,174, which are both hereby incorporated by reference in their entirety. The agents having sialidase activity can remove sialic acid residues from the surface of cells and reduce infection by certain viruses, e.g., hMPV.

In some embodiments, the severity of the infection is reduced with the treatment of the compounds. The reduction of the severity of the infection can be measured by the reduction of one or more symptoms which present with the infection.

The compounds of the present disclosure have sialidase activity. In some instances, the compounds having sialidase activity are a fusion protein in which the portion having sialidase activity is fused to a protein or protein fragment not having sialidase activity. In some instances the portion having sialidase activity is fused to an anchoring domain. In some instances the anchoring domain is GAG.

DAS181 (SEQ ID NOS: 13 and 14) is a fusion protein compound comprising the catalytic domain of a sialidase (A. viscosus) and an anchoring domain that is a human amphiregulin GAG-binding domain. In some instances of the present disclosure, DAS 181 could be used to treat (and/or reduce the risk of) infection by hMPV and disorders associated therewith.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Generally, the nomenclature used herein and the manufacture or laboratory procedures described below are well known and commonly employed in the art. Conventional methods are used for these procedures, such as those provided in the art and various general references. Where a term is provided in the singular, the inventors also contemplate the plural of that term. Where there are discrepancies in terms and definitions used in references that are incorporated by reference, the terms used in this application shall have the definitions given herein. As employed throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

A "target cell" is any cell that can be infected by hMPV, such as a lung cell.
A "domain that can anchor said at least one sialidase domain to the membrane of a target cell," also called an "extracellular anchoring domain" or simply, "anchoring domain" refers to a moiety that can interact with a entity that is at or on the exterior surface of a target cell or is in close proximity to the exterior surface of a target cell. An extracellular anchoring domain can be reversibly or irreversibly linked to one or more moieties, such as, preferably, one or more sialidase domains, and thereby cause the one or more attached therapeutic moieties to be retained at or in close proximity to the exterior surface of a eukaryotic cell. Preferably, an extracellular anchoring domain interacts with at least one molecule on the surface of a target cell or at least one molecule found in close association with the surface of a target cell. For example, an extracellular anchoring domain can bind a molecule covalently or noncovalently associated with the cell membrane of a target cell, or can bind a molecule present in the extracellular matrix surrounding a target cell. An extracellular anchoring domain preferably is a peptide, polypeptide, or protein, and can also comprise any additional type of chemical entity, including one or more additional proteins, polypeptides, or peptides, a nucleic acid, peptide nucleic acid, nucleic acid analogue, nucleotide, nucleotide analogue, small organic molecule, polymer, lipid, steroid, fatty acid, carbohydrate, or a combination of any of these.

As used herein, a protein or peptide sequence is "substantially homologous" to a reference sequence when it is either identical to a reference sequence, or comprises one or more amino acid deletions, one or more additional amino acids, or one or more conservative amino acid substitutions, and retains the same or essentially the same activity as the reference sequence. Conservative substitutions may be defined as exchanges within one of the following five groups:

1. Small, aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, Gly
2. Polar, negatively charged residues and their amides: Asp, Asn, Glu, Gin
3. Polar, positively charged residues: His, Arg, Lys
4. Large, aliphatic nonpolar residues: Met, Leu, Ile, Val, Cys
5. Large aromatic residues: Phe, Try, Trp

Within the foregoing groups, the following substitutions are considered to be "highly conservative": Asp/Glu, His/Arg/Lys, Phe/Tyr/Trp, and Met/Leu/Ile/Val. Semi-conservative substitutions are defined to be exchanges between two of groups (I)-(IV) above which are limited to supergroup (A), comprising (I), (II), and (III) above, or to supergroup (B), comprising (IV)
and (V) above. In addition, where hydrophobic amino acids are specified in the application, they refer to the amino acids Ala, Gly, Pro, Met, Leu, Ile, Val, Cys, Phe, and Tip, whereas hydrophilic amino acids refer to Ser, Thr, Asp, Asn, Glu, Gin, His, Arg, Lys, and Tyr.

As used herein, the phrase "therapeutically effective amount" refers to the amounts of active compounds or their combination that elicit the biological or medicinal response that is being sought in a tissue, system, animal, individual, or human by a researcher, veterinarian, medical doctor or other clinician, which includes one or more of the following:

(1) inhibiting the disease and its progression; for example, inhibiting a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., arresting further development of the pathology and/or symptomatology) such as in the case of hMPV infection; and

(2) ameliorating the disease; for example, ameliorating a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., reversing the pathology and/or symptomatology) such as in the case of hMPV infection.

As used herein, the phrase "treating (including treatment)" includes one or more of the following:

(1) inhibiting the disease and its progression; for example, inhibiting a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., arresting further development of the pathology and/or symptomatology); and

(2) ameliorating the disease; for example, ameliorating a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder.

A "sialidase" is an enzyme that can remove a sialic acid residue from a substrate molecule. The sialidases (N-acylneuraminosylglycohydrolases, EC 3.2.1.18) are a group of enzymes that hydrolytically remove sialic acid residues from sialo-glycoconjugates. Sialic acids are alpha-keto acids with 9-carbon backbones that are usually found at the outermost positions of the oligosaccharide chains that are attached to glycoproteins and glycolipids. One of the major types of sialic acids is N-acetylneuraminic acid (Neu5Ac), which is the biosynthetic precursor
for most of the other types. The substrate molecule can be, as nonlimiting examples, an
oligosaccharide, a polysaccharide, a glycoprotein, a ganglioside, or a synthetic molecule. For
example, a sialidase can cleave bonds having alpha (2,3)-Gal, alpha (2,6)-Gal, or alpha (2,8)-Gal
linkages between a sialic acid residue and the remainder of a substrate molecule. A sialidase can
also cleave any or all of the linkages between the sialic acid residue and the remainder of the
substrate molecule. Two major linkages between Neu5Ac and the penultimate galactose residues
of carbohydrate side chains are found in nature, Neu5Ac alpha (2,3)-Gal and Neu5Ac alpha
(2,6)-Gal. Both Neu5Ac alpha (2,3)-Gal and Neu5Ac alpha (2,6)-Gal molecules can be
recognized by influenza viruses as the receptor, although human viruses seem to prefer Neu5Ac
alpha (2,6)-Gal, and avian and equine viruses predominantly recognize Neu5Ac alpha (2,3)Gal.
A sialidase can be a naturally-occurring sialidase, an engineered sialidase (such as, but not
limited to a sialidase whose amino acid sequence is based on the sequence of a naturally-
occurring sialidase, including a sequence that is substantially homologous to the sequence of a
naturally-occurring sialidase). As used herein, "sialidase" can also mean the active portion of a
naturally-occurring sialidase, or a peptide or protein that comprises sequences based on the
active portion of a naturally-occurring sialidase.

A "fusion protein" is a protein comprising amino acid sequences from at least two
different sources. A fusion protein can comprise an amino acid sequence that is derived from a
naturally occurring protein or is substantially homologous to all or a portion of a naturally
occurring protein, and in addition can comprise from one to a very large number of amino acids
that are derived from or substantially homologous to all or a portion of a different naturally
occurring protein. In the alternative, a fusion protein can comprise an amino acid sequence that is
derived from a naturally occurring protein or is substantially homologous to all or a portion of a
naturally occurring protein, and in addition can comprise from one to a very large number of
amino acids that are synthetic sequences.

A "sialidase catalytic domain protein" is a protein that comprises the catalytic domain of
a sialidase, or an amino acid sequence that is substantially homologous to the catalytic domain of
a sialidase, but does not comprise the entire amino acid sequence of the sialidase the catalytic
domain is derived from, wherein the sialidase catalytic domain protein retains substantially the
same activity as the intact sialidase the catalytic domain is derived from. A sialidase catalytic
domain protein can comprise amino acid sequences that are not derived from a sialidase, but this is not required. A sialidase catalytic domain protein can comprise amino acid sequences that are derived from or substantially homologous to amino acid sequences of one or more other known proteins, or can comprise one or more amino acids that are not derived from or substantially homologous to amino acid sequences of other known proteins.

1. Composition for Preventing or Treating Infection by liMPV

The present disclosure relates to compounds (agents) that include a peptide. The compounds include all or a catalytic portion of a sialidase. In some cases the compound includes at least one domain that can associate the sialidase or portion thereof with a eukaryotic cell. By "peptide or protein-based" compounds, it is meant that a compound includes a portion having an amino acid framework, in which the amino acids are joined by peptide bonds. A peptide or protein-based compound can also have other chemical compounds or groups attached to the amino acid framework or backbone, including moieties that contribute to the anchoring activity of the anchoring domain, or moieties that contribute to the infection-preventing activity of the sialidase domain. For example, the protein-based therapeutics of the present disclosure can comprise compounds and molecules such as but not limited to: carbohydrates, fatty acids, lipids, steroids, nucleotides, nucleotide analogues, nucleic acid molecules, nucleic acid analogues, peptide nucleic acid molecules, small organic molecules, or even polymers. The protein-based therapeutics of the present disclosure can also comprise modified or non-naturally occurring amino acids. Non-amino acid portions of the compounds can serve any purpose, including but not limited to: facilitating the purification of the compound, improving the solubility or distribution of the compound (such as in a therapeutic formulation), linking domains of the compound or linking chemical moieties to the compound, contributing to the two-dimensional or three-dimensional structure of the compound, increasing the overall size of the compound, increasing the stability of the compound, and contributing to the anchoring activity or therapeutic activity of the compound.

The peptide or protein-based compounds of the present disclosure can also include protein or peptide sequences in addition to those that comprise anchoring domains or sialidase domains. The additional protein sequences can serve any purpose, including but not limited to
any of the purposes outlined above (facilitating the purification of the compound, improving the
solubility or distribution of the compound, linking domains of the compound or linking chemical
moieties to the compound, contributing to the two-dimensional or three-dimensional structure of
the compound, increasing the overall size of the compound, increasing the stability of the
compound, or contributing to the anchoring activity or therapeutic activity of the compound).
Preferably any additional protein or amino acid sequences are part of a single polypeptide or
protein chain that includes the sialidase domain or domains, but any feasible arrangement of
protein sequences is within the scope of the present disclosure.

The anchoring domain and sialidase domain can be arranged in any appropriate way that
allows the compound to bind at or near a target cell membrane such that the therapeutic sialidase
can exhibit an extracellular activity that prevents or impedes infection of the target cell by a
pathogen. The compound will preferably have at least one protein or peptide-based anchoring
domain and at least one peptide or protein-based sialidase domain. In this case, the domains can
be arranged linearly along the peptide backbone in any order. The anchoring domain can be N-
terminal to the sialidase domain, or can be C-terminal to the sialidase domain.

It is also possible to have one or more sialidase domains flanked by at least one anchoring
domain on each end. Alternatively, one or more anchoring domains can be flanked by at least
one sialidase domain on each end. Chemical, or preferably, peptide, linkers can optionally be
used to join some or all of the domains of a compound. It is also possible to have the domains in
a nonlinear, branched arrangement. For example, the sialidase domain can be attached to a
derivatized side chain of an amino acid that is part of a polypeptide chain that also includes, or is
linked to, the anchoring domain.

A compound of the present disclosure can have more than one anchoring domain. In
cases in which a compound has more than one anchoring domain, the anchoring domains can be
the same or different. A compound of the present disclosure can have more than one sialidase
domain. In cases in which a compound has more than one sialidase domain, the sialidase
domains can be the same or different. Where a compound comprises multiple anchoring
domains, the anchoring domains can be arranged in tandem (with or without linkers) or on
alternate sides of other domains, such as sialidase domains. Where a compound comprises...
multiple sialidase domains, the sialidase domains can be arranged in tandem (with or without linkers) or on alternate sides of other domains, such as, but not limited to, anchoring domains.

A peptide or protein-based compound of the present disclosure can be made by any appropriate way, including purifying naturally occurring proteins, optionally proteolytically cleaving the proteins to obtain the desired functional domains, and conjugating the functional domains to other functional domains. Peptides can also be chemically synthesized, and optionally chemically conjugated to other peptides or chemical moieties. Preferably, however, a peptide or protein-based compound of the present disclosure is made by engineering a nucleic acid construct to encode at least one anchoring domain and at least one sialidase domain together (with or without nucleic acid linkers) in a continuous polypeptide. The nucleic acid constructs, preferably having appropriate expression sequences, can be transfected into prokaryotic or eukaryotic cells, and the therapeutic protein-based compound can be expressed by the cells and purified. Any desired chemical moieties can optionally be conjugated to the peptide or protein-based compound after purification. In some cases, cell lines can be chosen for expressing the protein-based therapeutic for their ability to perform desirable post-translational modifications (such as, but not limited to glycosylation).

A great variety of constructs can be designed and their protein products tested for desirable activities (such as, for example, binding activity of an anchoring domain or catalytic activity of a sialidase domain). The protein products of nucleic acid constructs can also be tested for their efficacy in preventing or impeding infection of a target cell by a pathogen. *In vitro* and *in vivo* tests for the infectivity of pathogens are known in the art.

**Anchoring Domain**

As used herein, an "extracellular anchoring domain" or "anchoring domain" is any moiety that can interact with an entity that is at or on the exterior surface of a target cell or is in close proximity to the exterior surface of a target cell. An anchoring domain serves to retain a compound of the present disclosure at or near the external surface of a target cell. An extracellular anchoring domain preferably binds 1) a molecule expressed on the surface of a target cell, or a moiety, domain, or epitope of a molecule expressed on the surface of a target
cell, 2) a chemical entity attached to a molecule expressed on the surface of a target cell, or 3) a molecule of the extracellular matrix surrounding a target cell.

An anchoring domain is preferably a peptide or protein domain (including a modified or derivatized peptide or protein domain), or comprises a moiety coupled to a peptide or protein. A moiety coupled to a peptide or protein can be any type of molecule that can contribute to the interaction of the anchoring domain to an entity at or near the target cell surface, and is preferably an organic molecule, such as, for example, nucleic acid, peptide nucleic acid, nucleic acid analogue, nucleotide, nucleotide analogue, small organic molecule, polymer, lipid, steroid, fatty acid, carbohydrate, or any combination of any of these.

Target tissue or target cell type include the sites in an animal or human body where a pathogen invades or amplifies. For example, a target cell can be a lung cell that can be infected by hMPV. A compound or agent of the present disclosure can comprise an anchoring domain that can interact with a cell surface entity, for example, that is specific for the target cell type.

A compound for treating infection by a pathogen can comprise an anchoring domain that can bind at or near the surface of a target cell. For example, heparan sulfate, closely related to heparin, is a type of GAG that is ubiquitously present on cell membranes, including the surface of respiratory epithelium. Many proteins specifically bind to heparin/heparan sulfate, and the GAG-binding sequences in these proteins have been identified (Meyer, F.A., King, M. and Gelman, R.A. (1915) Biochimica et Biophysica Acta 392:223-232; Schauer, S. ed., pp 233, "Sialic Acids Chemistry, Metabolism and Function," Springer-Verlag, 1982). For example, the GAG-binding sequences of human platelet factor 4 (PF4) (SEQ ID NO: 2), human interleukin 8 (IL8) (SEQ ID NO: 3), human antithrombin III (AT III) (SEQ ID NO: 4), human apoprotein E (ApoE) (SEQ ID NO: 5), human angio-associated migratory cell protein (AAMP) (SEQ ID NO: 6), or human amphiregulin (SEQ ID NO: 7) have been shown to have very high affinity (in the nanomolar range) towards heparin (Lee, M.K. and Lander, A.D. (1991) Proc. Natl. Acad. Sci., USA 88:2768-2772; Goger, B., Halden, Y., Rek, A., Mosl, R., Pye, D., Gallagher, J. and Kungl, A. J. (2002) Biochem. 41:1640-1646; Witt, D P, and Lander A.D. (1994) Curr. Bio. 4:394-400; Weisgraber, K.H., Rail, S.C., Mahley, R W , Milne, R.W. and Marcel, Y. (1986) J. Bio. Chem. 261:2068-2076). These sequences, or other sequences that have been identified or are identified in the future as heparin/heparan sulfate binding sequences, or sequences substantially

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homologous to identified heparin/heparan sulfate binding sequences that have heparin/heparan sulfate binding activity, can be used as epithelium anchoring domains in compounds of the present disclosure.

5 **Sialidase Domain**

A sialidase that can cleave more than one type of linkage between a sialic acid residue and the remainder of a substrate molecule, in particular, a sialidase that can cleave both α(2,6)-Gal and α(2,3)-Gal linkages can be used in the compounds of the disclosure. Sialidases include the large bacterial sialidases that can degrade the receptor sialic acids Neu5Ac α(2,6)-Gal and Neu5Ac α(2,3)-Gal. For example, the bacterial sialidase enzymes from *Clostridium perfringens* (Genbank Accession Number X87369), *Actinomyces viscosus*, *Arthrobacter ureafaciens*, or *Micromonospora viridifaciens* (Genbank Accession Number D01045) can be used. Sialidase domains of compounds of the present disclosure can comprise all or a portion of the amino acid sequence of a large bacterial sialidase or can comprise amino acid sequences that are substantially homologous to all or a portion of the amino acid sequence of a large bacterial sialidase. In one preferred embodiment, a sialidase domain comprises a sialidase encoded by *Actinomyces viscosus*, such as that of SEQ ID NO: 12, or a sialidase sequence substantially homologous to SEQ ID NO: 12. In yet another preferred embodiment, a sialidase domain comprises the catalytic domain of the *Actinomyces viscosus* sialidase extending from amino acids 274-666 of SEQ ID NO: 12, or a substantially homologous sequence.

Additional sialidases include the human sialidases such as those encoded by the genes *NEU2* (SEQ ID NO: 8; Genbank Accession Number Y16535; Monti, E., Preti, Rossi, E., Ballabio, A. and Borsani G. (1999) *Genomics* 57:137-143) and *NEU4* (SEQ ID NO: 9; Genbank Accession Number NM080741; Monti, E., Preti, A., Venerando, Band, Borsani, G. (2002) *Neurochem. Res.* 27:646-663). Sialidase domains of compounds of the present disclosure can comprise all or a portion of the amino acid sequences of a sialidase or can comprise amino acid sequences that are substantially homologous to all or a portion of the amino acid sequences of a sialidase. Preferably, where a sialidase domain comprises a portion of the amino acid sequences of a naturally occurring sialidase, or sequences substantially homologous to a portion of the amino acid sequences of a naturally occurring sialidase, the portion comprises essentially the
same activity as the intact sialidase. The present disclosure also includes sialidase catalytic domain proteins. As used herein a "sialidase catalytic domain protein" comprises a catalytic domain of a sialidase but does not comprise the entire amino acid sequence of the sialidase from which the catalytic domain is derived. A sialidase catalytic domain protein has sialidase activity. Preferably, a sialidase catalytic domain protein comprises at least 10%, at least 20%, at least 50%, at least 70% of the activity of the sialidase from which the catalytic domain sequence is derived. More preferably, a sialidase catalytic domain protein comprises at least 90% of the activity of the sialidase from which the catalytic domain sequence is derived.

A sialidase catalytic domain protein can include other amino acid sequences, such as but not limited to additional sialidase sequences, sequences derived from other proteins, or sequences that are not derived from sequences of naturally occurring proteins. Additional amino acid sequences can perform any of a number of functions, including contributing other activities to the catalytic domain protein, enhancing the expression, processing, folding, or stability of the sialidase catalytic domain protein, or even providing a desirable size or spacing of the protein.

A preferred sialidase catalytic domain protein is a protein that comprises the catalytic domain of the A. viscosus sialidase. Preferably, an A. viscosus sialidase catalytic domain protein comprises amino acids 270-666 of the A. viscosus sialidase sequence (SEQ ID NO: 12). Preferably, an A. viscosus sialidase catalytic domain protein comprises an amino acid sequence that begins at any of the amino acids from amino acid 270 to amino acid 290 of the A. viscosus sialidase sequence (SEQ ID NO: 12) and ends at any of the amino acids from amino acid 665 to amino acid 901 of said A. viscosus sialidase sequence (SEQ ID NO: 12), and lacks any A. viscosus sialidase protein sequence extending from amino acid 1 to amino acid 269. (As used herein "lacks any A. viscosus sialidase protein sequence extending from amino acid 1 to amino acid 269" means lacks any stretch of four or more consecutive amino acids as they appear in the designated protein or amino acid sequence.)

In some preferred embodiments, an A. viscosus sialidase catalytic domain protein comprises amino acids 274-681 of the A. viscosus sialidase sequence (SEQ ID NO: 12) and lacks other A. viscosus sialidase sequences. In some preferred embodiments, an A. viscosus sialidase catalytic domain protein comprises amino acids 274-666 of the A. viscosus sialidase sequence (SEQ ID NO: 12) and lacks any other A. viscosus sialidase sequences. In some preferred
embodiments, an *A. viscosus* sialidase catalytic domain protein comprises amino acids 290-666 of the *A. viscosus* sialidase sequence (SEQ ID NO: 12) and lacks any other *A. viscosus* sialidase sequences. In yet other preferred embodiments, an *A. viscosus* sialidase catalytic domain protein comprises amino acids 290-681 of the *A. viscosus* sialidase sequence (SEQ ID NO: 12) and lacks any other *A. viscosus* sialidase sequences.

**Linkers**

A compound of the present disclosure can optionally include one or more linkers that can join domains of the compound. Linkers can be used to provide optimal spacing or folding of the domains of a compound. The domains of a compound joined by linkers can be sialidase domains, anchoring domains, or any other domains or moieties of the compound that provide additional functions such as enhancing compound stability, facilitating purification, etc. A linker used to join domains of compounds of the present disclosure can be a chemical linker or an amino acid or peptide linker. Where a compound comprises more than one linker, the linkers can be the same or different. Where a compound comprises more than one linker, the linkers can be of the same or different lengths.

Many chemical linkers of various compositions, polarity, reactivity, length, flexibility, and cleavability are known in the art of organic chemistry. Preferred linkers of the present disclosure include amino acid or peptide linkers. Peptide linkers are well known in the art. Preferably linkers are between one and one hundred amino acids in length, and more preferably between one and thirty amino acids in length, although length is not a limitation in the linkers of the compounds of the present disclosure. Preferably linkers comprise amino acid sequences that do not interfere with the conformation and activity of peptides or proteins encoded by monomers of the present disclosure. Some preferred linkers of the present disclosure are those that include the amino acid glycine. For example, linkers having the sequence: (GGGGS (SEQ ID NO: 10))$_n$, where $n$ is a whole number between 1 and 20, or more preferably between 1 and 12, can be used to link domains of therapeutic compounds of the present disclosure.

The present disclosure also includes nucleic acid molecules that encode protein-based compounds of the present disclosure that comprise at least one sialidase domain and at least one anchoring domain. The nucleic acid molecules can have codons optimized for expression in
particular cell types, such as, for example *E. coli* or human cells. The nucleic acid molecules of the present disclosure that encode protein-based compounds of the present disclosure that comprise at least one sialidase domain and at least one anchoring domain can also comprise other nucleic acid sequences, including but not limited to sequences that enhance gene expression. The nucleic acid molecules can be in vectors, such as but not limited to expression vectors.

**Administration**

The compound is administered so that it comes into contact with the target cells, but is preferably not administered systemically to the patient. Thus, in the case of infection of the lung, a composition comprising a sialidase (*e.g.*, a composition comprising DAS 181) can be administered by inhalation.

II. Pharmaceutical Compositions

The present disclosure includes compounds of the present disclosure formulated as pharmaceutical compositions. The pharmaceutical compositions comprise a pharmaceutically acceptable carrier prepared for storage and preferably subsequent administration, which have a pharmaceutically effective amount of the compound in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, 18th Ed., Mack Publishing Co., Easton, Pa. (1990). Preservatives, stabilizers, dyes and even flavoring agents can be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid can be added as preservatives. In addition, antioxidants and suspending agents can be used.

The pharmaceutically effective amount of a test compound required as a dose will depend on the route of administration, the type of animal or patient being treated, and the physical characteristics of the specific animal under consideration. The dose can be tailored to achieve a desired effect, but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize. In practicing the methods of the present disclosure, the pharmaceutical compositions can be used alone or in combination with
one another, or in combination with other therapeutic or diagnostic agents. These products can be utilized in vivo, preferably in a mammalian patient, preferably in a human, or in vitro. In employing them in vivo, the pharmaceutical compositions can be administered to the patient in a variety of ways, preferably topically to the target cells, topically to the locus of infection or topically to tissue comprising the target cells.

Accordingly, in some embodiments, the methods comprise administration of the agent and a pharmaceutically acceptable carrier. In some embodiments, the ophthalmic composition is a liquid composition, semi-solid composition, insert, film, microparticles or nanoparticles.

III. Method of Treating an Infection by hMPV

The method of the present disclosure includes: treating a subject that is infected with hMPV or at risk of being infected with hMPV with a pharmaceutical composition of the present disclosure that comprises a protein-based compound that comprises a sialidase activity. In some preferred embodiments the method includes applying a therapeutically effective amount of a pharmaceutical composition of the present disclosure to target cells of a subject. The sialidase activity can be an isolated naturally occurring sialidase protein, or a recombinant protein substantially homologous to at least a portion of a naturally occurring sialidase. A preferred pharmaceutical composition comprises a sialidase with substantial homology to the A. viscosus sialidase (SEQ ID NO: 12). The subject to be treated or prophylactically treated can be, for example, an infant, a child, or an immunocompromised patient. In yet another aspect, the method includes: treating a subject that is infected with hMPV with a pharmaceutical composition of the present disclosure that comprises a protein-based compound that comprises a sialidase catalytic domain. In some preferred embodiments, the method includes applying a therapeutically effective amount of a pharmaceutical composition of the present disclosure to epithelial cells of a subject. The sialidase catalytic domain is preferably substantially homologous to the catalytic domain of a naturally occurring sialidase. A preferred pharmaceutical composition comprises a sialidase catalytic domain with substantial homology to amino acids 274-666 of the A. viscosus sialidase (SEQ ID NO: 12). The subject to be treated can be an animal or human subject. In some cases the compound is DAS 181.
Dosage

As will be readily apparent to one skilled in the art, the useful in vivo dosage to be administered and the particular mode of administration will vary depending upon the age, weight and type of patient being treated, the particular pharmaceutical composition employed, and the specific use for which the pharmaceutical composition is employed. The determination of effective dosage levels, that is the dose levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine methods as discussed above. In non-human animal studies, applications of the pharmaceutical compositions are commenced at higher dose levels, with the dosage being decreased until the desired effect is no longer achieved or adverse side effects are reduced or disappear. The dosage for a compound of the present disclosure can range broadly depending upon the desired affects, the therapeutic indication, route of administration and purity and activity of the compound. Typically, human clinical applications of products are commenced at lower dosage levels, with dosage levels being increased until the desired effect is achieved. Alternatively, acceptable in vitro studies can be used to establish useful doses and routes of administration of the test compound. Typically, dosages can be between about 1 ng/kg and about 10 mg/kg, preferably between about 10 ng/kg and about 1 mg/kg, and more preferably between about 100 ng/kg and about 100 micrograms/kg.

In one preferred regimen, appropriate dosages are administered to each patient by either eyedrop, spray, or by aerosol. It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific salt or other form employed, the metabolic stability and length of action of that compound, the age of the patient, body weight of the patient, general health of the patient, sex of the patient, diet of the patient, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

Assessing Activity

In some embodiments, the effectiveness of the protein-based compound that comprises a sialidase catalytic domain in treating (including prophylactically treating) hMPV infection can be assessed in vitro and/or in vivo. Assays for such assessment are known to those of skill in the art.
and are known to correlate tested activities and results to therapeutic and in vivo activities. In one example, cells pre-treated with the protein-based compound that comprises a sialidase catalytic domain can be assessed in comparison to cells not treated with the protein-based compound that comprises a sialidase catalytic domain to determine the antiviral activity, i.e., the effect on hMPV infectivity, of the protein-based compound that comprises a sialidase catalytic domain. In vitro assays include any laboratory assay known to one of skill in the art, such as for example, cell-based assays including binding assays, protein assays, and molecular biology assays. In vivo assays include assays in animal models as well as administration to humans. In some embodiments, hMPV infection can be quantitated using a cell-based enzyme-linked immunosorbent assay (ELISA). In other embodiments, viral protein binding can be assessed by ELISA. The protein-based compounds that comprise a sialidase catalytic domain, such as those provided herein, also can be tested in vivo to assess an activity or property, such as therapeutic effect.

EXAMPLES

Example 1: Preparation of a Suspension of DAS181 Microparticles

Purification of DAS181

DAS181 is a fusion protein containing the heparin (glycosaminoglycan, or GAG)-binding domain from human amphiregulin fused via its N-terminus to the C-terminus of a catalytic domain of Actinomyces viscosus (e.g., sequence of amino acids set forth in SEQ ID NO: 13 (amino terminal methionine) and SEQ ID NO: 14 (no amino terminal methionine). The DAS181 protein used in the examples below was purified as described in Malakhov et al. (2006) Antimicrob. Agents Chemother. 50(4): 1470-1479, which is incorporated in its entirety by reference herein. Briefly, the DNA fragment coding for DAS181 was cloned into the plasmid vector pTrc99a (Pharmacia) under the control of an IPTG (isopropyl-B-D-thiogalactopyranoside)-inducible promoter. The resulting construct was expressed in the BL21 strain of Escherichia Coli (E.Coli). The E. coli cells expressing the DAS181 protein were washed by diafiltration in a fermentation harvest wash step using Toyopearl buffer 1, UFP-500-E55 hollow fiber cartridge (GE Healthcare) and a Watson-Marlow peristaltic pump. The
recombinant DAS 181 protein was then purified in bulk from the cells as described in US 20050004020 and US 20080075708, which are incorporated in their entirety by reference herein.

Activity of DAS 181

The sialidase activity of DAS181 was measured using the fluorogenic substrate 4-methylumbelliferyl -N-acetyl-a-D-neuraminic acid (4-MU-NANA; Sigma). One unit of sialidase is defined as the amount of enzyme that releases 10 nmol of MU from 4-MU-NANA in 10 minutes at 37°C (50 mM CH₃COOH-NaOH buffer, pH 5.5) in a reaction that contains 20 nmol of 4-MU-NANA in a 0.2 mL volume (Potier et al. (1979) Anal Biochem. 94:287-296). The specific activity of DAS181 was determined to be 1,300 U/mg protein (0.77 µg DAS181 protein per unit of activity).

Microparticle preparation

The following ingredients were then combined to form DAS181 microparticles in a large scale batch process:

(a) 75 mg/mL histidine, 0.107 M citric acid, pH 5.0 and 1 M trehalose stock solutions were sterile filtered into and combined in an Excipient Bottle.
(b) The contents of the Excipient Bottle were added, with mixing, to a Compounding Vessel containing 125 mg/mL DAS181 protein prepared as described above in Example 1.
(c) Isopropanol was sterile filtered into an Isopropanol Bag.
(d) The content of the Isopropanol Bag was pumped into the Compounding Vessel while mixing vigorously to form the Feedstock Solution. The final composition of the Feedstock Solution was as follows: 70 mg/mL DAS 181, 26% isopropanol, 9.8 mg/mL histidine, 9.8 mg/mL trehalose, 2.69 mg/mL citric acid, pH 5.0. The time between initiating the addition of isopropanol and starting the lyophilization cycle was between 90 minutes and 120 minutes.
(e) Stainless Steel trays that had undergone depyrogenation were each filled with 950 g of the Feedstock Solution, using a metering pump.
(f) The filled Stainless Steel trays were subjected to a Lyophilization Cycle as follows:
a. the Feedstock Solution in the lyophilization trays were gasketed and placed in
the lyophilizer shelves at 25°C for 5 minutes;
b. the temperature of the shelves was lowered to -55°C at a ramp rate of
-0.4°C/minute;
c. the trays were held at -55°C for between 60 and 180 minutes;
d. primary drying was accomplished by setting the condenser to < -60°C,
applying a vacuum of 125 mTorr with 250 mTorr dead band and increasing
the temperature to -40°C at a ramp rate of 0.125°C/minute and further to a
temperature of -30°C at 0.167°C/minute;
e. the temperature was held at -30°C for between 5000 and 6500 minutes;
f. secondary drying was accomplished by increasing the temperature to 15°C at
a ramp rate of 0.5°C/minute, holding at 15°C for 30 minutes, then further
ramping up to a temperature of 30°C at a ramp rate of 0.5°C/minute;
g. the temperature was held at 30°C for between 300 and 500 minutes; and
h. the vacuum was released and the lyophilizer was backfilled with nitrogen to
prevent oxidation of the microparticle formulations before transferring into
bottles for bulk mixing and aliquoting the bulk powder for storage at ≤ -15°C.

Physical Parameters:
The DAS181 dry powder microparticles prepared according to the above method have a
mass median aerodynamic diameter (MMAD) of about 10 microns and a GSD of between 1 and
2. Such particles are suitable for use in inhalers for treatment of respiratory infection.

Example 2: Antiviral activity of DAS181 in vitro

Cell and virus preparation
The human epithelial tumor cell line, HEp-2, and rhesus monkey kidney cells (LLC-
MK2) were grown in Medium 199 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal
bovine serum (FBS). Stocks of hMPV were prepared by inoculation of LLC-MK2 cells with
hMPV and incubation for 14-21 days at 37°C in 5% CO2. hMPV stocks used for infectivity
assays were as follows: V47041 (Bl strain), V32748 (BA strain), V50569 (A2 strain), V52283
(B2 strain), and V51200 (A2 strain). The B2 (V52283), A2 (V50569), and B1 (V47041) strains of hMPV were isolated from clinical samples by the Virology Laboratory, Flinders Medical Centre (FMC). These samples were positive only for hMPV and were not co-infected with influenza A, influenza B, RSV, adenovirus, or parainfluenza 1, 2 or 3. All virus stocks were stored at -70°C until use. hMPV infectivity titer was determined using an immunofluorescence assay. Briefly, cells were incubated with dilutions of the virus and counted after staining with a monoclonal Ab (mAb) to hMPV matrix protein (Chemicon, Temecula, CA) and FITC-labelled secondary antibody. The virus titer was calculated assuming each fluorescent focus represented 1 infectious unit of virus and was reported as fluorescent focus forming units (FFU) per mL.

hMPV Infectivity ELISA

The effect of DAS 181 on hMPV infectivity was examined using a cell infectivity ELISA. DAS 181 was prepared as described above in Example 1. As a control for sialidase specific activity, a mutated sialidase expressing molecule, DAS 185, was used at identical concentrations. DAS 185 is a mutated sialidase expressing construct that has the identical amphiregulin tag, but exhibits 400-fold reduced sialidase activity compared to DAS181. Both DAS 181 and DAS 185 were dry powders solubilized in sterile PBS to a stock concentration of 50 mg/mL before use.

HHeP-2 cell monolayers in 96-well plates (Linbro, ICN Biomedicals, Aurora, OH) were treated with 4 g/mL of DAS181 or DAS185 for 2 h at 37°C before inoculation with hMPV. The wells were then inoculated with one of the hMPV isolates described above (V32748, V47041, V50569, V51200, or V52283) at a multiplicity of infection of 1 FFU per cell. Cells were washed with Medium 199 to remove unbound virus. Medium 199 containing 1 µg/mL trypsin was then added and cells were cultured for 48 h. Control wells were "mock" inoculated with EDB-BSA buffer containing no virus (10 mM sodium acetate, pH 6.0, 0.1 M NaCl, 10 mM CaCb, 0.5 mM MgCh, 0.5% w/v BSA).

Viral infection was assessed 48 h post-inoculation. The medium was removed and cells were fixed with 1% paraformaldehyde in PBS for 30 min at room temperature. Cells were washed twice with PBS, and permeabilized with 0.02% Triton X-100/PBS for 30 min at 4°C, followed by two washes with PBS. Non-specific sites were blocked with 5% skim milk/PBS for 1 h. The wells were then incubated with hMPV matrix protein mAb diluted 1:320 (v/v) in 0.5%
 Tween 20-PBS followed by 1:10,000 (v/v) horseradish peroxidase (HRP)-conjugated sheep anti-mouse IgG (Chemicon). Each incubation was for 60 min at 37°C and the wells were washed four times with PBS after each step. O-phenylenediamine substrate (OPD; Sigma) was added, and after 30 min IN H2SO4 was added and the absorbance at 490 nm was determined. Wells were inoculated in triplicate and each experiment was performed at least two times. The optical density values (490 nm) of each hMPV isolate in HEp-2 cells pre-treated with either DAS181, DAS 185 or no virus (control) are shown in Table 1.

Table 1. Optical density values of hMPV isolates in HEp-2 cells pre-treated with 10 µg/mL DAS181 or DAS185

<table>
<thead>
<tr>
<th>hMPV isolate</th>
<th>Control (no virus)</th>
<th>DAS181</th>
<th>DAS185</th>
</tr>
</thead>
<tbody>
<tr>
<td>V32748 (B1)</td>
<td>0.814</td>
<td>0.154</td>
<td>0.667</td>
</tr>
<tr>
<td>V47041 (B1)</td>
<td>1.871</td>
<td>0.271</td>
<td>1.169</td>
</tr>
<tr>
<td>V50569 (A2)</td>
<td>1.882</td>
<td>0.181</td>
<td>1.412</td>
</tr>
<tr>
<td>V51200 (A2)</td>
<td>1.313</td>
<td>0.348</td>
<td>1.342</td>
</tr>
<tr>
<td>V52283 (B2)</td>
<td>1.518</td>
<td>0.318</td>
<td>1.773</td>
</tr>
</tbody>
</table>

hMPV infection was greatly inhibited by pre-treatment with DAS 181. As depicted in Table 1 and shown in Figure 1A, there was a linear relationship between virus input and optical density over a greater than 100 fold range of virus inoculums. The sialidase-defective DAS 185 showed little to no activity under similar conditions. Additionally, DAS181 had no effect on the growth or viability of HEp-2 cells at a concentration of 50µg/mL, indicating that the decreased infection was not due to cell cytotoxicity (data not shown).

Infectivity Inhibition Assays

The effect of concentration of DAS 181 on hMPV infectivity was determined by a modification of the hMPV infectivity ELISA. HEp-2 cells in 96-well tissue culture plates were pre-treated with 10-fold serial dilutions of DAS181 in EBD-BSA buffer (0.00064 ng/mL, 0.0032
ng/mL, 0.016 ng/mL, 0.08 ng/mL, 0.4 ng/mL, 2 ng/mL, 10 ng/mL, and 50 ng/mL) for 2 h at 37°C. The DAS181-containing media was removed, the plate was washed once with Medium 199, inoculated with $1.5 \times 10^5$ IFU/mL hMPV, and then incubated for 48 h at 37°C, 5% CO$_2$. hMPV infection of HEp-2 cells was then investigated using the ELISA assay as above. Table 2 depicts the % inhibition of hMPV infectivity of the HEp-2 cells upon pre-treatment with various concentrations of DAS 181.

**Table 2. Dose-dependent inhibition of hMPV infectivity by DAS181**

<table>
<thead>
<tr>
<th>DAS181 (ng/mL)</th>
<th>% hMPV inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>96.71</td>
</tr>
<tr>
<td>10</td>
<td>95.23</td>
</tr>
<tr>
<td>2</td>
<td>70.63</td>
</tr>
<tr>
<td>0.4</td>
<td>60.18</td>
</tr>
<tr>
<td>0.08</td>
<td>74.23</td>
</tr>
<tr>
<td>0.016</td>
<td>54.03</td>
</tr>
<tr>
<td>0.0032</td>
<td>51.54</td>
</tr>
<tr>
<td>0.00064</td>
<td>18.95</td>
</tr>
</tbody>
</table>

As depicted in Table 2 and shown in Figure IB, DAS 181 treatment decreased hMPV infectivity in a dose-dependent manner, with concentrations as low as 0.5 ng/mL (10 pM) exhibiting more than 50% inhibition of infection (results are expressed relative to virus infectivity of untreated HEp-2 cells).

**Example 3: Effect of DAS181 on hMPV G protein binding to cells**

The effect of DAS 181 on hMPV G protein binding to HEp-2 cells was evaluated by ELISA. Recombinant hMPV G protein was expressed in *Pichia pastoris* X33 cells after
methanol induction for 3 to 4 days and purified from culture supernatants using Hi-Trap nickel affinity chromatography (see, e.g., Thammawat et al., J. Virol. (2008) 82(23): 11767-1 1774).

Triplicate HEp-2 monolayers in 96-well plates were treated with either 5 μg/mL or 500 ng/mL of DAS181 in EDB-BSA buffer for 2 h at 37 °C; the solutions from each well were then removed and the cells washed twice with PBS. Both the sialidase-treated and untreated cells (control) were incubated with 100 μg/mL of biotinylated hMPV G protein at 37°C. After 1 h incubation, unbound protein was removed by washing with 50 mM phosphate buffer, pH 7.4 (PB). Cells were then incubated with 1:1,000 (v/v) HRP-conjugated streptavidin (Sigma) in 1% skim milk in PB at 37°C for 1 h. OPD substrate was added and OD490 nm was determined. The OD of wells without hMPV G protein was subtracted as background. Table 3 lists the % binding of hMPV G protein in HEp-2 cells pre-treated with 450 ng/mL DAS 181 as compared to a control (no virus).

<table>
<thead>
<tr>
<th></th>
<th>Binding (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>DAS181</td>
<td>25</td>
</tr>
</tbody>
</table>

As depicted in Table 3 and shown in Figure 2, pre-treatment of HEp-2 cells with DAS181 markedly inhibited viral G protein binding to cells, with a concentration of 450 ng/mL inhibiting binding by 75% (results are expressed as percentage binding relative to G protein binding of untreated cells).
CLAIMS

1. A method of treating infection by hMPV in a patient, the method comprising administering to the patient an effective amount of an agent having sialidase activity.

2. A method of reducing the risk of infection by hMPV or the severity of infection by hMPV, the method comprising administering to the patient an effective amount of an agent having sialidase activity.

3. The method of claim 1 or claim 2, wherein the agent having sialidase activity is administered to the patient prior to infection by hMPV.

4. The method of claim 1 or claim 2, wherein the agent having sialidase activity is administered to the patient before the patient exhibits a symptom of infection by hMPV.

5. The method of any of claims 1-4, wherein the agent having sialidase activity is a polypeptide comprising all or a portion of a sialidase having sialidase activity.

6. The method of claim 5, wherein the polypeptide comprises or consists of a fusion protein, wherein the fusion protein comprises at least a first portion comprising all or a portion of a sialidase having sialidase activity and the second portion binds to a glycosaminoglycan (GAG).

7. The method of claim 5, wherein the polypeptide comprises or consists of a fusion protein, wherein the fusion protein comprises at least a first portion comprising all or a portion of a sialidase having sialidase activity and the second portion has a net positive charge at physiological pH.

8. The method of claim 6, wherein the portion that binds to a GAG is selected from a group comprising: human platelet factor 4 (SEQ ID NO: 2), human interleukin 8 (SEQ ID NO: 3),
human antithrombin III (SEQ ID NO: 4), human apoprotein E (SEQ ID NO: 5), human angio-
associated migratory protein (SEQ ID NO: 6), and human amphiregulin (SEQ ID NO: 7).

9. The method of claim 5, wherein the sialidase is a bacterial sialidase.

10. The method of claim 9, wherein the bacterial sialidase is selected from a group comprising: *Vibrio cholera, Arthrobacter ureafaciens, Clostridium perfringens, Actinomyces viscosus*, and *Micromonospora viridifaciens*.

11. The method of claim 5, wherein the sialidase is a human sialidase.

12. The method of any of claims 1-11, wherein the agent is administered to the lung.

13. The method of any of claims 1-11, wherein the agent is administered by inhalation.

14. The method of any of claims 1-13, wherein the agent having sialidase activity is DAS181.

15. The method of any of claims 1-14, comprising administering a composition comprising microparticles comprising DAS181.

16. The method of any of claims 1-4, comprising administering a composition comprising a polypeptide comprising the amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 14.

17. The method of claim 2, wherein the patient is not infected by hMPV when the agent is first administered.

18. The method of claim 2 or claim 17, wherein the patient is immunocompromised.
FIGURE 1A
FIGURE 1B
FIGURE 2
INTERNATIONAL SEARCH REPORT

INTERNATIONAL SEARCH REPORT

PCT/US 16/36419

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/54; A61K 38/46; A61K 9/50; A61K 38/00 (2016.01)

CPC - C12N 9/96; A61K 38/46; A61K 9/14; C07K 14/195; A61K 38/00

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 38/54; A61K 38/46; A61K 9/50; A61K 38/00 (2016.01); USPC - 424/94.3; 424/94.6; 424/499; 514/3.7; 514/1 .1

CPC - C12N 9/96; A61K 38/46; A61K 9/14; C07K 14/195; A61K 38/00

Documented searched other than minimum documentation to the extent that such documents are included in the fields searched

IPC(8) - A61K 38/54; A61K 38/46; A61K 9/50; A61K 38/00 (2016.01); USPC - 424/94.3; 424/94.6; 424/499; 514/3.7; 514/1 .1

CPC - C12N 9/96; A61K 38/46; A61K 9/14; C07K 14/195; A61K 38/00 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Medline; Google: DAS181, human metapneumovirus, hMPV, treating, reducing, preventing, risk, severe, prophylaxis, infection, respiratory tract, virus, viral, patient, immunocompromised, subject, siadilase, DAS181, catalyzing, glycosyl, administer, effective, therapeutic, amount, prior, before, symptom, recombi

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*: Citation of document, with indication, where appropriate, of the relevant passages


Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"A" document member of the same family patent

Date of the actual completion of the international search 28 July 2016

Date of mailing of the international search report 01 SEP 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helper: 571-272-4300

PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)
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<td>1.</td>
<td>With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:</td>
</tr>
<tr>
<td>a.</td>
<td>forming part of the international application as filed:</td>
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<td></td>
<td>- [x] in the form of an Annex C/ST.25 text file.</td>
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<td></td>
<td>- [ ] on paper or in the form of an image file.</td>
</tr>
<tr>
<td>b.</td>
<td>furnished together with the international application under PCT Rule 13ter. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.</td>
</tr>
<tr>
<td>c.</td>
<td>furnished subsequent to the international filing date for the purposes of international search only:</td>
</tr>
<tr>
<td></td>
<td>- [ ] in the form of an Annex C/ST.25 text file (Rule 13ter. 1(a)).</td>
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<tr>
<td></td>
<td>- [ ] on paper or in the form of an image file (Rule 13/er. 1(b) and Administrative Instructions, Section 713).</td>
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<td>2.</td>
<td>In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.</td>
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<td>3.</td>
<td>Additional comments:</td>
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Form PCT/ISA/210 (continuation of first sheet (1)) (January 2015)
**INTERNATIONAL SEARCH REPORT**

<table>
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<tr>
<td>1.</td>
<td>Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:</td>
</tr>
<tr>
<td>2.</td>
<td>Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:</td>
</tr>
<tr>
<td>3.</td>
<td>☑ Claims Nos.: 5-16 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).</td>
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<table>
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<th>Observations where unity of invention is lacking (Continuation of item 3 of first sheet)</th>
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<tbody>
<tr>
<td></td>
<td>This International Searching Authority found multiple inventions in this international application, as follows:</td>
</tr>
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</table>

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.