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Coronavirus IRNA compositions and methods of use thereof

Abstract

The present invention relates to RNAi agents, e.g., dsRNA agents, targeting the coronavirus genome. The invention also relates to methods of using such RNAi agents to inhibit expression of a coronavirus genome and to methods of treating or preventing a coronavirus-associated disease in a subject.

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Background/Summary

RELATED APPLICATIONS (1) This application is a continuation of U.S. patent application Ser. No. 17/321,561, filed May 17, 2021, which is a 35 § U.S.C. 111(a) continuation application which claims the benefit of priority to PCT/US2021/024038, filed on Mar. 25, 2021, which, in turn, claims the benefit of priority to U.S. Provisional Application No. 62/994,907, filed on Mar. 26, 2020; U.S. Provisional Application No. 63/001,580, filed on Mar. 30, 2020; U.S. Provisional Application No. 63/019,481, filed on May 4, 2020; and U.S. Provisional Application No. 63/124,910, filed on Dec. 14, 2020. The entire contents of each of the foregoing applications are incorporated herein by reference.

SEQUENCE LISTING

(1) The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Oct. 29, 2021, is named 121301_12206_SL.txt and is 577,274 bytes in size.

BACKGROUND OF THE INVENTION

(2) Coronaviruses (CoV) are a large family of viruses that cause diseases in mammals and birds. Coronaviruses constitute the subfamily Orthocoronavirinae, in the family Coronaviridae. They are enveloped viruses with a positive-sense single-stranded RNA genome and a nucleocapsid of helical symmetry. The genome size of coronaviruses ranges from approximately 27 to 34 kilobases. The name coronavirus is derived from the Latin corona, meaning “crown” or “halo”, which refers to the characteristic appearance reminiscent of a crown or a solar corona around the virions (virus particles) when viewed under two-dimensional transmission electron microscopy, due to the surface covering in club-shaped protein spikes.

(3) Coronaviruses can cause illness ranging from the common cold to more severe diseases. For example, infections with the human coronavirus strains CoV-229E, CoV-OC43, CoV-NL63 and CoV-HKU1 usually result in mild, self-limiting upper respiratory tract infections, such as a common cold, e.g., runny nose, sneezing, headache, cough, sore throat or fever (Zumla A. et al., *Nature Reviews Drug Discovery* 15(5): 327-47, 2016; (Cheng V. C., et al., *Clin. Microbial. Rev.* 20:

660-694, 2007; Chan J. F. et al., *Clin. Microbial. Rev.* 28: 465-522, 2015). Other infections may result in more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV), diseases associated with pneumonia, severe acute respiratory syndrome, kidney failure and death.

(4) MERS-CoV and SARS-CoV have received global attention over the past decades owing to their ability to cause community and health-care-associated outbreaks of severe infections in human populations. MERS-CoV is a viral respiratory disease that was first reported in Saudi Arabia in 2012 and has since spread to more than 27 other countries, according to the World Health Organization (de Groot, R. J. et al., *J. Virol.* 87: 7790-7792, 2013). SARS was first reported in Asia in 2003, and quickly spread to about two dozen countries before being contained after about four months (Lee N. et al., *N. Engl. J. Med.* 348: 1986-1994, 2003; Peiris J. S. et al., *Lancet* 36: 1319-1325, 2003). Detailed investigations found that SARS-CoV was transmitted from civet cats to humans and MERS-CoV from dromedary camels to humans (Cheng V. C., et al., *Clin. Microbial. Rev.* 20: 660-694, 2007; Chan J. F. et al., *Clin. Microbial. Rev.* 28: 465-522, 2015).

(5) A recent outbreak of respiratory disease caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in Wuhan City, China. This disease, named by the World Health Organization as coronavirus disease 2019 ("COVID-19"), presents a major threat to public health worldwide. As of Feb. 24, 2020, there were more than 79,000 confirmed cases and 2,600 deaths across the world.

(6) Coronaviruses viruses pose major challenges to clinical management because many questions regarding transmission and control remain unanswered. Moreover, there is currently no vaccine to prevent infections by coronavirus, and there are no specific antiviral treatments available or proven to be effective to treat or prevent coronavirus infection in subjects.

(7) Accordingly, there exists an immediate need for therapeutics to treat coronavirus infections.

BRIEF SUMMARY OF THE INVENTION

(8) The present disclosure provides RNAi agent compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA genome and RNA transcripts of coronavirus genes. The coronavirus genome may be within a cell, e.g., a cell within a subject, such as a human. The present disclosure also provides methods of using the RNAi agent compositions of the disclosure for inhibiting the expression of a coronavirus genome or for treating a subject who would benefit from inhibiting or reducing the expression of a coronavirus genome, e.g., a subject having a coronavirus-associated disorder, e.g., a subject having a coronavirus infection, e.g., a subject having Severe Acute Respiratory Syndrome 2 (SARS-CoV-2; COVID-19), Severe Acute Respiratory Syndrome (SARS-CoV), or Middle East Respiratory Syndrome (MERS-CoV).

(9) Accordingly, in one aspect, the instant disclosure provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of a coronavirus genome, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of the nucleotide sequence of SEQ ID NO:1, or a nucleotide sequence having at least 90% nucleotide sequence identity to a portion of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO:2, or a nucleotide sequence having at least 90% nucleotide sequence identity to a portion of the nucleotide sequence of SEQ ID NO:2; and wherein the sense strand or the antisense strand is conjugated to one or more lipophilic moieties.

(10) In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of coronavirus genome in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of the nucleotide sequence of SEQ ID NO:2, or a nucleotide

sequence having at least 90% nucleotide sequence identity to a portion of the nucleotide sequence of SEQ ID NO:2, and the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO:1, or a nucleotide sequence having at least 90% nucleotide sequence identity to a portion of the nucleotide sequence of SEQ ID NO:1; and wherein the sense strand or the antisense strand is conjugated to one or more lipophilic moieties.

(11) In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of coronavirus genome in a cell, comprising a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region complementary to part of an mRNA encoding a coronavirus genome (SEQ ID NO:1), wherein each strand independently is 14 to 30 nucleotides in length; and wherein the sense strand or the antisense strand is conjugated to one or more lipophilic moieties.

(12) In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of coronavirus genome in a cell, comprising a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region complementary to part of a reverse complement of an mRNA encoding a coronavirus genome (SEQ ID NO:2), wherein each strand independently is 14 to 30 nucleotides in length; and wherein the sense strand or the antisense strand is conjugated to one or more lipophilic moieties.

(13) In yet another aspect, the present invention provides a double stranded RNAi agent for inhibiting expression of a coronavirus genome in a cell, comprising a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-5, wherein each strand independently is 14 to 30 nucleotides in length; and wherein the sense strand or the antisense strand is conjugated to one or more lipophilic moieties.

(14) In one embodiment, the sense strand or the antisense strand is a sense strand or an antisense strand selected from the group consisting of any of the sense strands and antisense strands in any one of Table 2-5. In one embodiment, the sense strand or the antisense strand is selected from the sense strand or antisense strand of a duplex selected from the group consisting of AD-1184137, AD-1184147, AD-1184150, AD-1184210, AD-1184270, AD-1184233, AD-1184271, AD-1184212, AD-1184228, AD-1184223, AD-1231490, AD-1231513, AD-1231485, AD-1231507, AD-1231471, AD-1231494, AD-1231496, and AD-1231497. In another embodiment, the sense strand or the antisense strand is selected from the sense strand or antisense strand of a duplex selected from the group consisting of AD-1184137, AD-1184147, AD-1184150, AD-1231490, AD-1231513, AD-1231485, AD-1231471, AD-1231496, and AD-1231497. In one embodiment, the sense strand or the antisense strand is selected from the sense strand or antisense strand of a duplex selected from the group consisting of AD-1184137 and AD-1184150. In one embodiment, the sense strand and the antisense strand are the sense strand and antisense strand of AD-1184137. In another embodiment, the sense strand and the antisense strand are the sense strand and antisense strand of AD-1184150.

(15) In one embodiment, both the sense strand and the antisense strand is conjugated to one or more lipophilic moieties.

(16) In one embodiment, the lipophilic moiety is conjugated to one or more positions in the double stranded region of the dsRNA agent.

(17) In one embodiment, the lipophilic moiety is conjugated via a linker or a carrier.

(18) In one embodiment, lipophilicity of the lipophilic moiety, measured by log Kow, exceeds 0.

(19) In one embodiment, the hydrophobicity of the double-stranded RNAi agent, measured by the unbound fraction in a plasma protein binding assay of the double-stranded RNAi agent, exceeds 0.2.

(20) In one embodiment, the plasma protein binding assay is an electrophoretic mobility shift assay

using human serum albumin protein.

(21) In one embodiment, the dsRNA agent comprises at least one modified nucleotide.

(22) In one embodiment, no more than five of the sense strand nucleotides and no more than five of the nucleotides of the antisense strand are unmodified nucleotides

(23) In another embodiment, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

(24) In one embodiment, at least one of the modified nucleotides is selected from the group a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5'-methylphosphonate group, a nucleotide comprising a 5' phosphate or 5' phosphate mimic, a nucleotide comprising vinyl phosphonate, a nucleotide comprising adenosine-glycol nucleic acid (GNA), a nucleotide comprising thymidine-glycol nucleic acid (GNA)S-Isomer, a nucleotide comprising 2-hydroxymethyl-tetrahydrofuran-5-phosphate, a nucleotide comprising 2'-deoxythymidine-3'-phosphate, a nucleotide comprising 2'-deoxyguanosine-3'-phosphate, a 2'-O-hexadecyl nucleotide, a nucleotide comprising a 2'-phosphate, a cytidine-2'-phosphate nucleotide, a guanosine-2'-phosphate nucleotide, a 2'-O-hexadecyl-cytidine-3'-phosphate nucleotide, a 2'-O-hexadecyl-adenosine-3'-phosphate nucleotide, a 2'-O-hexadecyl-guanosine-3'-phosphate nucleotide, a 2'-O-hexadecyl-uridine-3'-phosphate nucleotide, a 5'-vinyl phosphonate (VP), a 2'-deoxyadenosine-3'-phosphate nucleotide, a 2'-deoxycytidine-3'-phosphate nucleotide, a 2'-deoxyguanosine-3'-phosphate nucleotide, a 2'-deoxythymidine-3'-phosphate nucleotide, a 2'-deoxyuridine nucleotide, and a terminal nucleotide linked to a cholesteryl derivative and a dodecanoic acid bisdecylamide group; and combinations thereof.

(25) In another embodiment, modified nucleotide is selected from the group consisting of a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, 3'-terminal deoxy-thymine nucleotides (dT), a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

(26) In another embodiment, the modified nucleotide comprises a short sequence of 3'-terminal deoxy-thymine nucleotides (dT).

(27) In yet another embodiment, the modifications on the nucleotides are 2'-O-methyl modifications, 2'-deoxy-modifications, and 2'fluoro modifications.

(28) In one embodiment, the dsRNA agent further comprises at least one phosphorothioate internucleotide linkage.

(29) In one embodiment, the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages.

(30) In one embodiment, each strand is no more than 30 nucleotides in length.

(31) In one embodiment, at least one strand comprises a 3' overhang of at least 1 nucleotide.

(32) In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides.

(33) The double stranded region may be 15-30 nucleotide pairs in length; 17-23 nucleotide pairs in length; 17-25 nucleotide pairs in length; 23-27 nucleotide pairs in length; 19-21 nucleotide pairs in length; or 21-23 nucleotide pairs in length.

(34) Each strand of the dsRNA agent may be has 19-30 nucleotides in length; 19-23 nucleotides in length; or 21-23 nucleotides in length.

(35) In one embodiment, one or more lipophilic moieties are conjugated to one or more internal

positions on at least one strand.

(36) In one embodiment, the one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand via a linker or carrier.

(37) In one embodiment, the internal positions include all positions except the terminal two positions from each end of the at least one strand.

(38) In another embodiment, the internal positions include all positions except the terminal three positions from each end of the at least one strand.

(39) In another embodiment, the internal positions exclude a cleavage site region of the sense strand.

(40) In yet another embodiment, the internal positions include all positions except positions 9-12, counting from the 5'-end of the sense strand.

(41) In one embodiment, the internal positions include all positions except positions 11-13, counting from the 3'-end of the sense strand.

(42) In one embodiment, the internal positions exclude a cleavage site region of the antisense strand.

(43) In one embodiment, the internal positions include all positions except positions 12-14, counting from the 5'-end of the antisense strand.

(44) In one embodiment, the internal positions include all positions except positions 11-13 on the sense strand, counting from the 3'-end, and positions 12-14 on the antisense strand, counting from the 5'-end.

(45) In one embodiment, the one or more lipophilic moieties are conjugated to one or more of the internal positions selected from the group consisting of positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'-end of each strand.

(46) In one embodiment, the one or more lipophilic moieties are conjugated to one or more of the internal positions selected from the group consisting of positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'-end of each strand.

(47) In one embodiment, the positions in the double stranded region exclude a cleavage site region of the sense strand.

(48) In one embodiment, the sense strand is 21 nucleotides in length, the antisense strand is 23 nucleotides in length, and the lipophilic moiety is conjugated to position 21, position 20, position 15, position 1, position 7, position 6, or position 2 of the sense strand or position 16 of the antisense strand.

(49) In one embodiment, the lipophilic moiety is conjugated to position 21, position 20, position 15, position 1, or position 7 of the sense strand.

(50) In one embodiment, the lipophilic moiety is conjugated to position 21, position 20, or position 15 of the sense strand.

(51) In one embodiment, the lipophilic moiety is conjugated to position 20 or position 15 of the sense strand.

(52) In one embodiment, the lipophilic moiety is conjugated to position 16 of the antisense strand.

(53) In one embodiment, the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound.

(54) In one embodiment, the lipophilic moiety is selected from the group consisting of lipid, cholesterol, retinoic acid, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-bis-O(hexadecyl)glycerol, geranyloxyhexanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine.

(55) In one embodiment, the lipophilic moiety contains a saturated or unsaturated C4-C30 hydrocarbon chain, and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyl.

(56) In one embodiment, the lipophilic moiety contains a saturated or unsaturated C6-C18 hydrocarbon chain.

(57) In one embodiment, the lipophilic moiety contains a saturated or unsaturated C16 hydrocarbon chain.

(58) In one embodiment, the saturated or unsaturated C16 hydrocarbon chain is conjugated to position 6, counting from the 5'-end of the strand.

(59) In one embodiment, the lipophilic moiety is conjugated via a carrier that replaces one or more nucleotide(s) in the internal position(s) or the double stranded region.

(60) In one embodiment, the carrier is a cyclic group selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl; or is an acyclic moiety based on a serinol backbone or a diethanolamine backbone.

(61) In one embodiment, the lipophilic moiety is conjugated to the double-stranded iRNA agent via a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction, or carbamate.

(62) In one embodiment, the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

(63) In one embodiment, the lipophilic moiety or a targeting ligand is conjugated via a bio-cleavable linker selected from the group consisting of DNA, RNA, disulfide, amide, functionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, mannose, and combinations thereof.

(64) In one embodiment, the 3' end of the sense strand is protected via an end cap which is a cyclic group having an amine, said cyclic group being selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl.

(65) In one embodiment, the dsRNA agent further comprises a targeting ligand that targets a liver tissue.

(66) In one embodiment, the targeting ligand is a GalNAc conjugate.

(67) In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occurring at the first internucleotide linkage at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration, a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp configuration or Sp configuration.

(68) In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occurring at the first and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration, a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

(69) In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occurring at the first, second and third internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration, a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

(70) In one embodiment, the dsRNA agent further comprises a terminal, chiral modification

occurring at the first, and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration, a terminal, chiral modification occurring at the third internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

(71) In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occurring at the first, and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration, a terminal, chiral modification occurring at the first, and second internucleotide linkages at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

(72) In one embodiment, the dsRNA agent further comprises a phosphate or phosphate mimic at the 5'-end of the antisense strand.

(73) In one embodiment, the phosphate mimic is a 5'-vinyl phosphonate (VP).

(74) In one embodiment, the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.

(75) In one embodiment, the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

(76) In one embodiment, the sense strand comprises the nucleotide sequence 5'-UAACAAUGUUGC UUUUCAAAC-3' (SEQ ID NO: 5) and the antisense strand comprises the nucleotide sequence 5'-GUUUGAAAAGCAACAUUGUUAGU-3' (SEQ ID NO: 6).

(77) In another embodiment, the sense strand comprises the nucleotide sequence 5'-ACUGUACAGUCUAAAAUGUCA-3' (SEQ ID NO: 7) and the antisense strand comprises the nucleotide sequence 5'-UGACA UUUUAGACUGUACAGUGG-3' (SEQ ID NO: 8).

(78) In one embodiment, the sense strand comprises the sense strand nucleotide sequence 5'-usasaca(Ahd)UfgUfUfGfcuuucaaasasa-3' (SEQ ID NO: 9) and the antisense strand comprises the nucleotide sequence 5'-VPusUfsuugAfaaagcaaCfaUfuguuagsu-3' (SEQ ID NO: 10), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U; s is a phosphorothioate linkage; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; and VP is Vinyl-phosphonate.

(79) In another embodiment, the sense strand comprises the nucleotides sequence 5'-ascsgu(Ahd)CfaGfUfCfuuaaauguscsa-3' (SEQ ID NO: 11) and the antisense strand comprises the nucleotide sequence 5'-VPusGfsacaUfuuuagacUfgUfacagusgs-3' (SEQ ID NO: 12), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U; s is a phosphorothioate linkage; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; and VP is Vinyl-phosphonate.

(80) The present invention further provides cells, pharmaceutical compositions for inhibiting expression of a coronavirus genome, and pharmaceutical composition comprising a lipid formulation. comprising the dsRNA agents of the invention.

(81) In one aspect, the present invention provides a composition comprising two or more, e.g., 2, 3, or 4, double stranded RNAi agents for inhibiting expression of coronavirus genome in a cell, wherein each double stranded RNAi agent independently comprises a sense strand and an antisense strand forming a double stranded region, wherein each of the sense strands independently comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of the nucleotide sequence of SEQ ID NO:1, or a nucleotide sequence having at least 90% nucleotide sequence identity to a portion of the nucleotide sequence of SEQ ID

NO:1, and each of the antisense strands independently comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO:2, or a nucleotide sequence having at least 90% nucleotide sequence identity to a portion of the nucleotide sequence of SEQ ID NO:2.

(82) In another aspect, the present invention provides a composition comprising two or more, e.g., 2, 3, or 4, double stranded ribonucleic acid (dsRNA) agents for inhibiting expression of coronavirus genome in a cell, wherein each dsRNA agent independently comprises a sense strand and an antisense strand forming a double stranded region, wherein each of the sense strands independently comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of the nucleotide sequence of SEQ ID NO:2, or a nucleotide sequence having at least 90% nucleotide sequence identity to a portion of the nucleotide sequence of SEQ ID NO:2, and each of the antisense strands independently comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO:1, or a nucleotide sequence having at least 90% nucleotide sequence identity to a portion of the nucleotide sequence of SEQ ID NO:1.

(83) In yet another aspect, the present invention provides a composition comprising two or more, e.g., 2, 3, or 4, double stranded ribonucleic acid (dsRNA) agents for inhibiting expression of coronavirus genome in a cell, wherein each dsRNA agent independently comprises a sense strand and an antisense strand forming a double stranded region, wherein each of the antisense strands independently comprises a region complementary to part of an mRNA encoding a coronavirus genome (SEQ ID NO:1), wherein each of the sense strands or each of the antisense strands are independently 14 to 30 nucleotides in length.

(84) In one aspect, the present invention provides a composition comprising two or more, e.g., 2, 3, or 4, double stranded ribonucleic acid (dsRNA) agents for inhibiting expression of coronavirus genome in a cell, wherein each dsRNA agent independently comprises a sense strand and an antisense strand forming a double stranded region, wherein each of the antisense strands independently comprises a region complementary to part of a reverse complement of an mRNA encoding a coronavirus genome (SEQ ID NO:2), wherein each of the sense strands or each of the antisense strands are independently 14 to 30 nucleotides in length.

(85) In another aspect, the present invention provides a composition comprising two or more, e.g., 2, 3, or 4, double stranded RNAi agents for inhibiting expression of a coronavirus genome in a cell, wherein each double stranded RNAi agent independently comprises a sense strand and an antisense strand forming a double stranded region, wherein each of the antisense strands independently comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-5, wherein each of the sense strands or each of the antisense strands are independently 14 to 30 nucleotides in length.

(86) In one embodiment, each of the sense strands or each of the antisense strands is a sense strand or an antisense strand independently selected from the group consisting of any of the sense strands and antisense strands in any one of Table 2-5.

(87) In another embodiment, each of the sense strands or each of the antisense strands is a sense strand or an antisense strand independently selected from the sense strand or antisense strand of a duplex selected from the group consisting of AD-1184137, AD-1184147, AD-1184150, AD-1184210, AD-1184270, AD-1184233, AD-1184271, AD-1184212, AD-1184228, AD-1184223, AD-1231490, AD-1231513, AD-1231485, AD-1231507, AD-1231471, AD-1231494, AD-1231496, and AD-1231497.

(88) In yet another embodiment, each of the sense strands or each of the antisense strands is a sense strand or an antisense strand of a duplex independently selected from the group consisting of AD-1184137, AD-1184147, AD-1184150, AD-1231490, AD-1231513, AD-1231485, AD-1231471, AD-1231496, and AD-1231497.

(89) In one embodiment, each of the sense strands and each of the antisense strands is a sense

strand and an antisense strand of a duplex independently selected from the group consisting of AD-1184137 and AD-1184150.

(90) In one embodiment, at least one of said sense strands or at least one of said antisense strands is independently conjugated to one or more lipophilic moieties

(91) In one embodiment, all of the sense strands or all of the antisense strand of each of the dsRNA agents are independently conjugated to one or more lipophilic moieties.

(92) In one embodiment, each lipophilic moiety is independently conjugated to one or more positions in the double stranded region of the dsRNA agent.

(93) In one embodiment, each lipophilic moiety is independently conjugated via a linker or a carrier.

(94) In one embodiment, lipophilicity of each lipophilic moiety, measured by log Kow, independently exceeds 0.

(95) In another embodiment, the hydrophobicity of each double-stranded RNAi agent, measured by the unbound fraction in a plasma protein binding assay of the double-stranded RNAi agent, independently exceeds 0.2.

(96) In one embodiment, the plasma protein binding assay is an electrophoretic mobility shift assay using human serum albumin protein.

(97) In one embodiment, each of the dsRNA agents independently comprises at least one modified nucleotide.

(98) In one embodiment, each sense strand and each antisense strand of each dsRNA agent independently comprises no more than five unmodified nucleotides.

(99) In one embodiment, all of the nucleotides of each sense strand and all of the nucleotides of each antisense strand independently comprise a modification.

(100) In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5'-methylphosphonate group, a nucleotide comprising a 5' phosphate or 5' phosphate mimic, a nucleotide comprising vinyl phosphonate, a nucleotide comprising adenosine-glycol nucleic acid (GNA), a nucleotide comprising thymidine-glycol nucleic acid (GNA)S-Isomer, a nucleotide comprising 2-hydroxymethyl-tetrahydrofuran-5-phosphate, a nucleotide comprising 2'-deoxythymidine-3'-phosphate, a nucleotide comprising 2'-deoxyguanosine-3'-phosphate, a 2'-O-hexadecyl nucleotide, a nucleotide comprising a 2'-phosphate, a cytidine-2'-phosphate nucleotide, a guanosine-2'-phosphate nucleotide, a 2'-O-hexadecyl-cytidine-3'-phosphate nucleotide, a 2'-O-hexadecyl-adenosine-3'-phosphate nucleotide, a 2'-O-hexadecyl-guanosine-3'-phosphate nucleotide, a 2'-O-hexadecyl-uridine-3'-phosphate nucleotide, a 5'-vinyl phosphonate (VP), a 2'-deoxyadenosine-3'-phosphate nucleotide, a 2'-deoxycytidine-3'-phosphate nucleotide, a 2'-deoxyguanosine-3'-phosphate nucleotide, a 2'-deoxythymidine-3'-phosphate nucleotide, a 2'-deoxyuridine nucleotide, and a terminal nucleotide linked to a cholesteryl derivative and a dodecanoic acid bisdecylamide group; and combinations thereof.

(101) In another embodiment, the modified nucleotide is independently selected from the group consisting of a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, 3'-terminal deoxy-thymine nucleotides (dT), a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

- (102) In yet another embodiment, the modified nucleotide comprises a short sequence of 3'-terminal deoxy-thymine nucleotides (dT).
- (103) In one embodiment, the modifications on the nucleotides are each independently selected from the group consisting of 2'-O-methyl modifications, 2'-deoxy-modifications, or 2'fluoro modifications.
- (104) In one embodiment, at least one of the dsRNA agents further comprises at least one phosphorothioate internucleotide linkage.
- (105) In one embodiment, at least one of the dsRNA agents comprises 6-8 phosphorothioate internucleotide linkages.
- (106) In one embodiment, each strand of each dsRNA agent is independently no more than 30 nucleotides in length.
- (107) In one embodiment, at least one strand of at least one dsRNA agent independently comprises a 3' overhang of at least 1 nucleotide.
- (108) In another embodiment, at least one strand of at least one dsRNA agent independently comprises a 3' overhang of at least 2 nucleotides.
- (109) In one embodiment, the double stranded region of each dsRNA agent is independently 15-30 nucleotide pairs in length.
- (110) In another embodiment, the double stranded region of each dsRNA agent is independently is 17-23 nucleotide pairs in length.
- (111) In yet another embodiment, the double stranded region of each dsRNA agent is independently is 17-25 nucleotide pairs in length.
- (112) In one embodiment, double stranded region of each dsRNA agent is independently is 23-27 nucleotide pairs in length.
- (113) In another embodiment, the double stranded region of each dsRNA agent is independently is 19-21 nucleotide pairs in length.
- (114) In one embodiment, the double stranded region of each dsRNA agent is independently is 21-23 nucleotide pairs in length.
- (115) In one embodiment, each strand of each dsRNA agent independently has 19-30 nucleotides.
- (116) In another embodiment, each strand of each dsRNA agent independently has 19-23 nucleotides.
- (117) In yet another embodiment, wherein each strand of each dsRNA agent independently has 21-23 nucleotides.
- (118) In one embodiment, each dsRNA agent comprises one or more lipophilic moieties independently conjugated to one or more internal positions on at least one strand.
- (119) In one embodiment, the one or more lipophilic moieties are each independently conjugated to one or more internal positions on at least one strand via a linker or carrier.
- (120) In one embodiment, each of the internal positions independently include all positions except the terminal two positions from each end of the at least one strand.
- (121) In one embodiment, each of the internal positions independently include all positions except the terminal three positions from each end of the at least one strand.
- (122) In one embodiment, each of the internal positions independently exclude a cleavage site region of the sense strand.
- (123) In one embodiment, each of the internal positions independently include all positions except positions 9-12, counting from the 5'-end of the sense strand.
- (124) In one embodiment, each of the internal positions independently include all positions except positions 11-13, counting from the 3'-end of the sense strand.
- (125) In one embodiment, each of the internal positions independently exclude a cleavage site region of the antisense strand.
- (126) In one embodiment, each of the internal positions independently include all positions except positions 12-14, counting from the 5'-end of the antisense strand.

- (127) In another embodiment, each of the internal positions independently include all positions except positions 11-13 on the sense strand, counting from the 3'-end, and positions 12-14 on the antisense strand, counting from the 5'-end.
- (128) In one embodiment, each of the one or more lipophilic moieties are independently conjugated to one or more of the internal positions selected from the group consisting of positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'-end of each strand.
- (129) In another embodiment, the one or more lipophilic moieties are each independently conjugated to one or more of the internal positions selected from the group consisting of positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'-end of each strand.
- (130) In one embodiment, each of the positions in the double stranded region independently exclude a cleavage site region of the sense strand.
- (131) In one embodiment, each of the sense strands is independently 21 nucleotides in length, each of the antisense strands is independently 23 nucleotides in length, and each of the lipophilic moieties is independently conjugated to position 21, position 20, position 15, position 1, position 7, position 6, or position 2 of the sense strand or position 16 of the antisense strand.
- (132) In one embodiment, each of the lipophilic moieties is independently conjugated to position 21, position 20, position 15, position 1, or position 7 of the sense strand.
- (133) In another embodiment, each of the lipophilic moieties is independently conjugated to position 21, position 20, or position 15 of the sense strand.
- (134) In yet another embodiment, each of the lipophilic moieties is independently conjugated to position 20 or position 15 of the sense strand.
- (135) In one embodiment, each of the lipophilic moieties is independently conjugated to position 16 of the antisense strand.
- (136) In one embodiment, each of the lipophilic moieties is independently an aliphatic, alicyclic, or polyalicyclic compound.
- (137) In one embodiment, each of the lipophilic moieties is independently selected from the group consisting of lipid, cholesterol, retinoic acid, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-bis-O(hexadecyl)glycerol, geranyloxyhexanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, 03-(oleoyl)lithocholic acid, 03-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine.
- (138) In one embodiment, each of the lipophilic moieties independently contains a saturated or unsaturated C4-C30 hydrocarbon chain, and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.
- (139) In one embodiment, each of the lipophilic moieties independently contains a saturated or unsaturated C6-C18 hydrocarbon chain.
- (140) In one embodiment, each of the lipophilic moieties independently contains a saturated or unsaturated C16 hydrocarbon chain.
- (141) In one embodiment, each of the saturated or unsaturated C16 hydrocarbon chain is independently conjugated to position 6, counting from the 5'-end of the strand.
- (142) In one embodiment, each of the lipophilic moieties is independently conjugated via a carrier that replaces one or more nucleotide(s) in the internal position(s) or the double stranded region.
- (143) In one embodiment, each of the carriers is independently a cyclic group selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl; or is an acyclic moiety based on a serinol backbone or a diethanolamine backbone.
- (144) In one embodiment, each of the lipophilic moieties is independently conjugated to the double-stranded iRNA agent via a linker containing an ether, thioether, urea, carbonate, amine,

amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction, or carbamate.

(145) In one embodiment, each of the lipophilic moieties is independently conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

(146) In one embodiment, each of the lipophilic moieties or one or more targeting ligands is independently conjugated via a bio-cleavable linker selected from the group consisting of DNA, RNA, disulfide, amide, functionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, mannose, and combinations thereof.

(147) In one embodiment, the 3' end of at least one of the sense strands is independently protected via an end cap which is a cyclic group having an amine, said cyclic group being selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl.

(148) In one embodiment, at least one of the dsRNA agents further comprises a targeting ligand that targets a liver tissue.

(149) In one embodiment, each of the targeting ligands is independently a GalNAc conjugate.

(150) In one embodiment, at least one of the dsRNA agents further comprises a terminal, chiral modification occurring at the first internucleotide linkage at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration, a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp configuration or Sp configuration.

(151) In another embodiment, at least one of the dsRNA agents further comprises a terminal, chiral modification occurring at the first and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration, a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

(152) In yet another embodiment, at least one of the dsRNA agents further comprises a terminal, chiral modification occurring at the first, second and third internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration, a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

(153) In one embodiment, at least one of the dsRNA agents further comprises a terminal, chiral modification occurring at the first, and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration, a terminal, chiral modification occurring at the third internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration, and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

(154) In another embodiment, at least one of the dsRNA agents further comprises a terminal, chiral modification occurring at the first, and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration, a terminal, chiral modification occurring at the first, and second internucleotide linkages at the 5' end of the antisense strand,

having the linkage phosphorus atom in Rp configuration, and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

(155) In one embodiment, at least one of the dsRNA agents further comprises a phosphate or phosphate mimic at the 5'-end of the antisense strand.

(156) In one embodiment, each of the phosphate mimic is independently a 5'-vinyl phosphonate (VP).

(157) In one embodiment, the base pair at the 1 position of the 5'-end of the antisense strand of at least one of the duplex is independently an AU base pair.

(158) In one embodiment, each of the sense strands independently has a total of 21 nucleotides and each of the antisense strands independently has a total of 23 nucleotides.

(159) In one embodiment, the composition comprises a first dsRNA agent comprising the sense strand nucleotide sequence 5'-UAACAAUGUUGCUUUUCAAC-3' (SEQ ID NO: 5) and an antisense strand comprising the nucleotide sequence 5'-GUUUGAAAAGCAACAUGUUAGU-3' (SEQ ID NO: 6); and a second dsRNA agent comprising the sense strand nucleotide sequence 5'-ACUGUACAGUCUAAAUGUCA-3' (SEQ ID NO: 7) and an antisense strand comprising the nucleotide sequence 5'-UGACAUUUUAGACUGUACAGUGG-3' (SEQ ID NO: 8).

(160) In one embodiment, the sense strand of the first dsRNA agent comprises the sense strand nucleotide sequence 5'-usasaca(Ahd)UfgUfUfGfcuuuuaasasa-3' (SEQ ID NO: 9) and the antisense strand of the first dsRNA agent comprises the nucleotide sequence 5'-VPusUfsuugAfaaagcaaCfaUfuguuasgsu-3' (SEQ ID NO: 10); and the sense strand of the second dsRNA agent comprises the nucleotides sequence 5'-ascsugu(Ahd)CfaGfUfCfuaaaauguscsa-3' (SEQ ID NO: 11) and the antisense strand of the second dsRNA agent comprises the nucleotide sequence 5'-VPusGfsacaUfuuuagacUfgUfacagusgsg-3' (SEQ ID NO: 12), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U; s is a phosphorothioate linkage; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; and VP is Vinyl-phosphonate.

(161) The present invention further provides isolated cells, comprising the compositions of the invention.

(162) In one embodiment, the compositions of the invention are pharmaceutical compositions. In another embodiment, the compositions of the invention are pharmaceutical composition comprising a lipid formulation.

(163) In one aspect, the present invention provides a method of inhibiting expression of a coronavirus genome in a cell. The method includes contacting the cell with the dsRNA agent of the invention, the composition of the invention, or the pharmaceutical composition of the invention; and maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the coronavirus genome, thereby inhibiting expression of the coronavirus genome in the cell.

(164) In one embodiment, the cell is contacted with two or more, e.g., 2, 3, or 4, of the dsRNA agents of the invention.

(165) In one embodiment, the cell is within a subject.

(166) In one embodiment, the subject is a human.

(167) In one embodiment, the expression of the coronavirus genome is inhibited by at least 50%.

(168) In one aspect, the present invention provides a method of inhibiting replication of a coronavirus in a cell. The method includes contacting the cell with the dsRNA agent of the invention, the composition of the invention, or the pharmaceutical composition of the invention; and maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the RNA transcript of the coronavirus genome, thereby inhibiting replication of the coronavirus in a cell.

(169) In one embodiment, the cell is contacted with two or more, e.g., 2, 3, or 4, of the dsRNA agents of the invention.

(170) In one embodiment, the cell is within a subject.

- (171) In one embodiment, the subject is a human.
- (172) In one embodiment, the expression of the coronavirus genome is inhibited by at least 50%.
- (173) In one aspect, the present invention provides a method of treating a subject having a coronavirus infection. The method includes administering to the subject a therapeutically effective amount of the dsRNA agent of the invention, the composition of the invention, or the pharmaceutical composition of the invention, thereby treating the subject.
- (174) In one embodiment, the subject is administered two or more, e.g., 2, 3, or 4, dsRNA agents of the invention.
- (175) In one embodiment, the subject is a human, e.g., an immunocompromised human.
- (176) In one embodiment, the subject having the coronavirus infection is infected with a severe acute respiratory syndrome (SARS) virus, a Middle East respiratory syndrome (MERS) virus, or a severe acute respiratory syndrome 2 (SARS-2) virus.
- (177) In one embodiment, treating comprises amelioration of at least one sign or symptom of the disease.
- (178) In one embodiment, the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.
- (179) In one embodiment, the administration of the dsRNA is pulmonary system administration, e.g., intranasal administration, or oral inhalative administration.
- (180) In one embodiment, the double-stranded RNAi agent is administered intranasally.
- (181) By pulmonary system administration, e.g., intranasal administration or oral inhalative administration, of the double-stranded RNAi agent, the method can reduce the expression of a coronavirus genome in a pulmonary system tissue, e.g., a nasopharynx tissue, an oropharynx tissue, a laryngopharynx tissue, a larynx tissue, a trachea tissue, a carina tissue, a bronchi tissue, a bronchiole tissue, or an alveoli tissue.
- (182) In one embodiment, the dsRNA agent is administered to the subject subcutaneously.
- (183) In one embodiment, the method further comprises administering to the subject an additional agent or a therapy suitable for treatment or prevention of a coronavirus-associated disorder.
- (184) In one embodiment, the additional therapeutic agent is selected from the group consisting of an antiviral agent, an immune stimulator, a therapeutic vaccine, a viral entry inhibitor, and a combination of any of the foregoing.
- (185) In one aspect the present invention provides a method of treating a subject having a coronavirus infection. The method includes administering to the subject via pulmonary system administration a therapeutically effective amount of a first dsRNA agent comprising a first sense strand and a first antisense strand forming a first double stranded region, and a therapeutically effective amount of a second dsRNA agent comprising a second sense strand and a second antisense strand forming a second double stranded region, wherein the first sense strand comprises the nucleotide sequence 5'-UAACAAUGUUGCUUUUCAAAC-3' (SEQ ID NO: 5) and the first antisense strand comprises the nucleotide sequence 5'-GUUUGAAAAGCAACAUUGUUAGU-3' (SEQ ID NO: 6); and the second sense strand comprises the nucleotide sequence 5'-ACUGUACAGUCUAAA AUGUCA-3' (SEQ ID NO: 7) and the second antisense strand comprises the nucleotide sequence 5'-UGACAUUUUAGACUGUACAGUGG-3' (SEQ ID NO: 8).
- (186) In another aspect, the present invention provides a method of treating a subject having a coronavirus infection. The method includes administering to the subject via pulmonary system administration a therapeutically effective amount of a composition for inhibiting expression of a coronavirus genome in a cell, said composition comprising: a first dsRNA agent comprising a first sense strand comprising the nucleotide sequence 5'-UAACAAUGUUGCUUUUCAAAC-3' (SEQ ID NO: 5) and a first antisense strand comprising the nucleotide sequence 5'-GUUUGAAAAGCAACAUUGUUAGU-3' (SEQ ID NO: 6), and a second dsRNA agent comprising a second sense strand comprising the nucleotide sequence 5'-ACUGUACAGUCUAAA AUGUCA-3' (SEQ ID NO: 7) and a second antisense strand comprising

the nucleotide sequence 5'-UGACAUUUUAGACUGUACAGUGG-3' (SEQ ID NO: 8), thereby treating the subject.

(187) In one embodiment, the first and second dsRNA agents are present in a composition.

(188) In one embodiment, the first and second dsRNA agents are present in separate compositions.

(189) In another embodiment, the first and second dsRNA agents are present in the same composition.

(190) In one embodiment, the compositions are administered to the subject at the same time.

(191) In another embodiment, the compositions are administered to the subject at different times.

(192) In one embodiment, the composition is a pharmaceutical composition.

(193) In one embodiment, the first sense strand comprises the nucleotide sequence 5'-usasaca(Ahd)UfgUfUfGfcuuuucasasa-3' (SEQ ID NO: 9) and the first antisense strand comprises the nucleotide sequence 5'-VPusUfsuugAfaaagcaaCfaUfuguuasgsu-3' (SEQ ID NO: 10); and the second sense strand comprises the nucleotides sequence 5'-ascugu(Ahd)CfaGfUfCfuaaaaugusca-3' (SEQ ID NO: 11) and the second antisense strand comprises the nucleotide sequence 5'-VPusGfsacaUfuuuagacUfgUfacagusgsg-3' (SEQ ID NO: 12), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U; s is a phosphorothioate linkage; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; and VP is Vinyl-phosphonate.

(194) In one embodiment, the subject is a human.

(195) In one embodiment, the subject having the coronavirus infection is infected with a severe acute respiratory syndrome (SARS) virus, a Middle East respiratory syndrome (MERS) virus, or a severe acute respiratory syndrome 2 (SARS-2)-CoV-2 virus.

(196) In one embodiment, treating comprises amelioration of at least on sign or symptom of the disease.

(197) In one embodiment, the first and second dsRNA agents are independently administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

(198) In one embodiment, the pulmonary system administration is via inhalation or intranasally.

(199) In one embodiment, the methods further comprise administering to the subject an additional agent or a therapy suitable for treatment or prevention of a coronavirus-associated disorder.

(200) In one embodiment, the additional therapeutic agent is selected from the group consisting of an antiviral agent, an immune stimulator, a therapeutic vaccine, a viral entry inhibitor, and a combination of any of the foregoing.

(201) The present invention is further illustrated by the following detailed description and drawings.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

(1) FIG. 1 depicts the genomes and structures of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), and the Middle East Respiratory Syndrome coronavirus (MERS-CoV).

(2) FIG. 2 schematically depicts the concatemers and the assay used for the single dose screens of the dsRNA agents of the invention.

(3) FIG. 3 are graphs depicting the effect of the indicated siRNAs on extracellular SARS-CoV-2 genomes determined by RT-qPCR (top graph) and the effect on of the indicated siRNAs on intracellular viral nucleocapsid protein determined by in-cell ELISA (bottom graph).

(4) FIG. 4 depicts the effect of the indicated siRNAs on resistance selected cells infected with SARS-CoV-2 determined by RT-qPCR.

(5) FIG. 5 depicts the effect of the indicated siRNAs on resistance selected cells infected with SARS-CoV-2 determined in a focus-forming assay.

(6) FIG. 6 are images of an immunofluorescence assay depicting SARS-CoV-2 nucleocapsid

protein staining in a focus-forming assay in the presence of 10 nM of the indicated siRNAs.

(7) FIG. 7 is a graph depicting the effect of intranasal administration of a combination of AD-1184150 and AD-1184137 on the body weight of hamsters challenged with SARS-CoV-2.

(8) FIG. 8 is a graph depicting the effect of intranasal administration as compared to subcutaneous administration of a combination of AD-1184150 and AD-1184137 on the body weight of hamsters challenged with SARS-CoV-2.

DETAILED DESCRIPTION OF THE INVENTION

(9) The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA genomes and RNA transcripts of coronavirus genes, e.g., a SARS-CoV-2 gene. The iRNAs of the invention have been designed to target a human coronavirus genome, e.g., a Severe Acute Respiratory Syndrome 2 (SARS-CoV-2) genome, including portions of a coronavirus genome, e.g., a Severe Acute Respiratory Syndrome 2 (SARS-CoV-2) genome, that are cross-reactive with a Severe Acute Respiratory Syndrome (SARS-CoV) gene, and/or Middle East Respiratory Syndrome (MERS-CoV) gene. The coronavirus genome may be within a cell, e.g., a cell within a subject, such as a human. The use of these iRNAs enables the targeted degradation of mRNAs of the corresponding genome (a coronavirus genome) in mammals. The present disclosure also provides methods of using the RNAi compositions of the disclosure, e.g., compositions comprising one or more, e.g., 2, 3, or 4, dsRNA agents of the invention, for inhibiting the expression of coronavirus genes or genome for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a coronavirus genome, e.g., a coronavirus-associated disorder, e.g., a subject having a coronavirus infection, e.g., a subject having Severe Acute Respiratory Syndrome 2 (SARS-CoV-2; COVID-19), Severe Acute Respiratory Syndrome (SARS-CoV), or Middle East Respiratory Syndrome (MERS-CoV).

(10) The iRNAs of the invention include an RNA strand (the antisense strand) having a region which is up to about 30 nucleotides or less in length, e.g., 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of a coronavirus genome. In certain embodiments, the RNAi agents of the disclosure include an RNA strand (the antisense strand) having a region which is about 21-23 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of a coronavirus genome (either of a positive-sense genomic RNA or a negative-sense genomic RNA of a coronavirus).

(11) In certain embodiments, one or both of the strands of the double stranded RNAi agents of the invention is up to 66 nucleotides in length, e.g., 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length, with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA of a coronavirus genome (either of a positive-sense genomic RNA or a negative-sense genomic RNA of a coronavirus). In some embodiments, such iRNA agents having longer length antisense strands preferably may include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

(12) The use of iRNAs of the invention enables the targeted degradation of mRNAs or RNA genomes of the corresponding viral genes (coronavirus gene) in mammals. Thus, methods and compositions including these iRNAs are useful for treating a subject having a coronavirus-associated disorder, e.g., a subject having a coronavirus infection, e.g., a subject having Severe Acute Respiratory Syndrome 2 (SARS-CoV-2; COVID-19), Severe Acute Respiratory Syndrome (SARS-CoV), or Middle East Respiratory Syndrome (MERS-CoV).

(13) In certain embodiments, the administration of the dsRNA to a subject results in an improvement of lung function, or a stoppage or reduction of the rate of loss of lung function, reduction of fever, reduction of cough.

(14) The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a coronavirus genomes as well as compositions, uses, and methods for treating subjects that would benefit from inhibition and/or reduction of the expression of a coronavirus genome, e.g., subjects susceptible to or diagnosed with a coronavirus-associated disorder.

I. Definitions

(15) In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

(16) The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element, e.g., a plurality of elements.

(17) The term “including” is used herein to mean, and is used interchangeably with, the phrase “including but not limited to”.

(18) The term “or” is used herein to mean, and is used interchangeably with, the term “and/or,” unless context clearly indicates otherwise.

(19) The term “about” is used herein to mean within the typical ranges of tolerances in the art. For example, “about” can be understood as about 2 standard deviations from the mean. In certain embodiments, about means $\pm 10\%$. In certain embodiments, about means $\pm 5\%$. When about is present before a series of numbers or a range, it is understood that “about” can modify each of the numbers in the series or range.

(20) The term “at least” prior to a number or series of numbers is understood to include the number adjacent to the term “at least”, and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, “at least 19 nucleotides of a 21 nucleotide nucleic acid molecule” means that 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that “at least” can modify each of the numbers in the series or range.

(21) As used herein, “no more than” or “less than” is understood as the value adjacent to the phrase and logical lower values or integers, as logical from context, to zero. For example, a duplex with an overhang of “no more than 2 nucleotides” has a 2, 1, or 0 nucleotide overhang. When “no more than” is present before a series of numbers or a range, it is understood that “no more than” can modify each of the numbers in the series or range. As used herein, ranges include both the upper and lower limit.

(22) As used herein, methods of detection can include determination that the amount of analyte present is below the level of detection of the method.

(23) In the event of a conflict between an indicated target site and the nucleotide sequence for a sense or antisense strand, the indicated sequence takes precedence.

(24) In the event of a conflict between a sequence and its indicated site on a transcript or other sequence, the nucleotide sequence recited in the specification takes precedence.

(25) As used herein, the term “coronavirus,” (“CoV”; subfamily Coronavirinae, family Coronaviridae, order Nidovirales), refers to a group of highly diverse, enveloped, positive-sense, single-stranded RNA viruses that cause respiratory, enteric, hepatic and neurological diseases of varying severity in a broad range of animal species, including humans. Coronaviruses are subdivided into four genera: Alphacoronavirus, Betacoronavirus (13CoV), Gammacoronavirus and Deltacoronavirus.

(26) Any coronavirus that infects humans and animals is encompassed by the term “coronavirus” as used herein. Exemplary coronaviruses encompassed by the term include the coronaviruses that cause a common cold-like respiratory illness, e.g., human coronavirus 229E (HCoV-229E), human

coronavirus NL63 (HCoV-NL63), human coronavirus OC43 (HCoV-OC43), and human coronavirus HKU1 (HCoV-HKU1); the coronavirus that causes avian infectious bronchitis virus (IBV); the coronavirus that causes murine hepatitis virus (MHV); the coronavirus that causes porcine transmissible gastroenteritis virus PRCoV; the coronavirus that causes porcine respiratory coronavirus and bovine coronavirus; the coronavirus that causes Severe Acute Respiratory Syndrome (SARS), the coronavirus that causes the Middle East respiratory syndrome (MERS), and the coronavirus that causes Severe Acute Respiratory Syndrome 2 (SARS-CoV-2; COVID-19).

(27) The coronavirus (CoV) genome is a single-stranded, non-segmented RNA genome, which is approximately 26-32 kb. It contains 5'-methylated caps and 3'-polyadenylated tails and is arranged in the order of 5', replicase genes, genes encoding structural proteins (spike glycoprotein (S), envelope protein (E), membrane protein (M) and nucleocapsid protein (N)), polyadenylated tail and then the 3' end. The partially overlapping 5'-terminal open reading frame 1a/b (ORF1a/b) is within the 5' two-thirds of the CoV genome and encodes the large replicase polyprotein 1a (pp1a) and pp1ab. These polyproteins are cleaved by papain-like cysteine protease (PLpro) and 3C-like serine protease (3CLpro) to produce non-structural proteins, including RNA-dependent RNA polymerase (RdRp) and helicase (Hel), which are important enzymes involved in the transcription and replication of CoVs. The 3' one-third of the CoV genome encodes the structural proteins (S, E, M and N), which are essential for virus-cell-receptor binding and virion assembly, and other non-structural proteins and accessory proteins that may have immunomodulatory effects. (Peiris J S., et al., 2003, *Nat. Med.* 10 (Suppl. 12): 88-97).

(28) As a coronavirus is a positive-sense, single-stranded RNA virus having a 5' methylated cap and a 3' polyadenylated tail, once the virus enters the cell and is uncoated, the viral RNA genome attaches to the host cell's ribosome for direct translation. The host ribosome translates the initial overlapping open reading frame of the virus genome and forms a long polyprotein. The polyprotein has its own proteases which cleave the polyprotein into multiple nonstructural proteins.

(29) A number of the nonstructural proteins coalesce to form a multi-protein replicase-transcriptase complex (RTC). The main replicase-transcriptase protein is the RNA-dependent RNA polymerase (RdRp). It is directly involved in the replication and transcription of RNA from an RNA strand. The other nonstructural proteins in the complex assist in the replication and transcription process. The exoribonuclease non-structural protein for instance provides extra fidelity to replication by providing a proofreading function which the RNA-dependent RNA polymerase lacks.

(30) One of the main functions of the complex is to replicate the viral genome. RdRp directly mediates the synthesis of negative-sense genomic RNA from the positive-sense genomic RNA. This is followed by the replication of positive-sense genomic RNA from the negative-sense genomic RNA. The other important function of the complex is to transcribe the viral genome. RdRp directly mediates the synthesis of negative-sense subgenomic RNA molecules from the positive-sense genomic RNA. This is followed by the transcription of these negative-sense subgenomic RNA molecules to their corresponding positive-sense mRNAs

(31) The replicated positive-sense genomic RNA becomes the genome of the progeny viruses.

(32) As use herein, the term "severe acute respiratory syndrome coronavirus" or "SARS-CoV", refers to a coronavirus that was first discovered in 2003, which causes severe acute respiratory syndrome (SARS). SARS-CoV represents the prototype of a new lineage of coronaviruses capable of causing outbreaks of clinically significant and frequently fatal human disease. The complete genome of SARS-CoV has been identified, as well as common variants thereof. The genome of SARS-CoV is a 29,727-nucleotide polyadenylated RNA, has 11 open reading frames, and 41% of the residues are G or C (see, e.g., FIG. 1). The genomic organization is typical of coronaviruses, with the characteristic gene order (5'-replicase (rep), spike (S), envelope (E), membrane (M), nucleocapsid (N)-3' and short untranslated regions at both termini. The SARS-CoV rep gene, which comprises about two-thirds of the genome, is predicted to encode two polyproteins that undergo co-translational proteolytic processing. There are four open reading frames (ORFs) downstream of rep

that are predicted to encode the structural proteins S, E, M and N. The hemagglutinin-esterase gene, which is present between ORF1b and S in group 2 and some group 3 coronaviruses was not found.

(33) The amino acid and complete coding sequences of the SARS-CoV genomes are known may be found in for example, GenBank Accession Nos. AY502923.1; AP006559.1; AP006558.1; AY313906.1; AY345986.1; AY502931.1; AY282752.2; AY559097.1; AY559081.1; DQ182595.1; AY291451.1; AY568539.1; AY613947.1; and AY390556.1, the entire contents of each of which are incorporated herein by reference.

(34) The term “SARS-CoV,” as used herein, also refers to naturally occurring RNA sequence variations of the SARS-CoV genome.

(35) As use herein, the term “the Middle East respiratory syndrome coronavirus” or “MERS-CoV”, refers to a coronavirus that causes the Middle East respiratory syndrome (MERS), which was first identified in 2012. MERS-CoV is closely related to severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV). Clinically similar to SARS, MERS-CoV infection leads to severe respiratory illness with renal failure. The genomic structure of MERS-CoV is shown in FIG. 1.

(36) The amino acid and complete coding sequences of the MERS-CoV genomes are known and may be found in for example, GenBank Accession Nos. MK462243.1; MK462244.1; MK462245.1; MK462246.1; MK462247.1; MK462248.1; MK462249.1; MK462250.1; MK462251.1; MK462252.1; MK462253.1; MK462254.1; MK462255.1; MK462256.1; MK483839.1; and MH822886.1, the entire contents of each of which are incorporated herein by reference.

(37) The term “MERS-CoV,” as used herein, also refers to naturally occurring RNA sequence variations of the MERS-CoV genome.

(38) As use herein, the terms “severe acute respiratory syndrome coronavirus 2,” “SARS-CoV-2,” “2019-nCoV,” refer to the novel coronavirus that caused a pneumonia outbreak first reported in Wuhan, China in December 2019 (“COVID-19”). Phylogenetic analysis of the complete viral genome (29,903 nucleotides) revealed that SARS-CoV-2 was most closely related (89.1% nucleotide similarity similarity) to SARS-CoV.

(39) The amino acid and complete coding sequences of the SARS-CoV-2 genomes are known and may be found in for example, the GISAID EpiCoV™ Database (db.cngb.org/gisaid/), including Accession nos. EPI_ISL_402119; EPI_ISL_402120; EPI_ISL_402121; EPI_ISL_402123; EPI_ISL_402124; EPI_ISL_402125; EPI_ISL_402127; EPI_ISL_402128; EPI_ISL_402129; EPI_ISL_402130; EPI_ISL_402132; EPI_ISL_403928; EPI_ISL_403929; EPI_ISL_403930; EPI_ISL_403931; EPI_ISL_403932; EPI_ISL_403933; EPI_ISL_403934; EPI_ISL_403935; EPI_ISL_403936; EPI_ISL_403937; EPI_ISL_403962; EPI_ISL_404228; EPI_ISL_404253; and EPI_ISL_404895, the entire contents of watch of which are incorporated herein by reference.

(40) The term “SARS-CoV-2,” as used herein, also refers to naturally occurring RNA sequence variations of the SARS-CoV-2 genome.

(41) Additional examples of coronavirus genome and mRNA sequences are readily available using publicly available databases, e.g., GenBank, UniProt, and OMIM.

(42) As used herein, “target sequence” refers to a contiguous portion of the nucleotide sequence of an RNA molecule, such as a coronavirus positive-sense RNA molecule or a coronavirus negative-sense RNA molecule, including mRNA that is a product of RNA processing of a primary transcription product. The target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an RNA molecule, such as a coronavirus positive-sense RNA molecule or a coronavirus negative-sense RNA molecule. In one embodiment, the target sequence is within the protein coding region of a coronavirus genome.

(43) The target sequence may be from about 19-36 nucleotides in length, e.g., preferably about 19-30 nucleotides in length. For example, the target sequence can be about 19-30 nucleotides, 19-30,

19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. In some embodiments, the target sequence is about 19 to about 30 nucleotides in length. In other embodiments, the target sequence is about 19 to about 25 nucleotides in length. In still other embodiments, the target sequence is about 19 to about 23 nucleotides in length. In some embodiments, the target sequence is about 21 to about 23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

(44) As used herein, the term “strand comprising a sequence” refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

(45) “G,” “C,” “A,” “T,” and “U” each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that the term “ribonucleotide” or “nucleotide” can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, e.g., Table 1). The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

(46) The terms “iRNA”, “RNAi agent,” “iRNA agent,” “RNA interference agent” as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript via an RNA-induced silencing complex (RISC) pathway. RNA interference (RNAi) is a process that directs the sequence-specific degradation of mRNA. RNAi modulates, e.g., inhibits, the expression of a coronavirus genome in a cell, e.g., a cell within a subject, such as a mammalian subject.

(47) In one embodiment, an RNAi agent of the disclosure includes a single stranded RNAi that interacts with a target RNA sequence, e.g., a coronavirus target mRNA sequence, either a coronavirus positive-sense RNA molecule or a coronavirus negative-sense RNA molecule, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into double-stranded short interfering RNAs (siRNAs) comprising a sense strand and an antisense strand by a Type III endonuclease known as Dicer (Sharp et al. (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes these dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, et al., (2001) *Nature* 409:363). These siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, et al., (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, et al., (2001) *Genes Dev.* 15:188). Thus, in one aspect the disclosure relates to a single stranded RNA (ssRNA) (the antisense strand of a siRNA duplex) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target genome, i.e., a coronavirus genome or gene. Accordingly, the term “siRNA” is also used herein to refer to an RNAi as described above.

(48) In another embodiment, the RNAi agent may be a single-stranded RNA that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents bind to the RISC endonuclease, Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are

generally 15-30 nucleotides and are chemically modified. The design and testing of single-stranded RNAs are described in U.S. Pat. No. 8,101,348 and in Lima et al., (2012) *Cell* 150:883-894, the entire contents of each of which are hereby incorporated herein by reference. Any of the antisense nucleotide sequences described herein may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima et al., (2012) *Cell* 150:883-894.

(49) In another embodiment, a “RNAi agent” for use in the compositions and methods of the disclosure is a double stranded RNA and is referred to herein as a “double stranded RNAi agent,” “double stranded RNA (dsRNA) molecule,” “dsRNA agent,” or “dsRNA”. The term “dsRNA” refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having “sense” and “antisense” orientations with respect to a target RNA, i.e., either a coronavirus positive-sense RNA molecule or a coronavirus negative-sense RNA molecule. In some embodiments of the disclosure, a double stranded RNA (dsRNA) triggers the degradation of a target RNA, e.g., an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

(50) In general, a dsRNA molecule can include ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, e.g., a deoxyribonucleotide, a modified nucleotide. In addition, as used in this specification, an “RNAi agent” may include ribonucleotides with chemical modifications; an RNAi agent may include substantial modifications at multiple nucleotides.

(51) As used herein, the term “modified nucleotide” refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, or a modified nucleobase. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, e.g., a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the disclosure include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by “RNAi agent” for the purposes of this specification and claims.

(52) In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide—which is acknowledged as a naturally occurring form of nucleotide—if present within a RNAi agent can be considered to constitute a modified nucleotide.

(53) The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 9 to 36 base pairs in length, e.g., about 15-30 base pairs in length, for example, about 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, or 36 base pairs in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

(54) The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a “hairpin loop.” A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23 or more unpaired nucleotides or nucleotides not directed to the target site of the dsRNA. In some embodiments, the hairpin loop can be 10 or fewer nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 nucleotides.

(55) In certain embodiment, the two strands of double-stranded oligomeric compound can be linked together. The two strands can be linked to each other at both ends, or at one end only. By linking at one end is meant that 5'-end of first strand is linked to the 3'-end of the second strand or 3'-end of first strand is linked to 5'-end of the second strand. When the two strands are linked to each other at both ends, 5'-end of first strand is linked to 3'-end of second strand and 3'-end of first strand is linked to 5'-end of second strand. The two strands can be linked together by an oligonucleotide linker including, but not limited to, (N)_n; wherein N is independently a modified or unmodified nucleotide and n is 3-23. In some embodiments, n is 3-10, e.g., 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the oligonucleotide linker is selected from the group consisting of GNRA, (G)₄, (U)₄, and (dT)₄, wherein N is a modified or unmodified nucleotide and R is a modified or unmodified purine nucleotide. Some of the nucleotides in the linker can be involved in base-pair interactions with other nucleotides in the linker. The two strands can also be linked together by a non-nucleoside linker, e.g. a linker described herein. It will be appreciated by one of skill in the art that any oligonucleotide chemical modifications or variations describe herein can be used in the oligonucleotide linker.

(56) Hairpin and dumbbell type oligomeric compounds will have a duplex region equal to or at least 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotide pairs. The duplex region can be equal to or less than 200, 100, or 50, in length. In some embodiments, ranges for the duplex region are 15-30, 17 to 23, 19 to 23, and 19 to 21 nucleotides pairs in length.

(57) The hairpin oligomeric compounds can have a single strand overhang or terminal unpaired region, in some embodiments at the 3', and in some embodiments on the antisense side of the hairpin. In some embodiments, the overhangs are 1-4, more generally 2-3 nucleotides in length. The hairpin oligomeric compounds that can induce RNA interference are also referred to as "shRNA" herein.

(58) Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs.

(59) In one embodiment, an RNAi agent of the invention is a dsRNA, each strand of which is 24-30 nucleotides in length, that interacts with a target RNA sequence, e.g., a coronavirus target RNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp et al. (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, et al., (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, et al., (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, et al., (2001) *Genes Dev.* 15:188).

(60) In one embodiment, an RNAi agent of the invention is a dsRNA agent, each strand of which comprises 19-23 nucleotides that interacts with a coronavirus RNA sequence to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp et al. (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, et al., (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex

(RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, et al., (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, et al., (2001) *Genes Dev.* 15:188). In one embodiment, an RNAi agent of the invention is a dsRNA of 24-30 nucleotides that interacts with a coronavirus RNA sequence to direct the cleavage of the target RNA.

(61) As used herein, the term “nucleotide overhang” refers to at least one unpaired nucleotide that protrudes from the duplex structure of a RNAi agent, e.g., a dsRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or vice versa, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively, the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA.

(62) In one embodiment of the dsRNA, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, e.g., 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, e.g., 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

(63) In one embodiment, the antisense strand of a dsRNA has a 1-10 nucleotide, e.g., 0-3, 1-3, 2-4, 2-5, 4-10, 5-10, e.g., a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, e.g., a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

(64) In certain embodiments, the overhang on the sense strand or the antisense strand, or both, can include extended lengths longer than 10 nucleotides, e.g., 1-30 nucleotides, 2-30 nucleotides, 10-30 nucleotides, or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the antisense strand of the duplex. In certain embodiments, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate. In certain embodiments, the overhang includes a self-complementary portion such that the overhang is capable of forming a hairpin structure that is stable under physiological conditions.

(65) The terms “blunt” or “blunt ended” as used herein in reference to a dsRNA mean that there are no unpaired nucleotides or nucleotide analogs at a given terminal end of a dsRNA, i.e., no nucleotide overhang. One or both ends of a dsRNA can be blunt. Where both ends of a dsRNA are blunt, the dsRNA is said to be blunt ended. To be clear, a “blunt ended” dsRNA is a dsRNA that is blunt at both ends, i.e., no nucleotide overhang at either end of the molecule. Most often such a molecule will be double stranded over its entire length.

(66) The term “antisense strand” or “guide strand” refers to the strand of an iRNA, e.g., a dsRNA, which includes a region that is substantially complementary to a target sequence, e.g., a coronavirus RNA, i.e., either of a coronavirus positive-sense RNA or a coronavirus negative-sense RNA.

(67) As used herein, the term “region of complementarity” refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, e.g., a coronavirus nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, e.g., within 5, 4, 3, or 2 nucleotides of the 5'- or 3'-terminus of the RNAi agent.

(68) In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the antisense strand. In some embodiments, the antisense strand of the double stranded RNA agent of the invention includes no more than 4 mismatches with the target mRNA, e.g., the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the target mRNA. In some embodiments, the antisense strand double stranded RNA agent of the invention includes no more than 4 mismatches with the sense strand, e.g., the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the sense strand. In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the sense strand. In some embodiments, the sense strand of the double stranded RNA agent of the invention includes no more than 4 mismatches with the antisense strand, e.g., the sense strand includes 4, 3, 2, 1, or 0 mismatches with the antisense strand. In some embodiments, the nucleotide mismatch is, for example, within 5, 4, 3 nucleotides from the 3'-end of the iRNA. In another embodiment, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide of the iRNA agent. In some embodiments, the mismatch(s) is not in the seed region.

(69) Thus, an RNAi agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, a RNAi agent as described herein contains no more than 3 mismatches (i.e., 3, 2, 1, or 0 mismatches). In one embodiment, an RNAi agent as described herein contains no more than 2 mismatches. In one embodiment, an RNAi agent as described herein contains no more than 1 mismatch. In one embodiment, an RNAi agent as described herein contains 0 mismatches. In certain embodiments, if the antisense strand of the RNAi agent contains mismatches to the target sequence, the mismatch can optionally be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, in such embodiments, for a 23 nucleotide RNAi agent, the strand which is complementary to a region of a coronavirus genome, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of a coronavirus genome or gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a coronavirus genome is important, especially if the particular region of complementarity in a coronavirus genome is known to mutate.

(70) The term “sense strand” or “passenger strand” as used herein, refers to the strand of a RNAi agent that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

(71) As used herein, “substantially all of the nucleotides are modified” are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

(72) As used herein, the term “cleavage region” refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

(73) As used herein, and unless otherwise indicated, the term “complementary,” when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide

comprising the second nucleotide sequence, as will be understood by the skilled person. Conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

(74) Complementary sequences within a RNAi agent, e.g., within a dsRNA as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as “fully complementary” with respect to each other herein. However, where a first sequence is referred to as “substantially complementary” with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3, or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, e.g., inhibition of gene expression via a RISC pathway. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as “fully complementary” for the purposes described herein.

(75) “Complementary” sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs or base pairs formed from non-natural and modified nucleotides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogsteen base pairing.

(76) The terms “complementary,” “fully complementary” and “substantially complementary” herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a RNAi agent and a target sequence, as will be understood from the context of their use.

(77) As used herein, a polynucleotide that is “substantially complementary to at least part of” a messenger RNA (mRNA) or target sequence refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest or target sequence (e.g., a coronavirus target sequence, either of a coronavirus positive-sense RNA or a coronavirus negative-sense RNA). For example, a polynucleotide is complementary to at least a part of a coronavirus RNA if the sequence is substantially complementary to a non-interrupted portion of a coronavirus RNA.

(78) Accordingly, in some embodiments, the antisense strand polynucleotides disclosed herein are fully complementary to the target coronavirus sequence, either of a coronavirus positive-sense RNA or a coronavirus negative-sense RNA.

(79) In other embodiments, the antisense strand polynucleotides disclosed herein are substantially complementary to the target coronavirus sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NO: 1, or a fragment of SEQ ID NO: 1, such as about 85%, about 90%, or about 95% complementary.

(80) In other embodiments, the antisense strand polynucleotides disclosed herein are substantially complementary to the target coronavirus sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NO: 2, or a fragment of SEQ ID NO: 2, such as about 85%, about 90%, or about 95% complementary.

(81) In other embodiments, the antisense polynucleotides disclosed herein are substantially

complementary to the target coronavirus sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 2-5, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2-5, such as about 85%, about 90%, or about 95% complementary.

(82) In one embodiment, an RNAi agent of the disclosure includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is the same as a target coronavirus sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NO: 2, or a fragment of any one of SEQ ID NO: 2, such as about 85%, about 90%, or about 95% complementary.

(83) In another embodiment, an RNAi agent of the disclosure includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is the same as a target coronavirus sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NO:1, or a fragment of any one of SEQ ID NO: 1, such as about 85%, about 90%, or about 95% complementary.

(84) In some embodiments, an iRNA of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target coronavirus sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the antisense strand nucleotide sequences in any one of any one of Tables 2-5, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 2-5, such as about 85%, about 90%, or about 95% complementary.

(85) In some embodiments, the double-stranded region of a double-stranded iRNA agent is equal to or at least, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, 30 or more nucleotide pairs in length.

(86) In some embodiments, the antisense strand of a double-stranded iRNA agent is equal to or at least 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

(87) In some embodiments, the sense strand of a double-stranded iRNA agent is equal to or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

(88) In one embodiment, the sense and antisense strands of the double-stranded iRNA agent are each independently 15 to 30 nucleotides in length.

(89) In one embodiment, the sense and antisense strands of the double-stranded iRNA agent are each independently 19 to 25 nucleotides in length.

(90) In one embodiment, the sense and antisense strands of the double-stranded iRNA agent are each independently 21 to 23 nucleotides in length.

(91) In one embodiment, the sense strand of the iRNA agent is 21-nucleotides in length, and the antisense strand is 23-nucleotides in length, wherein the strands form a double-stranded region of 21 consecutive base pairs having a 2-nucleotide long single stranded overhangs at the 3-end.

(92) In one aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense nucleic acid molecule that inhibits a target mRNA via an antisense inhibition mechanism. The single-stranded antisense RNA molecule is complementary to a sequence within the target mRNA. The single-stranded antisense oligonucleotides can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. et al., (2002) *Mol Cancer Ther* 1:347-355. The single-stranded antisense RNA molecule may be about 15 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense RNA molecule may comprise a sequence that is at least about 15, 16, 17, 18, 19, 20, or more contiguous

nucleotides from any one of the antisense sequences described herein.

(93) In one embodiment, at least partial suppression of the expression of a coronavirus genome, is assessed by a reduction of the amount of coronavirus genome which can be isolated from or detected in a first cell or group of cells in which a coronavirus genome is transcribed and which has or have been treated such that the expression of a coronavirus genome is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition may be expressed in terms of:

$$(94) \frac{(\text{genome in control cells}) - (\text{genome in treated cells})}{(\text{genome in control cells})} 100\%$$

(95) In one embodiment, inhibition of expression is determined by the dual luciferase method in Example 1 wherein the RNAi agent is present at 10 nM.

(96) The phrase “contacting a cell with an RNAi agent,” such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an RNAi agent includes contacting a cell in vitro with the RNAi agent or contacting a cell in vivo with the RNAi agent. The contacting may be done directly or indirectly. Thus, for example, the RNAi agent may be put into physical contact with the cell by the individual performing the method, or alternatively, the RNAi agent may be put into a situation that will permit or cause it to subsequently come into contact with the cell.

(97) Contacting a cell in vitro may be done, for example, by incubating the cell with the RNAi agent. Contacting a cell in vivo may be done, for example, via inhalation, intranasal administration, or intratracheal administration, by injecting the RNAi agent into or near the tissue where the cell is located, e.g., a pulmonary system cell, or by injecting the RNAi agent into another area, or to the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the RNAi agent may contain or be coupled to a ligand, e.g., a lipophilic moiety or moieties as described below and further detailed, e.g., in PCT Publication No. WO 2019/217459, which is incorporated herein by reference, that directs or otherwise stabilizes the RNAi agent at a site of interest, e.g., the pulmonary system. In some embodiments, the RNAi agent may contain or be coupled to a ligand, e.g., one or more GalNAc derivatives as described below, that directs or otherwise stabilizes the RNAi agent at a site of interest, e.g., the liver. In other embodiments, the RNAi agent may contain or be coupled to a lipophilic moiety or moieties and one or more GalNAc derivatives. Combinations of in vitro and in vivo methods of contacting are also possible. For example, a cell may also be contacted in vitro with an RNAi agent and subsequently transplanted into a subject.

(98) In one embodiment, contacting a cell with an RNAi agent includes “introducing” or “delivering the RNAi agent into the cell” by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of a RNAi agent can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. Introducing a RNAi agent into a cell may be in vitro or in vivo. For example, for in vivo introduction, a RNAi agent can be injected into a tissue site or administered systemically. In vitro introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or are known in the art.

(99) The term “lipophile” or “lipophilic moiety” broadly refers to any compound or chemical moiety having an affinity for lipids. One way to characterize the lipophilicity of the lipophilic moiety is by the octanol-water partition coefficient, log K_{sub.ow}, where K_{sub.ow} is the ratio of a chemical's concentration in the octanol-phase to its concentration in the aqueous phase of a two-phase system at equilibrium. The octanol-water partition coefficient is a laboratory-measured property of a substance. However, it may also be predicted by using coefficients attributed to the structural components of a chemical which are calculated using first-principle or empirical methods (see, for example, Tetko et al., *J. Chem. Inf Comput. Sci.* 41:1407-21 (2001), which is incorporated herein by reference in its entirety). It provides a thermodynamic measure of the tendency of the substance to prefer a non-aqueous or oily milieu rather than water (i.e. its hydrophilic/lipophilic balance). In principle, a chemical substance is lipophilic in character when its log K_{sub.ow}

exceeds 0. Typically, the lipophilic moiety possesses a log K_{sub.ow} exceeding 1, exceeding 1.5, exceeding 2, exceeding 3, exceeding 4, exceeding 5, or exceeding 10. For instance, the log K_{sub.ow} of 6-amino hexanol, for instance, is predicted to be approximately 0.7. Using the same method, the log K_{sub.ow} of cholesteryl N-(hexan-6-ol) carbamate is predicted to be 10.7.

(100) The lipophilicity of a molecule can change with respect to the functional group it carries. For instance, adding a hydroxyl group or amine group to the end of a lipophilic moiety can increase or decrease the partition coefficient (e.g., log K_{sub.ow}) value of the lipophilic moiety.

(101) Alternatively, the hydrophobicity of the double-stranded RNAi agent, conjugated to one or more lipophilic moieties, can be measured by its protein binding characteristics. For instance, in certain embodiments, the unbound fraction in the plasma protein binding assay of the double-stranded RNAi agent could be determined to positively correlate to the relative hydrophobicity of the double-stranded RNAi agent, which could then positively correlate to the silencing activity of the double-stranded RNAi agent.

(102) In one embodiment, the plasma protein binding assay determined is an electrophoretic mobility shift assay (EMSA) using human serum albumin protein. An exemplary protocol of this binding assay is illustrated in detail in, e.g., PCT Publication No. WO 2019/217459. The hydrophobicity of the double-stranded RNAi agent, measured by fraction of unbound siRNA in the binding assay, exceeds 0.15, exceeds 0.2, exceeds 0.25, exceeds 0.3, exceeds 0.35, exceeds 0.4, exceeds 0.45, or exceeds 0.5 for an enhanced in vivo delivery of siRNA.

(103) Accordingly, conjugating the lipophilic moieties to the internal position(s) of the double-stranded RNAi agent provides optimal hydrophobicity for the enhanced in vivo delivery of siRNA.

(104) The term “lipid nanoparticle” or “LNP” is a vesicle comprising a lipid layer encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, e.g., a RNAi agent or a plasmid from which a RNAi agent is transcribed. LNPs are described in, for example, U.S. Pat. Nos. 6,858,225, 6,815,432, 8,158,601, and 8,058,069, the entire contents of which are hereby incorporated herein by reference.

(105) As used herein, a “subject” is an animal, such as a mammal, including a primate (such as a human, a non-human primate, e.g., a monkey, and a chimpanzee), or a non-primate (such as a cow, a pig, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, or a mouse), or a bird that expresses the target gene, either endogenously or heterologously. In a preferred embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder, or condition that would benefit from reduction in coronavirus genome expression; a human at risk for a disease, disorder, or condition that would benefit from reduction in coronavirus genome expression; a human having a disease, disorder, or condition that would benefit from reduction in coronavirus genome expression; or human being treated for a disease, disorder, or condition that would benefit from reduction in coronavirus genome expression as described herein. In some embodiments, the subject is a female human. In other embodiments, the subject is a male human. In one embodiment, the subject is an adult subject. In another embodiment, the subject is a pediatric subject.

(106) As used herein, the terms “treating” or “treatment” refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more signs or symptoms associated with coronavirus genome expression or coronavirus protein production, e.g., a coronavirus-associated disease, e.g., viral replication. Treatment also includes a reduction of one or more sign or symptoms associated with unwanted coronavirus genome expression; diminishing the extent of unwanted coronavirus genome activation or stabilization; amelioration or palliation of unwanted coronavirus genome activation or stabilization. “Treatment” can also mean prolonging survival as compared to expected survival in the absence of treatment.

(107) The term “lower” in the context of the level of a coronavirus genome in a subject or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%,

80%, 85%, 90%, 95%, or more. In certain embodiments, a decrease is at least 20%. In certain embodiments, the decrease is at least 50% in a disease marker, e.g., protein or gene expression level. “Lower” in the context of the level of a coronavirus genome in a subject is preferably down to a level accepted as within the range of normal for an individual without such disorder. In certain embodiments, the expression of the target is normalized, i.e., decreased towards or to a level accepted as within the range of normal for an individual without such disorder, e.g., viral load, blood oxygen level, white blood cell count, kidney function, liver function. As used here, “lower” in a subject can refer to lowering of gene expression or protein production in a cell in a subject does not require lowering of expression in all cells or tissues of a subject. For example, as used herein, lowering in a subject can include lowering of gene expression or protein production or viral replication in a subject.

(108) The term “lower” can also be used in association with normalizing a symptom of a disease or condition, i.e. decreasing the difference between a level in a subject suffering from a coronavirus-associated disease towards or to a level in a normal subject not suffering from a coronavirus-associated disease. As used herein, if a disease is associated with an elevated value for a symptom, “normal” is considered to be the upper limit of normal. If a disease is associated with a decreased value for a symptom, “normal” is considered to be the lower limit of normal.

(109) As used herein, “prevention” or “preventing,” when used in reference to a disease, disorder, or condition thereof, that would benefit from a reduction in expression of a coronavirus genome or production of a coronavirus protein, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, e.g., a symptom of a coronavirus-associated disease. The failure to develop a disease, disorder, or condition, or the reduction in the development of a symptom associated with such a disease, disorder, or condition, e.g., pneumonia (e.g., by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed symptoms delayed (e.g., by days, weeks, months or years) is considered effective prevention.

(110) As used herein, the term “coronavirus-associated disease,” is a disease or disorder that is caused by, or associated with a coronavirus infection, coronavirus genome expression or coronavirus protein production. The term “coronavirus-associated disease” includes a disease, disorder or condition that would benefit from a decrease in coronavirus genome expression, replication, or protein activity. Non-limiting examples of coronavirus-associated diseases include, for example, disease or disorders caused by infection with human coronavirus 229E (HCoV-229E), human coronavirus NL63 (HCoV-NL63), human coronavirus OC43 (HCoV-OC43), human coronavirus HKU1 (HCoV-HKU1), severe acute respiratory syndrome coronavirus (SARS), the Middle East respiratory syndrome coronavirus (MERS), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or COVID-19). The symptoms for a coronavirus-associated disease depend on the type of coronavirus and how serious the infection is. Patients with a mild to moderate upper-respiratory infection may develop symptoms such as runny nose, sneezing, headache, cough, sore throat, fever, or short of breath. In more severe cases, coronavirus infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death. Further details regarding signs and symptoms of the various diseases or conditions are provided herein and are well known in the art.

(111) “Therapeutically effective amount,” as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a coronavirus-associated disease, is sufficient to effect treatment of the disease (e.g., by diminishing, ameliorating, or maintaining the existing disease or one or more symptoms of disease). The “therapeutically effective amount” may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

(112) “Prophylactically effective amount,” as used herein, is intended to include the amount of a

RNAi agent that, when administered to a subject having a coronavirus-associated disorder, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease.

Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The “prophylactically effective amount” may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

(113) A “therapeutically-effective amount” or “prophylactically effective amount” also includes an amount of a RNAi agent that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. A RNAi agent employed in the methods of the present disclosure may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

(114) The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human subjects and animal subjects without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

(115) The phrase “pharmaceutically-acceptable carrier” as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

(116) The term “sample,” as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, bronchial fluids, sputum, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from a nasal swab. In certain embodiments, samples may be derived from a throat swab. In certain embodiments, samples may be derived from the lung, or certain types of cells in the lung. In some embodiments, the samples may be derived from the bronchioles. In some embodiments, the samples may be derived from the bronchus. In some embodiments, the samples may be derived from the alveoli. In other embodiments, a “sample derived from a subject” refers to liver tissue (or subcomponents thereof) derived from the subject. In some embodiments, a “sample derived from a subject” refers to blood drawn from the subject or plasma or serum derived

therefrom. In further embodiments, a “sample derived from a subject” refers to pulmonary system tissue (or subcomponents thereof) derived from the subject.

II. RNAi Agents of the Disclosure

(117) Described herein are RNAi agents which inhibit the expression of a coronavirus genome. In one embodiment, the RNAi agent includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a coronavirus genome in a cell, such as a cell within a subject, e.g., a mammal, such as a human, e.g., a subject having a coronavirus-associated disorder, e.g., a subject having a coronavirus infection, e.g., a subject having Severe Acute Respiratory Syndrome 2 (SARS-CoV-2; COVID-19), Severe Acute Respiratory Syndrome (SARS-CoV), or Middle East Respiratory Syndrome (MERS-CoV). The dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of a target coronavirus RNA, e.g., an mRNA formed in the expression of a coronavirus genome. The region of complementarity is about 15-30 nucleotides or less in length. Upon contact with a cell expressing the coronavirus genome, the RNAi agent inhibits the expression of the coronavirus genome (e.g., a human gene, a primate gene, a non-primate gene) by at least 50% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting or flowcytometric techniques. In preferred embodiments, inhibition of expression is by at least 50% as assayed by the Dual-Glo luciferase assay in Example a where the siRNA is at a 10 nM concentration.

(118) A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. For example, the target sequence can be derived from the sequence of an mRNA formed during the expression of a coronavirus genome. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

(119) Generally, the duplex structure is 15 to 30 base pairs in length, e.g., 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. In certain preferred embodiments, the duplex structure is 18 to 25 base pairs in length, e.g., 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-25, 20-24, 20-23, 20-22, 20-21, 21-25, 21-24, 21-23, 21-22, 22-25, 22-24, 22-23, 23-25, 23-24 or 24-25 base pairs in length, for example, 19-21 basepairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

(120) Similarly, the region of complementarity to the target sequence is 15 to 30 nucleotides in length, e.g., 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, for example 19-23 nucleotides in length or 21-23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

(121) In some embodiments, the dsRNA is 15 to 23 nucleotides in length, or 25 to 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well known in the art that dsRNAs longer than about 21-23 nucleotides can serve as

substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a “part” of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (i.e., cleavage through a RISC pathway).

(122) One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, e.g., a duplex region of about 15 to 36 base pairs, e.g., 15-36, 15-35, 15-34, 15-33, 15-32, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs, for example, 19-21 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of e.g., 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, a RNAi agent useful to target coronavirus expression is not generated in the target cell by cleavage of a larger dsRNA.

(123) A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs e.g., 1, 2, 3, or 4 nucleotides. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA. In certain embodiments, longer, extended overhangs are possible.

(124) A dsRNA can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

(125) iRNA compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide strands comprising unnatural or modified nucleotides can be easily prepared. Single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

(126) An siRNA can be produced, e.g., in bulk, by a variety of methods. Exemplary methods include: organic synthesis and RNA cleavage, e.g., in vitro cleavage.

(127) An siRNA can be made by separately synthesizing a single stranded RNA molecule, or each respective strand of a double-stranded RNA molecule, after which the component strands can then be annealed.

(128) A large bioreactor, e.g., the OligoPilot II from Pharmacia Biotec AB (Uppsala Sweden), can be used to produce a large amount of a particular RNA strand for a given siRNA. The OligoPilotII reactor can efficiently couple a nucleotide using only a 1.5 molar excess of a phosphoramidite nucleotide. To make an RNA strand, ribonucleotides amidites are used. Standard cycles of monomer addition can be used to synthesize the 21 to 23 nucleotide strand for the siRNA. Typically, the two complementary strands are produced separately and then annealed, e.g., after release from the solid support and deprotection.

(129) Organic synthesis can be used to produce a discrete siRNA species. The complementary of the species to a coronavirus genome can be precisely specified. For example, the species may be complementary to a region that includes a polymorphism, e.g., a single nucleotide polymorphism. Further the location of the polymorphism can be precisely defined. In some embodiments, the

polymorphism is located in an internal region, e.g., at least 4, 5, 7, or 9 nucleotides from one or both of the termini.

(130) In one embodiment, RNA generated is carefully purified to remove endsiRNA is cleaved in vitro into siRNAs, for example, using a Dicer or comparable RNase III-based activity. For example, the dsRNA can be incubated in an in vitro extract from *Drosophila* or using purified components, e.g., a purified RNase or RISC complex (RNA-induced silencing complex). See, e.g., Ketting et al. *Genes Dev* 2001 Oct. 15; 15(20):2654-9 and Hammond *Science* 2001 Aug. 10; 293(5532):1146-50.

(131) dsRNA cleavage generally produces a plurality of siRNA species, each being a particular 21 to 23 nucleotide fragment of a source dsRNA molecule. For example, siRNAs that include sequences complementary to overlapping regions and adjacent regions of a source dsRNA molecule may be present.

(132) Regardless of the method of synthesis, the siRNA preparation can be prepared in a solution (e.g., an aqueous or organic solution) that is appropriate for formulation. For example, the siRNA preparation can be precipitated and redissolved in pure double-distilled water, and lyophilized. The dried siRNA can then be resuspended in a solution appropriate for the intended formulation process.

(133) In one aspect, a dsRNA of the disclosure includes at least two nucleotide sequences, a sense sequence and an antisense sequence. The sense strand sequence for coronavirus may be selected from the group of sequences provided in any one of Tables 2-5, and the corresponding nucleotide sequence of the antisense strand of the sense strand may be selected from the group of sequences of any one of Tables 2-5. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of a target coronavirus RNA, e.g., an mRNA generated in the expression of a coronavirus genome. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand (passenger strand) in any one of Tables 2-5, and the second oligonucleotide is described as the corresponding antisense strand (guide strand) of the sense strand in any one of Tables 2-5 for coronavirus.

(134) In certain embodiments of the invention, the sense strand or the antisense strand of the dsRNA agent is selected from the sense strand or antisense strand of a duplex selected from the group consisting of AD-1184137, AD-1184147, AD-1184150, AD-1184210, AD-1184270, AD-1184233, AD-1184271, AD-1184212, AD-1184228, AD-1184223, AD-1231490, AD-1231513, AD-1231485, AD-1231507, AD-1231471, AD-1231494, AD-1231496, and AD-1231497. In another embodiment, the sense strand or the antisense strand of the dsRNA agent is selected from the sense strand or antisense strand of a duplex selected from the group consisting of AD-1184137, AD-1184147, AD-1184150, AD-1231490, AD-1231513, AD-1231485, AD-1231471, AD-1231496, and AD-1231497. In another embodiment, the sense strand or the antisense strand of the dsRNA agent is selected from the sense strand or antisense strand of a duplex selected from the group consisting of AD-1184137 and AD-1184150. In one embodiment, the sense strand or the antisense strand of the dsRNA agent is the sense strand or antisense strand of duplex AD-1184137. In another embodiment, the sense strand or the antisense strand of the dsRNA agent is the sense strand or antisense strand of duplex AD-1184150.

(135) In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

(136) It will be understood that, although the sequences provided herein are described as modified or conjugated sequences, the RNA of the RNAi agent of the disclosure e.g., a dsRNA of the disclosure, may comprise any one of the sequences set forth in any one of Tables 2-5 that is unmodified, un-conjugated, or modified or conjugated differently than described therein. One or more lipophilic ligands or one or more GalNAc ligands can be included in any of the positions of the

RNAi agents provided in the instant application.

(137) The skilled person is well aware that dsRNAs having a duplex structure of about 20 to 23 base pairs, e.g., 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir et al., (2001) *EMBO J.*, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim et al. (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided herein, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences provided herein, and differing in their ability to inhibit the expression of a coronavirus genome by not more than 10, 15, 20, 25, or 30% inhibition from a dsRNA comprising the full sequence using the in vitro assay with Cos7 and a 10 nM concentration of the RNA agent and the PCR assay as provided in the examples herein, are contemplated to be within the scope of the present disclosure.

(138) In addition, the RNAs described herein identify a site(s) in a coronavirus transcript that is susceptible to RISC-mediated cleavage. As such, the present disclosure further features RNAi agents that target within this site(s). As used herein, a RNAi agent is said to target within a particular site of an RNA transcript if the RNAi agent promotes cleavage of the transcript anywhere within that particular site. Such a RNAi agent will generally include at least about 15 contiguous nucleotides, preferably at least 19 nucleotides, from one of the sequences provided herein coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a coronavirus genome.

(139) An RNAi agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, an RNAi agent as described herein contains no more than 3 mismatches (i.e., 3, 2, 1, or 0 mismatches). In one embodiment, an RNAi agent as described herein contains no more than 2 mismatches. In one embodiment, an RNAi agent as described herein contains no more than 1 mismatch. In one embodiment, an RNAi agent as described herein contains 0 mismatches. In certain embodiments, if the antisense strand of the RNAi agent contains mismatches to the target sequence, the mismatch can optionally be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, in such embodiments, for a 23 nucleotide RNAi agent, the strand which is complementary to a region of a coronavirus genome generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of a coronavirus genome. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a coronavirus genome is important, especially if the particular region of complementarity in a coronavirus genome is known to mutate.

III. Modified RNAi Agents of the Disclosure

(140) In one embodiment, the RNA of the RNAi agent of the disclosure e.g., a dsRNA, is unmodified, and does not comprise, e.g., chemical modifications or conjugations known in the art and described herein. In preferred embodiments, the RNA of an RNAi agent of the disclosure, e.g., a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the disclosure, substantially all of the nucleotides of an RNAi agent of the disclosure are modified. In other embodiments of the disclosure, all of the nucleotides of an RNAi agent of the disclosure are modified. RNAi agents of the disclosure in which "substantially all of the nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides. In still other embodiments of the disclosure, RNAi agents of the disclosure can include not more than 5, 4, 3, 2 or 1 modified nucleotides.

(141) The nucleic acids featured in the disclosure can be synthesized or modified by methods well

established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S. L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Modifications include, for example, end modifications, e.g., 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, etc.); base modifications, e.g., replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (e.g., at the 2'-position or 4'-position) or replacement of the sugar; or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of RNAi agents useful in the embodiments described herein include, but are not limited to, RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified RNAi agent will have a phosphorus atom in its internucleoside backbone.

(142) Modified RNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, e.g., sodium salts, mixed salts and free acid forms are also included.

(143) Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6,239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and U.S. Pat. RE39464, the entire contents of each of which are hereby incorporated herein by reference.

(144) Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

(145) Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

(146) In other embodiments, suitable RNA mimetics are contemplated for use in RNAi agents, in which both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are

replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the RNAi agents of the disclosure are described in, for example, in Nielsen et al., *Science*, 1991, 254, 1497-1500.

(147) Some embodiments featured in the disclosure include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular $\text{—CH.sub.2—NH—CH.sub.2—}$, $\text{—CH.sub.2—N(CH.sub.3)—O—CH.sub.2—}$ [known as a methylene (methylimino) or MMI backbone], $\text{—CH.sub.2—O—N(CH.sub.3)—CH.sub.2—}$, $\text{—CH.sub.2—N(CH.sub.3)—N(CH.sub.3)—CH.sub.2—}$ and $\text{—N(CH.sub.3)—CH.sub.2—CH.sub.2—}$ [wherein the native phosphodiester backbone is represented as —O—P—O—CH.sub.2—] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

(148) Modified RNAs can also contain one or more substituted sugar moieties. The RNAi agents, e.g., dsRNAs, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C.sub.1 to C.sub.10 alkyl or C.sub.2 to C.sub.10 alkenyl and alkynyl. Exemplary suitable modifications include $\text{O[(CH.sub.2).sub.nO].sub.mCH.sub.3}$, $\text{O(CH.sub.2).sub.nOCH.sub.3}$, $\text{O(CH.sub.2).sub.nNH.sub.2}$, $\text{O(CH.sub.2).sub.nCH.sub.3}$, $\text{O(CH.sub.2).sub.nONH.sub.2}$, and $\text{O(CH.sub.2).sub.nON[(CH.sub.2).sub.nCH.sub.3].sub.2}$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C.sub.1 to C.sub.10 lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH.sub.3, OCN, Cl, Br, CN, CF.sub.3, OCF.sub.3, SOCH.sub.3, SO.sub.2CH.sub.3, ONO.sub.2, NO.sub.2, N.sub.3, NH.sub.2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of a RNAi agent, or a group for improving the pharmacodynamic properties of a RNAi agent, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O—CH.sub.2CH.sub.2OCH.sub.3, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chim. Acta*, 1995, 78:486-504) i.e., an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminoethoxy, i.e., a $\text{O(CH.sub.2).sub.2ON(CH.sub.3).sub.2}$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., $\text{2'-O—CH.sub.2—O—CH.sub.2—N(CH.sub.2).sub.2}$. Further exemplary modifications include: 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

(149) Other modifications include 2'-methoxy (2'-OCH.sub.3), 2'-aminopropoxy (2'-OCH.sub.2CH.sub.2CH.sub.2NH.sub.2), 2'-O-hexadecyl, and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the RNA of a RNAi agent, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. RNAi agents can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800;

5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application. The entire contents of each of the foregoing are hereby incorporated herein by reference.

(150) An RNAi agent of the disclosure can also include nucleobase (often referred to in the art simply as “base”) modifications or substitutions. As used herein, “unmodified” or “natural” nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in *Modified Nucleosides in Biochemistry, Biotechnology and Medicine*, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J. L., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., (1991) *Angewandte Chemie, International Edition*, 30:613, and those disclosed by Sanghvi, Y S., Chapter 15, *dsRNA Research and Applications*, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the disclosure. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., *dsRNA Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

(151) Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

(152) An RNAi agent of the disclosure can also be modified to include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. This structure effectively “locks” the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. et al., (2005) *Nucleic Acids Research* 33(1):439-447; Mook, O R. et al., (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. et al., (2003) *Nucleic Acids Research* 31(12):3185-3193).

(153) An RNAi agent of the disclosure can also be modified to include one or more bicyclic sugar moieties. A “bicyclic sugar” is a furanosyl ring modified by the bridging of two atoms. A “bicyclic nucleoside” (“BNA”) is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring. Thus, in some embodiments an agent of the disclosure may include one or more locked nucleic acids (LNA). A locked nucleic acid

is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH₂-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. et al., (2005) *Nucleic Acids Research* 33(1):439-447; Mook, O R. et al., (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. et al., (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the disclosure include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the disclosure include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to 4'-(CH₂)-O-2' (LNA); 4'-(CH₂)-S-2'; 4'-(CH₂)₂-O-2' (ENA); 4'-CH(CH₃)-O-2' (also referred to as "constrained ethyl" or "cEt") and 4'-CH(CH₂OCH₃)-O-2' (and analogs thereof; see, e.g., U.S. Pat. No. 7,399,845); 4'-C(CH₃)(CH₃)-O-2' (and analogs thereof; see e.g., U.S. Pat. No. 8,278,283); 4'-CH₂-N(OCH₃)-2' (and analogs thereof; see e.g., U.S. Pat. No. 8,278,425); 4'-CH₂-O-N(CH₃)-2' (see, e.g., U.S. Patent Publication No. 2004/0171570); 4'-CH₂-N(R)—O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (see, e.g., U.S. Pat. No. 7,427,672); 4'-CH₂-C(H)(CH₃)-2' (see, e.g., Chattopadhyaya et al., *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂-C(=CH₂)-2' (and analogs thereof; see, e.g., U.S. Pat. No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

(154) Additional representative US Patents and US Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Pat. Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133; 7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

(155) Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α -L-ribofuranose and β -D-ribofuranose (see WO 99/14226).

(156) An RNAi agent of the disclosure can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)-O-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

(157) An RNAi agent of the disclosure may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2' and C4' carbons of ribose or the -C3' and -C5' carbons of ribose. CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

(158) Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, US 2013/0190383; and WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

(159) In some embodiments, a RNAi agent of the disclosure comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'-C4' have been removed (i.e. the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (i.e. the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed (see *Nuc. Acids Symp. Series*, 52, 133-134 (2008) and Fluiter et al., *Mol. Biosyst.*, 2009, 10, 1039 hereby incorporated by reference).

(160) Representative U.S. publications that teach the preparation of UNA include, but are not limited to, U.S. Pat. No. 8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

(161) Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3''-phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in WO 2011/005861.

(162) Other modifications of a RNAi agent of the disclosure include a 5' phosphate or 5' phosphate mimic, e.g., a 5'-terminal phosphate or phosphate mimic on the antisense strand of a RNAi agent. Suitable phosphate mimics are disclosed in, for example US 2012/0157511, the entire contents of which are incorporated herein by reference.

(163) A. Modified RNAi agents Comprising Motifs of the Disclosure

(164) In certain aspects of the disclosure, the double-stranded RNAi agents of the disclosure include agents with chemical modifications as disclosed, for example, in WO 2013/075035, the entire contents of which are incorporated herein by reference. As shown herein and in WO 2013/075035, a superior result may be obtained by introducing one or more motifs of three identical modifications on three consecutive nucleotides into a sense strand or antisense strand of an RNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the RNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The RNAi agent may be optionally conjugated with a lipophilic ligand, e.g., a C16 ligand, for instance on the sense strand. The RNAi agent may be optionally modified with a (S)-glycol nucleic acid (GNA) modification, for instance on one or more residues of the antisense strand. The resulting RNAi agents present superior gene silencing activity.

(165) Accordingly, the disclosure provides double stranded RNAi agents capable of inhibiting the expression of a target genome or gene (i.e., a coronavirus genome or gene) in vivo. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may be 15-30 nucleotides in length. For example, each strand may be 16-30 nucleotides in length, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 17-23 nucleotides in length, 17-21 nucleotides in length, 17-19 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length. In certain embodiments, each strand is 19-23 nucleotides in length.

(166) The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as an "RNAi agent." The duplex region of an RNAi agent may be 15-30 nucleotide pairs in length. For example, the duplex region can be 16-30 nucleotide pairs in length, 17-30 nucleotide pairs in length, 27-30 nucleotide pairs in length, 17-23 nucleotide pairs in length, 17-21 nucleotide pairs in length, 17-19 nucleotide pairs in length, 19-25 nucleotide pairs in length, 19-23 nucleotide pairs in length, 19-21 nucleotide pairs in length, 21-25 nucleotide pairs in length, or 21-23 nucleotide pairs in length. In another example, the duplex region is selected from 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27 nucleotides in length. In preferred embodiments, the duplex region is 19-21 nucleotide pairs in length.

(167) In one embodiment, the RNAi agent may contain one or more overhang regions or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5 nucleotides in length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. In preferred embodiments, the nucleotide overhang region is 2 nucleotides in length. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang

can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence. The first and second strands can also be joined, e.g., by additional bases to form a hairpin, or by other non-base linkers.

(168) In one embodiment, the nucleotides in the overhang region of the RNAi agent can each independently be a modified or unmodified nucleotide including, but not limited to 2'-sugar modified, such as, 2-F, 2'-O-methyl, thymidine (T), and any combinations thereof.

(169) For example, TT can be an overhang sequence for either end on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence.

(170) The 5'- or 3'-overhangs at the sense strand, antisense strand or both strands of the RNAi agent may be phosphorylated. In some embodiments, the overhang region(s) contains two nucleotides having a phosphorothioate between the two nucleotides, where the two nucleotides can be the same or different. In one embodiment, the overhang is present at the 3'-end of the sense strand, antisense strand, or both strands. In one embodiment, this 3'-overhang is present in the antisense strand. In one embodiment, this 3'-overhang is present in the sense strand.

(171) The RNAi agent may contain only a single overhang, which can strengthen the interference activity of the RNAi, without affecting its overall stability. For example, the single-stranded overhang may be located at the 3-terminal end of the sense strand or, alternatively, at the 3-terminal end of the antisense strand. The RNAi may also have a blunt end, located at the 5'-end of the antisense strand (or the 3'-end of the sense strand) or vice versa. Generally, the antisense strand of the RNAi has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. While not wishing to be bound by theory, the asymmetric blunt end at the 5'-end of the antisense strand and 3'-end overhang of the antisense strand favor the guide strand loading into RISC process.

(172) In one embodiment, the RNAi agent is a double ended bluntmer of 19 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 7, 8, 9 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

(173) In another embodiment, the RNAi agent is a double ended bluntmer of 20 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 8, 9, 10 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

(174) In yet another embodiment, the RNAi agent is a double ended bluntmer of 21 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

(175) In one embodiment, the RNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang. Preferably, the 2 nucleotide overhang is at the 3'-end of the antisense strand. When the 2 nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In one

embodiment, every nucleotide in the sense strand and the antisense strand of the RNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In one embodiment each residue is independently modified with a 2'-O-methyl or 3'-fluoro, e.g., in an alternating motif. Optionally, the RNAi agent further comprises a ligand (e.g., a lipophilic ligand, optionally a C16 ligand).

(176) In one embodiment, the RNAi agent comprises a sense and an antisense strand, wherein the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1) positions 1 to 23 of the first strand comprise at least 8 ribonucleotides; the antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, comprises at least 8 ribonucleotides in the positions paired with positions 1-23 of sense strand to form a duplex; wherein at least the 3' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the double stranded nucleic acid is introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

(177) In one embodiment, the RNAi agent comprises sense and antisense strands, wherein the RNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11, 12, 13 from the 5' end; wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region which is at least 25 nucleotides in length, and the second strand is sufficiently complementary to a target mRNA along at least 19 nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein dicer cleavage of the RNAi agent preferentially results in an siRNA comprising the 3' end of the second strand, thereby reducing expression of the target gene in the mammal. Optionally, the RNAi agent further comprises a ligand.

(178) In one embodiment, the sense strand of the RNAi agent contains at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at the cleavage site in the sense strand.

(179) In one embodiment, the antisense strand of the RNAi agent can also contain at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at or near the cleavage site in the antisense strand.

(180) For an RNAi agent having a duplex region of 17-23 nucleotide in length, the cleavage site of the antisense strand is typically around the 10, 11 and 12 positions from the 5'-end. Thus the motifs of three identical modifications may occur at the 9, 10, 11 positions; 10, 11, 12 positions; 11, 12, 13 positions; 12, 13, 14 positions; or 13, 14, 15 positions of the antisense strand, the count starting from the 1st nucleotide from the 5'-end of the antisense strand, or, the count starting from the 1st paired nucleotide within the duplex region from the 5'-end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the RNAi from the 5'-end.

(181) The sense strand of the RNAi agent may contain at least one motif of three identical modifications on three consecutive nucleotides at the cleavage site of the strand; and the antisense strand may have at least one motif of three identical modifications on three consecutive nucleotides at or near the cleavage site of the strand. When the sense strand and the antisense strand form a dsRNA duplex, the sense strand and the antisense strand can be so aligned that one motif of the three nucleotides on the sense strand and one motif of the three nucleotides on the antisense strand have at least one nucleotide overlap, i.e., at least one of the three nucleotides of the motif in the sense strand forms a base pair with at least one of the three nucleotides of the motif in the antisense strand. Alternatively, at least two nucleotides may overlap, or all three nucleotides may overlap.

(182) In one embodiment, the sense strand of the RNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides. The first motif may occur at or near the cleavage site of the strand and the other motifs may be a wing modification. The term “wing modification” herein refers to a motif occurring at another portion of the strand that is separated from the motif at or near the cleavage site of the same strand. The wing modification is either adjacent to the first motif or is separated by at least one or more nucleotides. When the motifs are immediately adjacent to each other then the chemistry of the motifs are distinct from each other and when the motifs are separated by one or more nucleotide then the chemistries can be the same or different. Two or more wing modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

(183) Like the sense strand, the antisense strand of the RNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides, with at least one of the motifs occurring at or near the cleavage site of the strand. This antisense strand may also contain one or more wing modifications in an alignment similar to the wing modifications that may be present on the sense strand.

(184) In one embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end or both ends of the strand.

(185) In another embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two paired nucleotides within the duplex region at the 3'-end, 5'-end or both ends of the strand.

(186) When the sense strand and the antisense strand of the RNAi agent each contain at least one wing modification, the wing modifications may fall on the same end of the duplex region, and have an overlap of one, two or three nucleotides.

(187) When the sense strand and the antisense strand of the RNAi agent each contain at least two wing modifications, the sense strand and the antisense strand can be so aligned that two modifications each from one strand fall on one end of the duplex region, having an overlap of one, two or three nucleotides; two modifications each from one strand fall on the other end of the duplex region, having an overlap of one, two or three nucleotides; two modifications one strand fall on each side of the lead motif, having an overlap of one, two, or three nucleotides in the duplex region.

(188) In one embodiment, the RNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch may occur in the overhang region or the duplex region. The base pair may be ranked on the basis of their propensity to promote dissociation or melting (e.g., on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, e.g., non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

(189) In one embodiment, the RNAi agent comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'-end of the antisense strand independently selected from the group of: A:U, G:U, I:C, and mismatched pairs, e.g., non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

(190) In one embodiment, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from the group consisting of A, dA, dU, U, and dT. Alternatively, at least one of the first 1, 2 or 3 base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair.

(191) In another embodiment, the nucleotide at the 3'-end of the sense strand is deoxy-thymine (dT). In another embodiment, the nucleotide at the 3'-end of the antisense strand is deoxy-thymine (dT). In one embodiment, there is a short sequence of deoxy-thymine nucleotides, for example, two dT nucleotides on the 3'-end of the sense or antisense strand.

(192) In one embodiment, the sense strand sequence may be represented by formula (I):

$$5'n.sub.p-N.sub.a-(XXX).sub.i-N.sub.b-YYY-N.sub.b-(ZZZ).sub.j-N.sub.a-n.sub.q3' \quad (I)$$
wherein: i and j are each independently 0 or 1; p and q are each independently 0-6; each N.sub.a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides; each N.sub.b independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides; each n.sub.p and n.sub.q independently represent an overhang nucleotide; wherein Nb and Y do not have the same modification; and XXX, YYY and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. Preferably YYY is all 2'-F modified nucleotides.

(193) In one embodiment, the N.sub.a or N.sub.b comprise modifications of alternating pattern.

(194) In one embodiment, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (e.g.: can occur at positions 6, 7, 8, 7, 8, 9, 8, 9, 10, 9, 10, 11, 10, 11, 12 or 11, 12, 13) of—the sense strand, the count starting from the 1.sup.st nucleotide, from the 5'-end; or optionally, the count starting at the 1.sup.st paired nucleotide within the duplex region, from the 5'-end.

(195) In one embodiment, i is 1 and j is 0, or i is 0 and j is 1, or both i and j are 1. The sense strand can therefore be represented by the following formulas:

$$5'n.sub.p-N.sub.a-YYY-N.sub.b-ZZZ-N.sub.a-n.sub.q3' \quad (Ib);$$

$$5'n.sub.p-N.sub.a-XXX-N.sub.b-YYY-N.sub.a-n.sub.q3' \quad (Ic); \text{ or}$$

$$5'n.sub.p-N.sub.a-XXX-N.sub.b-YYY-N.sub.b-ZZZ-N.sub.a-n.sub.q3' \quad (Id).$$

(196) When the sense strand is represented by formula (Ib), N.sub.b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides.

(197) Each N.sub.a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

(198) When the sense strand is represented as formula (Ic), N.sub.b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N.sub.a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

(199) When the sense strand is represented as formula (Id), each N.sub.b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Preferably, N.sub.b is 0, 1, 2, 3, 4, 5 or 6. Each N.sub.a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

(200) Each of X, Y and Z may be the same or different from each other.

(201) In other embodiments, i is 0 and j is 0, and the sense strand may be represented by the formula:

5'n.sub.p'-N.sub.a'-YYY-N.sub.a-n.sub.q3' (Ia).

(202) When the sense strand is represented by formula (Ia), each N.sub.a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

(203) In one embodiment, the antisense strand sequence of the RNAi may be represented by formula (II):

5'n.sub.q'-N.sub.a'-(Z'Z'Z').sub.k-N.sub.b'-Y'Y'Y'-N.sub.b'X'X'X').sub.l-N'.sub.a-n.sub.p'3'

(II) wherein: k and l are each independently 0 or 1; p' and q' are each independently 0-6;

each N.sub.a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N.sub.b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n.sub.p' and n.sub.q' independently represent an overhang nucleotide;

wherein N.sub.b' and Y' do not have the same modification; and X'X'X', Y'Y'Y' and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

(204) In one embodiment, the N.sub.a' or N.sub.b' comprise modifications of alternating pattern.

(205) The Y'Y'Y' motif occurs at or near the cleavage site of the antisense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotide in length, the Y'Y'Y' motif can occur at positions 9, 10, 11; 10, 11, 12; 11, 12, 13; 12, 13, 14; or 13, 14, 15 of the antisense strand, with the count starting from the 1.sup.st nucleotide, from the 5'-end; or optionally, the count starting at the 1.sup.st paired nucleotide within the duplex region, from the 5'-end. Preferably, the Y'Y'Y' motif occurs at positions 11, 12, 13.

(206) In one embodiment, Y'Y'Y' motif is all 2'-OMe modified nucleotides.

(207) In one embodiment, k is 1 and l is 0, or k is 0 and l is 1, or both k and l are 1.

(208) The antisense strand can therefore be represented by the following formulas:

5'n.sub.q'-N.sub.a'-Z'Z'Z'-N.sub.b'-Y'Y'Y'-N.sub.a'-n.sub.p'3' (IIb);

5'n.sub.q'-N.sub.a'-Y'Y'Y'-N.sub.b'-X'X'X'-n.sub.p'3' (IIc); or

5'n.sub.q'-N.sub.a'-Z'Z'Z'-N.sub.b'-Y'Y'Y'-N.sub.b'-X'X'X'-N.sub.a'-n.sub.p'3' (IId).

(209) When the antisense strand is represented by formula (IIb), N.sub.b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides.

Each N.sub.a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

(210) When the antisense strand is represented as formula (IIc), N.sub.b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides.

Each N.sub.a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

(211) When the antisense strand is represented as formula (IId), each N.sub.b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N.sub.a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Preferably, N.sub.b is 0, 1, 2, 3, 4, 5 or 6.

(212) In other embodiments, k is 0 and l is 0 and the antisense strand may be represented by the formula:

5'n.sub.p'-N.sub.a'-Y'Y'Y'-N.sub.a'-n.sub.q'3' (Ia).

(213) When the antisense strand is represented as formula (IIa), each N.sub.a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

(214) Each of X', Y' and Z' may be the same or different from each other.

(215) Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-hydroxyl, or 2'-fluoro.

For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X, Y, Z, X', Y' and Z', in particular, may represent a 2'-O-

methyl modification or a 2'-fluoro modification.

(216) In one embodiment, the sense strand of the RNAi agent may contain YYY motif occurring at 9, 10 and 11 positions of the strand when the duplex region is 21 nt, the count starting from the 1.sup.st nucleotide from the 5'-end, or optionally, the count starting at the 1.sup.st paired nucleotide within the duplex region, from the 5'-end; and Y represents 2'-F modification. The sense strand may additionally contain XXX motif or ZZZ motifs as wing modifications at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-OMe modification or 2'-F modification.

(217) In one embodiment the antisense strand may contain Y'Y'Y' motif occurring at positions 11, 12, 13 of the strand, the count starting from the 1.sup.st nucleotide from the 5'-end, or optionally, the count starting at the 1.sup.st paired nucleotide within the duplex region, from the 5'-end; and Y' represents 2'-O-methyl modification. The antisense strand may additionally contain X'X'X' motif or Z'Z'Z' motifs as wing modifications at the opposite end of the duplex region; and X'X'X' and Z'Z'Z' each independently represents a 2'-OMe modification or 2'-F modification.

(218) The sense strand represented by any one of the above formulas (Ia), (Ib), (Ic), and (Id) forms a duplex with a antisense strand being represented by any one of formulas (IIa), (IIb), (IIc), and (IId), respectively.

(219) Accordingly, the RNAi agents for use in the methods of the disclosure may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the RNAi duplex represented by formula (III):

sense: 5'n.sub.p-N.sub.a-(XXX).sub.i-N.sub.b-YYY-N.sub.b-(ZZZ).sub.j-N.sub.b-n.sub.q3'
antisense: 3'n.sub.p'-N.sub.a'-(X'X'X').sub.k-N.sub.b'-Y'Y'Y'-N.sub.b'-(Z'Z'Z').sub.lN.sub.a'-
n.sub.q'5' (III) wherein: i, j, k, and l are each independently 0 or 1; p, p', q, and q' are each independently 0-6; each N.sub.a and N.sub.a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides; each N.sub.b and N.sub.b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides; wherein each n.sub.p', n.sub.p, n.sub.q', and n.sub.q, each of which may or may not be present, independently represents an overhang nucleotide; and XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

(220) In one embodiment, i is 0 and j is 0; or i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 0; or both i and j are 1. In another embodiment, k is 0 and l is 0; or k is 1 and l is 0; k is 0 and l is 1; or both k and l are 0; or both k and l are 1.

(221) Exemplary combinations of the sense strand and antisense strand forming a RNAi duplex include the formulas below:

5'n.sub.p-N.sub.a-YYY-N.sub.a-n.sub.q3'

3'n.sub.p'-N.sub.a'-Y'Y'Y'-N.sub.a'n.sub.q'5' (IIIa)

5'n.sub.p-N.sub.a-YYY-N.sub.b-ZZZ-N.sub.a-n.sub.q3'

3'n.sub.p'-N.sub.a'-Y'Y'Y'-N.sub.b'-Z'Z'Z'-N.sub.a'n.sub.q'5' (IIIb)

5'n.sub.p-N.sub.a-XXX-N.sub.b-YYY-N.sub.a-n.sub.q3'

3'n.sub.p'-N.sub.a'-X'X'X'-N.sub.b'-Y'Y'Y'-N.sub.a'-n.sub.q'5' (IIc)

5'n.sub.p-N.sub.a-XXX-N.sub.b-YYY-N.sub.b-ZZZ-N.sub.a-n.sub.q3'

3'n.sub.p'-N.sub.a'-X'X'X'-N.sub.b'-Y'Y'Y'-N.sub.b'-Z'Z'Z'-N.sub.a-n.sub.q'5' (IIId)

(222) When the RNAi agent is represented by formula (IIIa), each N.sub.a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

(223) When the RNAi agent is represented by formula (IIIb), each N.sub.b independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5 or 1-4 modified nucleotides.

Each N.sub.a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

(224) When the RNAi agent is represented as formula (IIId), each N.sub.b, N.sub.b' independently

represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N.sub.a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

(225) When the RNAi agent is represented as formula (IIId), each N.sub.b, N.sub.b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N.sub.a, N.sub.a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of N.sub.a, N.sub.a', N.sub.b and N.sub.b' independently comprises modifications of alternating pattern.

(226) In one embodiment, when the RNAi agent is represented by formula (IIId), the N.sub.a modifications are 2'-O-methyl or 2'-fluoro modifications. In another embodiment, when the RNAi agent is represented by formula (IIId), the N.sub.a modifications are 2'-O-methyl or 2'-fluoro modifications and n.sub.p>0 and at least one n.sub.p' is linked to a neighboring nucleotide a via phosphorothioate linkage. In yet another embodiment, when the RNAi agent is represented by formula (IIId), the N.sub.a modifications are 2'-O-methyl or 2'-fluoro modifications, n.sub.p>0 and at least one n.sub.p' is linked to a neighboring nucleotide via phosphorothioate linkage, and the sense strand is conjugated to one or more C16 (or related) moieties attached through a bivalent or trivalent branched linker (described below). In another embodiment, when the RNAi agent is represented by formula (IIId), the N.sub.a modifications are 2'-O-methyl or 2'-fluoro modifications, n.sub.p>0 and at least one n.sub.p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more lipophilic, e.g., C16 (or related) moieties, optionally attached through a bivalent or trivalent branched linker.

(227) In one embodiment, when the RNAi agent is represented by formula (IIIa), the N.sub.a modifications are 2'-O-methyl or 2'-fluoro modifications, n.sub.p>0 and at least one n.sub.p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more lipophilic, e.g., C16 (or related) moieties attached through a bivalent or trivalent branched linker.

(228) In one embodiment, the RNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb), (IIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

(229) In one embodiment, the RNAi agent is a multimer containing three, four, five, six or more duplexes represented by formula (III), (IIIa), (IIIb), (IIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

(230) In one embodiment, two RNAi agents represented by formula (III), (IIIa), (IIIb), (IIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends and are optionally conjugated to a ligand. Each of the agents can target the same gene or two different genes; or each of the agents can target same gene at two different target sites.

(231) Various publications describe multimeric RNAi agents that can be used in the methods of the disclosure. Such publications include WO2007/091269, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520; and U.S. Pat. No. 7,858,769, the entire contents of each of which are hereby incorporated herein by reference.

(232) In certain embodiments, the compositions and methods of the disclosure include a vinyl phosphonate (VP) modification of an RNAi agent as described herein. In exemplary embodiments, a vinyl phosphonate of the disclosure has the following structure:

(233) ##STR00001##

A vinyl phosphonate of the instant disclosure may be attached to either the antisense or the sense

strand of a dsRNA of the disclosure. In certain preferred embodiments, a vinyl phosphonate of the instant disclosure is attached to the antisense strand of a dsRNA, optionally at the 5' end of the antisense strand of the dsRNA.

(234) Vinyl phosphate modifications are also contemplated for the compositions and methods of the instant disclosure. An exemplary vinyl phosphate structure is:

(235) ##STR00002##

E. Thermally Destabilizing Modifications

(236) In certain embodiments, a dsRNA molecule can be optimized for RNA interference by incorporating thermally destabilizing modifications in the seed region of the antisense strand (i.e., at positions 2-9 of the 5'-end of the antisense strand) to reduce or inhibit off-target gene silencing. It has been discovered that dsRNAs with an antisense strand comprising at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5' end, of the antisense strand have reduced off-target gene silencing activity. Accordingly, in some embodiments, the antisense strand comprises at least one (e.g., one, two, three, four, five or more) thermally destabilizing modification of the duplex within the first 9 nucleotide positions of the 5' region of the antisense strand. In some embodiments, one or more thermally destabilizing modification(s) of the duplex is/are located in positions 2-9, or preferably positions 4-8, from the 5'-end of the antisense strand. In some further embodiments, the thermally destabilizing modification(s) of the duplex is/are located at position 6, 7 or 8 from the 5'-end of the antisense strand. In still some further embodiments, the thermally destabilizing modification of the duplex is located at position 7 from the 5'-end of the antisense strand. The term "thermally destabilizing modification(s)" includes modification(s) that would result with a dsRNA with a lower overall melting temperature (T_m) (preferably a T_m with one, two, three or four degrees lower than the T_m of the dsRNA without having such modification(s)). In some embodiments, the thermally destabilizing modification of the duplex is located at position 2, 3, 4, 5 or 9 from the 5'-end of the antisense strand.

(237) The thermally destabilizing modifications can include, but are not limited to, abasic modification; mismatch with the opposing nucleotide in the opposing strand; and sugar modification such as 2'-deoxy modification or acyclic nucleotide, e.g., unlocked nucleic acids (UNA) or glycol nucleic acid (GNA).

(238) Exemplified abasic modifications include, but are not limited to the following:

(239) ##STR00003##

Wherein $R=H$, Me, Et or OMe; $R' H$, Me, Et or OMe; $R''=H$, Me, Et or OMe

(240) ##STR00004##

wherein B is a modified or unmodified nucleobase.

(241) Exemplified sugar modifications include, but are not limited to the following:

(242) ##STR00005##

wherein B is a modified or unmodified nucleobase.

(243) In some embodiments the thermally destabilizing modification of the duplex is selected from the group consisting of:

(244) ##STR00006##

wherein B is a modified or unmodified nucleobase and the asterisk on each structure represents either R, S or racemic.

(245) The term "acyclic nucleotide" refers to any nucleotide having an acyclic ribose sugar, for example, where any of bonds between the ribose carbons (e.g., C1'-C2', C2'-C3', C3'-C4', C4'-O4', or C1'-O4') is absent or at least one of ribose carbons or oxygen (e.g., C1', C2', C3', C4', or O4') are independently or in combination absent from the nucleotide. In some embodiments, acyclic nucleotide is

(246) ##STR00007##

wherein B is a modified or unmodified nucleobase, $R_{sup.1}$ and $R_{sup.2}$ independently are H,

halogen, OR.sub.3, or alkyl; and R.sub.3 is H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar). The term “UNA” refers to unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked “sugar” residue. In one example, UNA also encompasses monomers with bonds between C1'-C4' being removed (i.e. the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (i.e. the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar is removed (see Mikhailov et. al., Tetrahedron Letters, 26 (17): 2059 (1985); and Fluiter et al., Mol. Biosyst., 10: 1039 (2009), which are hereby incorporated by reference in their entirety). The acyclic derivative provides greater backbone flexibility without affecting the Watson-Crick pairings. The acyclic nucleotide can be linked via 2'-5' or 3'-5' linkage.

(247) The term ‘GNA’ refers to glycol nucleic acid which is a polymer similar to DNA or RNA but differing in the composition of its “backbone” in that is composed of repeating glycerol units linked by phosphodiester bonds:

(248) ##STR00008##

(249) The thermally destabilizing modification of the duplex can be mismatches (i.e., noncomplementary base pairs) between the thermally destabilizing nucleotide and the opposing nucleotide in the opposite strand within the dsRNA duplex. Exemplary mismatch base pairs include G:G, G:A, G:U, G:T, A:A, A:C, C:C, C:U, C:T, U:U, T:T, U:T, or a combination thereof. Other mismatch base pairings known in the art are also amenable to the present invention. A mismatch can occur between nucleotides that are either naturally occurring nucleotides or modified nucleotides, i.e., the mismatch base pairing can occur between the nucleobases from respective nucleotides independent of the modifications on the ribose sugars of the nucleotides. In certain embodiments, the dsRNA molecule contains at least one nucleobase in the mismatch pairing that is a 2'-deoxy nucleobase; e.g., the 2'-deoxy nucleobase is in the sense strand.

(250) In some embodiments, the thermally destabilizing modification of the duplex in the seed region of the antisense strand includes nucleotides with impaired W—C H-bonding to complementary base on the target mRNA, such as:

(251) ##STR00009##

(252) More examples of abasic nucleotide, acyclic nucleotide modifications (including UNA and GNA), and mismatch modifications have been described in detail in WO 2011/133876, which is herein incorporated by reference in its entirety.

(253) The thermally destabilizing modifications may also include universal base with reduced or abolished capability to form hydrogen bonds with the opposing bases, and phosphate modifications.

(254) In some embodiments, the thermally destabilizing modification of the duplex includes nucleotides with non-canonical bases such as, but not limited to, nucleobase modifications with impaired or completely abolished capability to form hydrogen bonds with bases in the opposite strand. These nucleobase modifications have been evaluated for destabilization of the central region of the dsRNA duplex as described in WO 2010/0011895, which is herein incorporated by reference in its entirety. Exemplary nucleobase modifications are:

(255) ##STR00010##

(256) In some embodiments, the thermally destabilizing modification of the duplex in the seed region of the antisense strand includes one or more α -nucleotide complementary to the base on the target mRNA, such as:

(257) ##STR00011##

wherein R is H, OH, OCH.sub.3, F, NH.sub.2, NHMe, NMe.sub.2 or O-alkyl.

(258) Exemplary phosphate modifications known to decrease the thermal stability of dsRNA duplexes compared to natural phosphodiester linkages are:

(259) ##STR00012## R=alkyl

(260) The alkyl for the R group can be a C.sub.1-C.sub.6alkyl. Specific alkyls for the R group

include, but are not limited to methyl, ethyl, propyl isopropyl, butyl, pentyl and hexyl.

(261) As the skilled artisan will recognize, in view of the functional role of nucleobases is defining specificity of a RNAi agent of the disclosure, while nucleobase modifications can be performed in the various manners as described herein, e.g., to introduce destabilizing modifications into a RNAi agent of the disclosure, e.g., for purpose of enhancing on-target effect relative to off-target effect, the range of modifications available and, in general, present upon RNAi agents of the disclosure tends to be much greater for non-nucleobase modifications, e.g., modifications to sugar groups or phosphate backbones of polyribonucleotides. Such modifications are described in greater detail in other sections of the instant disclosure and are expressly contemplated for RNAi agents of the disclosure, either possessing native nucleobases or modified nucleobases as described above or elsewhere herein.

(262) In addition to the antisense strand comprising a thermally destabilizing modification, the dsRNA can also comprise one or more stabilizing modifications. For example, the dsRNA can comprise at least two (e.g., two, three, four, five, six, seven, eight, nine, ten or more) stabilizing modifications. Without limitations, the stabilizing modifications all can be present in one strand. In some embodiments, both the sense and the antisense strands comprise at least two stabilizing modifications. The stabilizing modification can occur on any nucleotide of the sense strand or antisense strand. For instance, the stabilizing modification can occur on every nucleotide on the sense strand or antisense strand; each stabilizing modification can occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand comprises both stabilizing modification in an alternating pattern. The alternating pattern of the stabilizing modifications on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the stabilizing modifications on the sense strand can have a shift relative to the alternating pattern of the stabilizing modifications on the antisense strand.

(263) In some embodiments, the antisense strand comprises at least two (e.g., two, three, four, five, six, seven, eight, nine, ten or more) stabilizing modifications. Without limitations, a stabilizing modification in the antisense strand can be present at any positions. In some embodiments, the antisense comprises stabilizing modifications at positions 2, 6, 8, 9, 14, and 16 from the 5'-end. In some other embodiments, the antisense comprises stabilizing modifications at positions 2, 6, 14, and 16 from the 5'-end. In still some other embodiments, the antisense comprises stabilizing modifications at positions 2, 14, and 16 from the 5'-end.

(264) In some embodiments, the antisense strand comprises at least one stabilizing modification adjacent to the destabilizing modification. For example, the stabilizing modification can be the nucleotide at the 5'-end or the 3'-end of the destabilizing modification, i.e., at position -1 or +1 from the position of the destabilizing modification. In some embodiments, the antisense strand comprises a stabilizing modification at each of the 5'-end and the 3'-end of the destabilizing modification, i.e., positions -1 and +1 from the position of the destabilizing modification.

(265) In some embodiments, the antisense strand comprises at least two stabilizing modifications at the 3'-end of the destabilizing modification, i.e., at positions +1 and +2 from the position of the destabilizing modification.

(266) In some embodiments, the sense strand comprises at least two (e.g., two, three, four, five, six, seven, eight, nine, ten or more) stabilizing modifications. Without limitations, a stabilizing modification in the sense strand can be present at any positions. In some embodiments, the sense strand comprises stabilizing modifications at positions 7, 10, and 11 from the 5'-end. In some other embodiments, the sense strand comprises stabilizing modifications at positions 7, 9, 10, and 11 from the 5'-end. In some embodiments, the sense strand comprises stabilizing modifications at positions opposite or complimentary to positions 11, 12, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some other embodiments, the sense strand comprises stabilizing modifications at positions opposite or complimentary to positions 11, 12, 13, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some embodiments, the

sense strand comprises a block of two, three, or four stabilizing modifications.

(267) In some embodiments, the sense strand does not comprise a stabilizing modification in position opposite or complimentary to the thermally destabilizing modification of the duplex in the antisense strand.

(268) Exemplary thermally stabilizing modifications include, but are not limited to, 2'-fluoro modifications. Other thermally stabilizing modifications include, but are not limited to, LNA.

(269) In some embodiments, the dsRNA of the disclosure comprises at least four (e.g., four, five, six, seven, eight, nine, ten, or more) 2'-fluoro nucleotides. Without limitations, the 2'-fluoro nucleotides all can be present in one strand. In some embodiments, both the sense and the antisense strands comprise at least two 2'-fluoro nucleotides. The 2'-fluoro modification can occur on any nucleotide of the sense strand or antisense strand. For instance, the 2'-fluoro modification can occur on every nucleotide on the sense strand or antisense strand; each 2'-fluoro modification can occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand comprises both 2'-fluoro modifications in an alternating pattern. The alternating pattern of the 2'-fluoro modifications on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the 2'-fluoro modifications on the sense strand can have a shift relative to the alternating pattern of the 2'-fluoro modifications on the antisense strand.

(270) In some embodiments, the antisense strand comprises at least two (e.g., two, three, four, five, six, seven, eight, nine, ten, or more) 2'-fluoro nucleotides. Without limitations, a 2'-fluoro modification in the antisense strand can be present at any positions. In some embodiments, the antisense comprises 2'-fluoro nucleotides at positions 2, 6, 8, 9, 14, and 16 from the 5'-end. In some other embodiments, the antisense comprises 2'-fluoro nucleotides at positions 2, 6, 14, and 16 from the 5'-end. In still some other embodiments, the antisense comprises 2'-fluoro nucleotides at positions 2, 14, and 16 from the 5'-end.

(271) In some embodiments, the antisense strand comprises at least one 2'-fluoro nucleotide adjacent to the destabilizing modification. For example, the 2'-fluoro nucleotide can be the nucleotide at the 5'-end or the 3'-end of the destabilizing modification, i.e., at position -1 or +1 from the position of the destabilizing modification. In some embodiments, the antisense strand comprises a 2'-fluoro nucleotide at each of the 5'-end and the 3'-end of the destabilizing modification, i.e., positions -1 and +1 from the position of the destabilizing modification.

(272) In some embodiments, the antisense strand comprises at least two 2'-fluoro nucleotides at the 3'-end of the destabilizing modification, i.e., at positions +1 and +2 from the position of the destabilizing modification.

(273) In some embodiments, the sense strand comprises at least two (e.g., two, three, four, five, six, seven, eight, nine, ten, or more) 2'-fluoro nucleotides. Without limitations, a 2'-fluoro modification in the sense strand can be present at any positions. In some embodiments, the antisense comprises 2'-fluoro nucleotides at positions 7, 10, and 11 from the 5'-end. In some other embodiments, the sense strand comprises 2'-fluoro nucleotides at positions 7, 9, 10, and 11 from the 5'-end. In some embodiments, the sense strand comprises 2'-fluoro nucleotides at positions opposite or complimentary to positions 11, 12, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some other embodiments, the sense strand comprises 2'-fluoro nucleotides at positions opposite or complimentary to positions 11, 12, 13, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some embodiments, the sense strand comprises a block of two, three or four 2'-fluoro nucleotides.

(274) In some embodiments, the sense strand does not comprise a 2'-fluoro nucleotide in position opposite or complimentary to the thermally destabilizing modification of the duplex in the antisense strand.

(275) In some embodiments, the dsRNA molecule of the disclosure comprises a 21 nucleotides (nt) sense strand and a 23 nucleotides (nt) antisense, wherein the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide occurs

in the seed region of the antisense strand (i.e., at position 2-9 of the 5'-end of the antisense strand), wherein one end of the dsRNA is blunt, while the other end is comprises a 2 nt overhang, and wherein the dsRNA optionally further has at least one (e.g., one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4, or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA comprises a blunt end at 5'-end of the antisense strand. Preferably, the 2 nt overhang is at the 3'-end of the antisense.

(276) In some embodiments, the dsRNA molecule of the disclosure comprising a sense and antisense strands, wherein: the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1), positions 1 to 23 of said sense strand comprise at least 8 ribonucleotides; antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, at least 8 ribonucleotides in the positions paired with positions 1-23 of sense strand to form a duplex; wherein at least the 3' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when said double stranded nucleic acid is introduced into a mammalian cell; and wherein the antisense strand contains at least one thermally destabilizing nucleotide, where at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (i.e. at position 2-9 of the 5'-end of the antisense strand). For example, the thermally destabilizing nucleotide occurs between positions opposite or complimentary to positions 14-17 of the 5'-end of the sense strand, and wherein the dsRNA optionally further has at least one (e.g., one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4, or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; and (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA comprises a duplex region of 12-30 nucleotide pairs in length.

(277) In some embodiments, the dsRNA molecule of the disclosure comprises a sense and antisense strands, wherein said dsRNA molecule comprises a sense strand having a length which is at least 25 and at most 29 nucleotides and an antisense strand having a length which is at most 30 nucleotides with the sense strand comprises a modified nucleotide that is susceptible to enzymatic degradation at position 11 from the 5' end, wherein the 3' end of said sense strand and the 5' end of said antisense strand form a blunt end and said antisense strand is 1-4 nucleotides longer at its 3' end than the sense strand, wherein the duplex region which is at least 25 nucleotides in length, and said antisense strand is sufficiently complementary to a target mRNA along at least 19 nt of said antisense strand length to reduce target gene expression when said dsRNA molecule is introduced into a mammalian cell, and wherein dicer cleavage of said dsRNA preferentially results in an siRNA comprising said 3' end of said antisense strand, thereby reducing expression of the target gene in the mammal, wherein the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the

antisense strand (i.e. at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (e.g., one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4, or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; and (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA has a duplex region of 12-29 nucleotide pairs in length.

(278) In some embodiments, every nucleotide in the sense strand and antisense strand of the dsRNA molecule may be modified. Each nucleotide may be modified with the same or different modification which can include one or more alteration of one or both of the non-linking phosphate oxygens or of one or more of the linking phosphate oxygens; alteration of a constituent of the ribose sugar, e.g., of the 2' hydroxyl on the ribose sugar; wholesale replacement of the phosphate moiety with "dephospho" linkers; modification or replacement of a naturally occurring base; and replacement or modification of the ribose-phosphate backbone.

(279) As nucleic acids are polymers of subunits, many of the modifications occur at a position which is repeated within a nucleic acid, e.g., a modification of a base, or a phosphate moiety, or a non-linking O of a phosphate moiety. In some cases, the modification will occur at all of the subject positions in the nucleic acid but in many cases it will not. By way of example, a modification may only occur at a 3' or 5' terminal position, may only occur in a terminal region, e.g., at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand. A modification may occur in a double strand region, a single strand region, or in both. A modification may occur only in the double strand region of an RNA or may only occur in a single strand region of an RNA. e.g., a phosphorothioate modification at a non-linking O position may only occur at one or both termini, may only occur in a terminal region, e.g., at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in double strand and single strand regions, particularly at termini. The 5' end or ends can be phosphorylated.

(280) It may be possible, e.g., to enhance stability, to include particular bases in overhangs, or to include modified nucleotides or nucleotide surrogates, in single strand overhangs, e.g., in a 5' or 3' overhang, or in both. E.g., it can be desirable to include purine nucleotides in overhangs. In some embodiments all or some of the bases in a 3' or 5' overhang may be modified, e.g., with a modification described herein. Modifications can include, e.g., the use of modifications at the 2' position of the ribose sugar with modifications that are known in the art, e.g., the use of deoxyribonucleotides, 2'-deoxy-2'-fluoro (2'-F) or 2'-O-methyl modified instead of the ribosugar of the nucleobase, and modifications in the phosphate group, e.g., phosphorothioate modifications. Overhangs need not be homologous with the target sequence.

(281) In some embodiments, each residue of the sense strand and antisense strand is independently modified with LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-deoxy, or 2'-fluoro. The strands can contain more than one modification. In some embodiments, each residue of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. It is to be understood that these modifications are in addition to the at least one thermally destabilizing modification of the duplex present in the antisense strand.

(282) At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'-deoxy, 2'-O-methyl or 2'-fluoro modifications, acyclic nucleotides or others. In some embodiments, the sense strand and antisense strand each comprises two differently modified nucleotides selected from 2'-O-methyl or 2'-deoxy. In some embodiments, each residue of the sense strand and antisense strand is independently modified with 2'-O-methyl nucleotide, 2'-deoxy nucleotide, 2'-deoxy-2'-fluoro nucleotide, 2'-O—N-methylacetamido (2'-O-NMA) nucleotide, a 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE) nucleotide, 2'-O-aminopropyl (2'-O-AP) nucleotide, or 2'-ara-F nucleotide. Again, it is to be

understood that these modifications are in addition to the at least one thermally destabilizing modification of the duplex present in the antisense strand.

(283) In some embodiments, the dsRNA molecule of the disclosure comprises modifications of an alternating pattern, particular in the B1, B2, B3, B1', B2', B3', B4' regions. The term "alternating motif" or "alternative pattern" as used herein refers to a motif having one or more modifications, each modification occurring on alternating nucleotides of one strand. The alternating nucleotide may refer to one per every other nucleotide or one per every three nucleotides, or a similar pattern. For example, if A, B and C each represent one type of modification to the nucleotide, the alternating motif can be "ABABABABAB . . .," "AABBAABBAABB . . .," "AABAABAABAAB . . .," "AAABAAABAAAB . . .," "AAABBBAAABBB . . .," or "ABCABCABCABC . . .," etc. The type of modifications contained in the alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on the nucleotide, the alternating pattern, i.e., modifications on every other nucleotide, may be the same, but each of the sense strand or antisense strand can be selected from several possibilities of modifications within the alternating motif such as "ABABAB . . .," "ACACAC . . ." "BDBDBD . . ." or "CDCDCD . . .," etc.

(284) In some embodiments, the dsRNA molecule of the disclosure comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and vice versa. For example, the sense strand when paired with the antisense strand in the dsRNA duplex, the alternating motif in the sense strand may start with "ABABAB" from 5'-3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 3'-5' of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5'-3' of the strand and the alternating motif in the antisense strand may start with "BBAABBAA" from 3'-5' of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

(285) The dsRNA molecule of the disclosure may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate internucleotide linkage modification may occur on any nucleotide of the sense strand or antisense strand or both in any position of the strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand comprises both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the internucleotide linkage modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand.

(286) In some embodiments, the dsRNA molecule comprises the phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region comprises two nucleotides having a phosphorothioate or methylphosphonate internucleotide linkage between the two nucleotides. Internucleotide linkage modifications also may be made to link the overhang nucleotides with the terminal paired nucleotides within duplex region. For example, at least 2, 3, 4, or all the overhang nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage, and optionally, there may be additional phosphorothioate or methylphosphonate internucleotide linkages linking the overhang nucleotide with a paired nucleotide that is next to the overhang nucleotide. For instance, there may be at least two phosphorothioate internucleotide linkages between the terminal three nucleotides, in which two of the three nucleotides are overhang nucleotides, and the third is a paired nucleotide

next to the overhang nucleotide. Preferably, these terminal three nucleotides may be at the 3'-end of the antisense strand.

(287) In some embodiments, the sense strand of the dsRNA molecule comprises 1-10 blocks of two to ten phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said sense strand is paired with an antisense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

(288) In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of two phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

(289) In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of three phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

(290) In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of four phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

(291) In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of five phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

(292) In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of six phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

(293) In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of seven phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, or 8 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of

phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

(294) In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of eight phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, or 6 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

(295) In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of nine phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, or 4 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

(296) In some embodiments, the dsRNA molecule of the disclosure further comprises one or more phosphorothioate or methylphosphonate internucleotide linkage modification within 1-10 of the termini position(s) of the sense or antisense strand. For example, at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage at one end or both ends of the sense or antisense strand.

(297) In some embodiments, the dsRNA molecule of the disclosure further comprises one or more phosphorothioate or methylphosphonate internucleotide linkage modification within 1-10 of the internal region of the duplex of each of the sense or antisense strand. For example, at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides may be linked through phosphorothioate methylphosphonate internucleotide linkage at position 8-16 of the duplex region counting from the 5'-end of the sense strand; the dsRNA molecule can optionally further comprise one or more phosphorothioate or methylphosphonate internucleotide linkage modification within 1-10 of the termini position(s).

(298) In some embodiments, the dsRNA molecule of the disclosure further comprises one to five phosphorothioate or methylphosphonate internucleotide linkage modification(s) within position 1-5 and one to five phosphorothioate or methylphosphonate internucleotide linkage modification(s) within position 18-23 of the sense strand (counting from the 5'-end), and one to five phosphorothioate or methylphosphonate internucleotide linkage modification at positions 1 and 2 and one to five within positions 18-23 of the antisense strand (counting from the 5'-end).

(299) In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification within position 1-5 and one phosphorothioate or methylphosphonate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and 2 and two phosphorothioate or methylphosphonate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

(300) In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and one phosphorothioate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

(301) In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and two phosphorothioate internucleotide linkage modifications within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at

positions 1 and one at position 21 of the antisense strand (counting from the 5'-end).

(311) In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification at position 1, and one phosphorothioate internucleotide linkage modification at position 21 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications at positions 20 and 21 the antisense strand (counting from the 5'-end).

(312) In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications at position 1 and 2, and two phosphorothioate internucleotide linkage modifications at position 21 and 22 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and one phosphorothioate internucleotide linkage modification at position 21 of the antisense strand (counting from the 5'-end).

(313) In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification at position 1, and one phosphorothioate internucleotide linkage modification at position 21 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications at positions 21 and 22 the antisense strand (counting from the 5'-end).

(314) In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications at position 1 and 2, and two phosphorothioate internucleotide linkage modifications at position 22 and 23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and one phosphorothioate internucleotide linkage modification at position 21 of the antisense strand (counting from the 5'-end).

(315) In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification at position 1, and one phosphorothioate internucleotide linkage modification at position 21 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications at positions 23 and 23 the antisense strand (counting from the 5'-end).

(316) In some embodiments, compound of the disclosure comprises a pattern of backbone chiral centers. In some embodiments, a common pattern of backbone chiral centers comprises at least 5 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 6 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 7 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 8 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 9 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 10 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 11 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 12 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 13 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 14 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 15 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 16 internucleotidic linkages in the Sp configuration. In

some embodiments, a common pattern of backbone chiral centers comprises at least 17 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 18 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 19 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 8 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 7 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 6 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 5 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 4 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 3 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 2 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 1 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 8 internucleotidic linkages which are not chiral (as a non-limiting example, a phosphodiester). In some embodiments, a common pattern of backbone chiral centers comprises no more than 7 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 5 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 4 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 3 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 2 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 1 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 10 internucleotidic linkages in the Sp configuration, and no more than 8 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 11 internucleotidic linkages in the Sp configuration, and no more than 7 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 12 internucleotidic linkages in the Sp configuration, and no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 13 internucleotidic linkages in the Sp configuration, and no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 14 internucleotidic linkages in the Sp configuration, and no more than 5 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 15 internucleotidic linkages in the Sp configuration, and no more than 4 internucleotidic linkages which are not chiral. In some embodiments, the internucleotidic linkages in the Sp configuration are optionally contiguous or not contiguous. In some embodiments, the internucleotidic linkages in the Rp configuration are optionally contiguous or not contiguous. In some embodiments, the internucleotidic linkages which are not chiral are optionally contiguous or not contiguous.

(317) In some embodiments, compound of the disclosure comprises a block is a stereochemistry block. In some embodiments, a block is an Rp block in that each internucleotidic linkage of the block is Rp. In some embodiments, a 5'-block is an Rp block. In some embodiments, a 3'-block is an Rp block. In some embodiments, a block is an Sp block in that each internucleotidic linkage of

the block is Sp. In some embodiments, a 5'-block is an Sp block. In some embodiments, a 3'-block is an Sp block. In some embodiments, provided oligonucleotides comprise both Rp and Sp blocks. In some embodiments, provided oligonucleotides comprise one or more Rp but no Sp blocks. In some embodiments, provided oligonucleotides comprise one or more Sp but no Rp blocks. In some embodiments, provided oligonucleotides comprise one or more PO blocks wherein each internucleotidic linkage in a natural phosphate linkage.

(318) In some embodiments, compound of the disclosure comprises a 5'-block is an Sp block wherein each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block is an Sp block wherein each of internucleotidic linkage is a modified internucleotidic linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block is an Sp block wherein each of internucleotidic linkage is a phosphorothioate linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block comprises 4 or more nucleoside units. In some embodiments, a 5'-block comprises 5 or more nucleoside units. In some embodiments, a 5'-block comprises 6 or more nucleoside units. In some embodiments, a 5'-block comprises 7 or more nucleoside units. In some embodiments, a 3'-block is an Sp block wherein each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block is an Sp block wherein each of internucleotidic linkage is a modified internucleotidic linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block is an Sp block wherein each of internucleotidic linkage is a phosphorothioate linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block comprises 4 or more nucleoside units. In some embodiments, a 3'-block comprises 5 or more nucleoside units. In some embodiments, a 3'-block comprises 6 or more nucleoside units. In some embodiments, a 3'-block comprises 7 or more nucleoside units.

(319) In some embodiments, compound of the disclosure comprises a type of nucleoside in a region or an oligonucleotide is followed by a specific type of internucleotidic linkage, e.g., natural phosphate linkage, modified internucleotidic linkage, Rp chiral internucleotidic linkage, Sp chiral internucleotidic linkage, etc. In some embodiments, A is followed by Sp. In some embodiments, A is followed by Rp. In some embodiments, A is followed by natural phosphate linkage (PO). In some embodiments, U is followed by Sp. In some embodiments, U is followed by Rp. In some embodiments, U is followed by natural phosphate linkage (PO). In some embodiments, C is followed by Sp. In some embodiments, C is followed by Rp. In some embodiments, C is followed by natural phosphate linkage (PO). In some embodiments, G is followed by Sp. In some embodiments, G is followed by Rp. In some embodiments, G is followed by natural phosphate linkage (PO). In some embodiments, C and U are followed by Sp. In some embodiments, C and U are followed by Rp. In some embodiments, C and U are followed by natural phosphate linkage (PO). In some embodiments, A and G are followed by Sp. In some embodiments, A and G are followed by Rp.

(320) In some embodiments, the antisense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (i.e., at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (e.g., one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.

(321) In some embodiments, the antisense strand comprises phosphorothioate internucleotide

linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (i.e., at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (e.g., one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the sense strand is conjugated with a ligand; (iii) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (iv) the sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (v) the dsRNA comprises at least four 2'-fluoro modifications; (vi) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.

(322) In some embodiments, the sense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (i.e., at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (e.g., one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 3, 4 or 5 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.

(323) In some embodiments, the sense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3, the antisense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (i.e., at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (e.g., one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the sense strand is conjugated with a ligand; (iii) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (iv) the sense strand comprises 3, 4 or 5 phosphorothioate internucleotide linkages; (v) the dsRNA comprises at least four 2'-fluoro modifications; (vi) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (vii) the dsRNA has a blunt end at 5'-end of the antisense strand.

(324) In some embodiments, the dsRNA molecule of the disclosure comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch can occur in the overhang region or the duplex region. The base pair can be ranked on the basis of their propensity to promote dissociation or melting (e.g., on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, e.g., non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

(325) In some embodiments, the dsRNA molecule of the disclosure comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'-end of the antisense strand can be chosen independently from the group of: A:U, G:U, I:C, and mismatched pairs, e.g., non-

canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

(326) In some embodiments, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from the group consisting of A, dA, dU, U, and dT.

Alternatively, at least one of the first 1, 2 or 3 base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair.

(327) It was found that introducing 4'-modified or 5'-modified nucleotide to the 3'-end of a phosphodiester (PO), phosphorothioate (PS), or phosphorodithioate (PS2) linkage of a dinucleotide at any position of single stranded or double stranded oligonucleotide can exert steric effect to the internucleotide linkage and, hence, protecting or stabilizing it against nucleases.

(328) In some embodiments, 5'-modified nucleoside is introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. For instance, a 5'-alkylated nucleoside may be introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. The alkyl group at the 5' position of the ribose sugar can be racemic or chirally pure R or S isomer. An exemplary 5'-alkylated nucleoside is 5'-methyl nucleoside. The 5'-methyl can be either racemic or chirally pure R or S isomer.

(329) In some embodiments, 4'-modified nucleoside is introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. For instance, a 4'-alkylated nucleoside may be introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. The alkyl group at the 4' position of the ribose sugar can be racemic or chirally pure R or S isomer. An exemplary 4'-alkylated nucleoside is 4'-methyl nucleoside. The 4'-methyl can be either racemic or chirally pure R or S isomer. Alternatively, a 4'-O-alkylated nucleoside may be introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. The 4'-O-alkyl of the ribose sugar can be racemic or chirally pure R or S isomer. An exemplary 4'-O-alkylated nucleoside is 4'-O-methyl nucleoside. The 4'-O-methyl can be either racemic or chirally pure R or S isomer.

(330) In some embodiments, 5'-alkylated nucleoside is introduced at any position on the sense strand or antisense strand of a dsRNA, and such modification maintains or improves potency of the dsRNA. The 5'-alkyl can be either racemic or chirally pure R or S isomer. An exemplary 5'-alkylated nucleoside is 5'-methyl nucleoside. The 5'-methyl can be either racemic or chirally pure R or S isomer.

(331) In some embodiments, 4'-alkylated nucleoside is introduced at any position on the sense strand or antisense strand of a dsRNA, and such modification maintains or improves potency of the dsRNA. The 4'-alkyl can be either racemic or chirally pure R or S isomer. An exemplary 4'-alkylated nucleoside is 4'-methyl nucleoside. The 4'-methyl can be either racemic or chirally pure R or S isomer.

(332) In some embodiments, 4'-O-alkylated nucleoside is introduced at any position on the sense strand or antisense strand of a dsRNA, and such modification maintains or improves potency of the dsRNA. The 5'-alkyl can be either racemic or chirally pure R or S isomer. An exemplary 4'-O-alkylated nucleoside is 4'-O-methyl nucleoside. The 4'-O-methyl can be either racemic or chirally pure R or S isomer.

(333) In some embodiments, the dsRNA molecule of the disclosure can comprise 2'-5' linkages (with 2'-H, 2'-OH and 2'-OMe and with P=O or P=S). For example, the 2'-5' linkages modifications can be used to promote nuclease resistance or to inhibit binding of the sense to the antisense strand, or can be used at the 5' end of the sense strand to avoid sense strand activation by RISC.

(334) In another embodiment, the dsRNA molecule of the disclosure can comprise L sugars (e.g., L ribose, L-arabinose with 2'-H, 2'-OH and 2'-OMe). For example, these L sugars modifications can be used to promote nuclease resistance or to inhibit binding of the sense to the antisense strand, or

can be used at the 5' end of the sense strand to avoid sense strand activation by RISC.

(335) Various publications describe multimeric siRNA which can all be used with the dsRNA of the disclosure. Such publications include WO2007/091269, U.S. Pat. No. 7,858,769, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520 which are hereby incorporated by their entirety.

(336) As described in more detail below, the RNAi agent that contains conjugations of one or more carbohydrate moieties to an RNAi agent can optimize one or more properties of the RNAi agent. In many cases, the carbohydrate moiety will be attached to a modified subunit of the RNAi agent. For example, the ribose sugar of one or more ribonucleotide subunits of a dsRNA agent can be replaced with another moiety, e.g., a non-carbohydrate (preferably cyclic) carrier to which is attached a carbohydrate ligand. A ribonucleotide subunit in which the ribose sugar of the subunit has been so replaced is referred to herein as a ribose replacement modification subunit (RRMS). A cyclic carrier may be a carbocyclic ring system, i.e., all ring atoms are carbon atoms, or a heterocyclic ring system, i.e., one or more ring atoms may be a heteroatom, e.g., nitrogen, oxygen, sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, e.g. fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

(337) The ligand may be attached to the polynucleotide via a carrier. The carriers include (i) at least one "backbone attachment point," preferably two "backbone attachment points" and (ii) at least one "tethering attachment point." A "backbone attachment point" as used herein refers to a functional group, e.g. a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier into the backbone, e.g., the phosphate, or modified phosphate, e.g., sulfur containing, backbone, of a ribonucleic acid. A "tethering attachment point" (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, e.g., a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point), that connects a selected moiety. The moiety can be, e.g., a carbohydrate, e.g. monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide and polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, e.g., an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, e.g., a ligand to the constituent ring.

(338) The RNAi agents may be conjugated to a ligand via a carrier, wherein the carrier can be cyclic group or acyclic group; preferably, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl and decalin; preferably, the acyclic group is selected from serinol backbone or diethanolamine backbone.

(339) In certain specific embodiments, the RNAi agent for use in the methods of the disclosure is an agent selected from the group of agents listed in any one of Tables 2-5. These agents may further comprise a ligand, such as one or more lipophilic moieties, one or more GalNAc derivatives, or both of one or more lipophilic moieties and one or more GalNAc derivatives.

IV. iRNAs Conjugated to Ligands

(340) Another modification of the RNA of an iRNA of the invention involves chemically linking to the iRNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the iRNA, e.g., into a cell. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86: 6553-6556), cholic acid (Manoharan et al., *Biorg. Med. Chem. Lett.*, 1994, 4:1053-1060), a thioether, e.g., beryl-S-tritylthiol (Manoharan et al., *Ann. N. Y. Acad. Sci.*, 1992, 660:306-309; Manoharan et al., *Biorg. Med. Chem. Lett.*, 1993, 3:2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20:533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J*, 1991, 10:1111-1118; Kabanov et al., *FEBS Lett.*, 1990, 259:327-330; Svinarchuk et

al., *Biochimie*, 1993, 75:49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36:3651-3654; Shea et al., *Nucl. Acids Res.*, 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14:969-973), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36:3651-3654), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277:923-937).

(341) In certain embodiments, a ligand alters the distribution, targeting or lifetime of an iRNA agent into which it is incorporated. In some embodiments, a ligand provides an enhanced affinity for a selected target, e.g., molecule, cell or cell type, compartment, e.g., a cellular or organ compartment, tissue, organ or region of the body, as, e.g., compared to a species absent such a ligand. Typical ligands will not take part in duplex pairing in a duplexed nucleic acid.

(342) Ligands can include a naturally occurring substance, such as a protein (e.g., human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand may also be a recombinant or synthetic molecule, such as a synthetic polymer, e.g., a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolid) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an a helical peptide.

(343) Ligands can also include targeting groups, e.g., a cell or tissue targeting agent, e.g., a lectin, glycoprotein, lipid or protein, e.g., an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, biotin, or an RGD peptide or RGD peptide mimetic. In certain embodiments, the ligand is a multivalent galactose, e.g., an N-acetyl-galactosamine.

(344) Other examples of ligands include dyes, intercalating agents (e.g. acridines), cross-linkers (e.g. psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (e.g., phenazine, dihydrophenazine), artificial endonucleases (e.g. EDTA), lipophilic molecules, e.g., cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine) and peptide conjugates (e.g., antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (e.g., PEG-40K), MPEG, [MPEG].sub.2, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (e.g. biotin), transport/absorption facilitators (e.g., aspirin, vitamin E, folic acid), synthetic ribonucleases (e.g., imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu³⁺ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

(345) Ligands can be proteins, e.g., glycoproteins, or peptides, e.g., molecules having a specific affinity for a co-ligand, or antibodies e.g., an antibody, that binds to a specified cell type such as a cancer cell, endothelial cell, or bone cell. Ligands may also include hormones and hormone

receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF- κ B.

(346) The ligand can be a substance, e.g., a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, e.g., by disrupting the cell's microtubules, microfilaments, or intermediate filaments. The drug can be, for example, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

(347) In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins etc. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin etc. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, e.g., oligonucleotides of about 5 bases, 10 bases, 15 bases or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (e.g. as PK modulating ligands). In addition, aptamers that bind serum components (e.g. serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

(348) Ligand-conjugated iRNAs of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

(349) The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems® (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

(350) In the ligand-conjugated oligonucleotides and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

(351) When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

(352) A. Lipid Conjugates

(353) In certain embodiments, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule can typically bind a serum protein, such as human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, e.g., a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For

example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, or (c) can be used to adjust binding to a serum protein, e.g., HSA.

(354) A lipid-based ligand can be used to modulate, e.g., control (e.g., inhibit) the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

(355) In certain embodiments, the lipid-based ligand binds HSA. For example, the ligand can bind HSA with a sufficient affinity such that distribution of the conjugate to a non-kidney tissue is enhanced. However, the affinity is typically not so strong that the HSA-ligand binding cannot be reversed.

(356) In certain embodiments, the lipid-based ligand binds HSA weakly or not at all, such that distribution of the conjugate to the kidney is enhanced. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid-based ligand.

(357) In another aspect, the ligand is a moiety, e.g., a vitamin, which is taken up by a target cell, e.g., a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, e.g., of the malignant or non-malignant type, e.g., cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin, e.g., folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by cancer cells. Also included are HSA and low density lipoprotein (LDL).

(358) B. Cell Permeation Agents

(359) In another aspect, the ligand is a cell-permeation agent, such as a helical cell-permeation agent. In certain embodiments, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopodia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent is typically an α -helical agent and can have a lipophilic and a lipophobic phase.

(360) The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

(361) A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (e.g., consisting primarily of Tyr, Trp, or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO:13). An RFGF analogue (e.g., amino acid sequence AALLPVLLAAP (SEQ ID NO:14)) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO:15)) and the *Drosophila* Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO:16)) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam et al., *Nature*, 354:82-84, 1991). Typically, the peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit is a cell targeting peptide such as an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide

moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

(362) An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, e.g., glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidomimetics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Preferred conjugates of this ligand target PECAM-1 or VEGF.

(363) An RGD peptide moiety can be used to target a particular cell type, e.g., a tumor cell, such as an endothelial tumor cell or a breast cancer tumor cell (Zitzmann et al., *Cancer Res.*, 62:5139-43, 2002). An RGD peptide can facilitate targeting of an dsRNA agent to tumors of a variety of other tissues, including the lung, kidney, spleen, or liver (Aoki et al., *Cancer Gene Therapy* 8:783-787, 2001). Typically, the RGD peptide will facilitate targeting of an iRNA agent to the kidney. The RGD peptide can be linear or cyclic, and can be modified, e.g., glycosylated or methylated to facilitate targeting to specific tissues. For example, a glycosylated RGD peptide can deliver an iRNA agent to a tumor cell expressing α .sub.v β .sub.3 (Haubner et al., *Jour. Nucl. Med.*, 42:326-336, 2001).

(364) A "cell permeation peptide" is capable of permeating a cell, e.g., a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α -helical linear peptide (e.g., LL-37 or Ceropin P1), a disulfide bond-containing peptide (e.g., α -defensin, β -defensin or bactenecin), or a peptide containing only one or two dominating amino acids (e.g., PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni et al., *Nucl. Acids Res.* 31:2717-2724, 2003).

(365) C. Carbohydrate Conjugates

(366) In some embodiments of the compositions and methods of the invention, an iRNA further comprises a carbohydrate. The carbohydrate conjugated iRNA are advantageous for the in vivo delivery of nucleic acids, as well as compositions suitable for in vivo therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate per se made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (e.g., C5, C6, C7, or C8) sugars; di- and tri-saccharides include sugars having two or three monosaccharide units (e.g., C5, C6, C7, or C8).

(367) In certain embodiments, a carbohydrate conjugate comprises a monosaccharide.

(368) In certain embodiments, the monosaccharide is an N-acetylgalactosamine (GalNAc). GalNAc conjugates, which comprise one or more N-acetylgalactosamine (GalNAc) derivatives, are described, for example, in U.S. Pat. No. 8,106,022, the entire content of which is hereby incorporated herein by reference. In some embodiments, the GalNAc conjugate serves as a ligand that targets the iRNA to particular cells. In some embodiments, the GalNAc conjugate targets the iRNA to liver cells, e.g., by serving as a ligand for the asialoglycoprotein receptor of liver cells (e.g., hepatocytes).

(369) In some embodiments, the carbohydrate conjugate comprises one or more GalNAc derivatives. The GalNAc derivatives may be attached via a linker, e.g., a bivalent or trivalent

branched linker. In some embodiments the GalNAc conjugate is conjugated to the 3' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (e.g., to the 3' end of the sense strand) via a linker, e.g., a linker as described herein. In some embodiments the GalNAc conjugate is conjugated to the 5' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (e.g., to the 5' end of the sense strand) via a linker, e.g., a linker as described herein.

(370) In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a trivalent linker. In other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a tetravalent linker.

(371) In certain embodiments, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (e.g., 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

(372) In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached via a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

(373) In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached via a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

(374) In some embodiments, the GalNAc conjugate is

(375) ##STR00013##

(376) In some embodiments, the RNAi agent is attached to the carbohydrate conjugate via a linker as shown in the following schematic, wherein X is O or S

(377) ##STR00014##

(378) In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below:

(379) ##STR00015##

(380) In certain embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:

(381) ##STR00016## ##STR00017## ##STR00018## ##STR00019## ##STR00020##
##STR00021## ##STR00022## ##STR00023##

(382) In certain embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In certain embodiments, the monosaccharide is an N-acetylgalactosamine, such as

(383) ##STR00024##

(384) Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,

(385) ##STR00025## when one of X or Y is an oligonucleotide, the other is a hydrogen.

(386) In some embodiments, a suitable ligand is a ligand disclosed in WO 2019/055633, the entire contents of which are incorporated herein by reference. In one embodiment the ligand comprises

the structure below:

(387) ##STR00026##

(388) In certain embodiments, the RNAi agents of the disclosure may include GalNAc ligands, even if such GalNAc ligands are currently projected to be of limited value for the preferred intrathecal/CNS delivery route(s) of the instant disclosure.

(389) In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a trivalent linker. In other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a tetravalent linker.

(390) In certain embodiments, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent, e.g., the 5' end of the sense strand of a dsRNA agent, or the 5' end of one or both sense strands of a dual targeting RNAi agent as described herein. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (e.g., 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

(391) In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached via a monovalent linker.

(392) In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator or a cell permeation peptide.

(393) Additional carbohydrate conjugates and linkers suitable for use in the present invention include those described in WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.

(394) D. Linkers

(395) In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

(396) The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, e.g., covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR₈, C(O), C(O)NH, SO, SO.sub.2, SO.sub.2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclalkyl, heterocyclalkenyl, heterocyclalkynyl, aryl, heteroaryl, heterocycl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclalkyl, alkylheterocyclalkenyl, alkylheterocyclalkynyl, alkenylheterocyclalkyl, alkenylheterocyclalkenyl, alkenylheterocyclalkynyl, alkynylheterocyclalkyl, alkynylheterocyclalkenyl, alkynylheterocyclalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylheteroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO.sub.2, N(R₈), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R₈ is hydrogen, acyl, aliphatic or substituted

aliphatic. In certain embodiments, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-16, or 8-16 atoms.

(397) A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times or more, or at least about 100 times faster in a target cell or under a first reference condition (which can, e.g., be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, e.g., be selected to mimic or represent conditions found in the blood or serum).

(398) Cleavable linking groups are susceptible to cleavage agents, e.g., pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, e.g., oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, e.g., those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

(399) A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

(400) A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

(401) Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

(402) In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, e.g., blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood or serum (or under in vitro conditions selected to mimic extracellular conditions).

(403) i. Redox Cleavable Linking Groups

(404) In certain embodiments, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (—S—S—). To determine if a candidate cleavable linking group is a

suitable “reductively cleavable linking group,” or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents known in the art, which mimic the rate of cleavage which would be observed in a cell, e.g., a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood (or under in vitro conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

(405) ii. Phosphate-Based Cleavable Linking Groups

(406) In certain embodiments, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are —O—P(O)(ORk)—O— , —O—P(S)(ORk)—O— , —O—P(S)(SRk)—O— , —S—P(O)(ORk)—O— , —O—P(O)(ORk)—S— , —S—P(O)(ORk)—S— , —O—P(S)(ORk)—S— , —S—P(S)(ORk)—O— , —O—P(O)(Rk)—O— , —O—P(S)(Rk)—O— , —S—P(O)(Rk)—O— , —S—P(S)(Rk)—O— , —S—P(O)(Rk)—S— , —O—P(S)(Rk)—S— . Preferred embodiments are —O—P(O)(OH)—O— , —O—P(S)(OH)—O— , —O—P(S)(SH)—O— , —S—P(O)(OH)—O— , —O—P(O)(OH)—S— , —S—P(O)(OH)—S— , —O—P(S)(OH)—S— , —S—P(S)(OH)—O— , —O—P(O)(H)—O— , —O—P(S)(H)—O— , —S—P(O)(H)—O— , —S—P(S)(H)—O— , —S—P(O)(H)—S— , —O—P(S)(H)—S— . A preferred embodiment is —O—P(O)(OH)—O— . These candidates can be evaluated using methods analogous to those described above.

(407) iii. Acid Cleavable Linking Groups

(408) In certain embodiments, a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (e.g., about 6.0, 5.75, 5.5, 5.25, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula —C=NN— , C(O)O , or —OC(O) . A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

(409) iv. Ester-Based Cleavable Linking Groups

(410) In certain embodiments, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula —C(O)O— , or —OC(O)— . These candidates can be evaluated using methods analogous to those described above.

(411) v. Peptide-Based Cleavable Linking Groups

(412) In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (e.g., dipeptides, tripeptides etc.) and polypeptides. Peptide-based

cleavable groups do not include the amide group (—C(O)NH—). The amide group can be formed between any alkylene, alkenylene or alkynylene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (i.e., the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula $\text{—NHCHRAC(O)NHCHRBC(O)—}$, where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

(413) In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include but are not limited to

(414) ##STR00027## ##STR00028## ##STR00029##

when one of X or Y is an oligonucleotide, the other is a hydrogen.

(415) In certain embodiments of the compositions and methods of the invention, a ligand is one or more “GalNAc” (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

(416) In certain embodiments, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XLV)-(XLVI):

(417) ##STR00030## wherein: q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different; P.sup.2A, P.sup.2B, P.sup.3A, P.sup.3B, P.sup.4A, P.sup.4B, P.sup.5A, P.sup.5B, P.sup.5C, T.sup.2A, T.sup.2B, T.sup.3A, T.sup.3B, T.sup.4A, T.sup.4B, T.sup.4A, T.sup.5B, T.sup.5C are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH.sub.2, CH.sub.2NH or CH.sub.2O; Q.sup.2A, Q.sup.2B, Q.sup.3A, Q.sup.3B, Q.sup.4A, Q.sup.4B, Q.sup.5A, Q.sup.5B, Q.sup.5C are independently for each occurrence absent, alkylene, substituted alkylene wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO.sub.2, N(R.sup.N), C(R')=C(R''), C≡C or C(O); R.sup.2A, R.sup.2B, R.sup.3A, R.sup.3B, R.sup.4A, R.sup.4B, R.sup.5A, R.sup.5B, R.sup.5C are each independently for each occurrence absent, NH, O, S, CH.sub.2, C(O)O, C(O)NH, NHCH(R.sup.a)C(O), —C(O)CH(R.sup.a)—NH—, CO, CH=N—O,

(418) ##STR00031## or heterocyclyl; L.sup.2A, L.sup.2B, L.sup.3A, L.sup.3B, L.sup.4A, L.sup.4B, L.sup.5A, L.sup.5B and L.sup.5C represent the ligand; i.e. each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; and R.sup.a is H or amino acid side chain. Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (XLIX):

(419) ##STR00032## wherein L.sup.5A, L.sup.5B and L.sup.5C represent a monosaccharide, such as GalNAc derivative.

(420) Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II, VII, XI, X, and XIII.

(421) Representative U.S. Patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928; 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931;

6,900,297; 7,037,646; and 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

(422) It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an iRNA. The present invention also includes iRNA compounds that are chimeric compounds.

(423) “Chimeric” iRNA compounds or “chimeras,” in the context of this invention, are iRNA compounds, preferably dsRNA agents, that contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of a dsRNA compound. These iRNAs typically contain at least one region wherein the RNA is modified so as to confer upon the iRNA increased resistance to nuclease degradation, increased cellular uptake, or increased binding affinity for the target nucleic acid. An additional region of the iRNA can serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of iRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter iRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxy dsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

(424) In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. et al., *Biochem. Biophys. Res. Comm.*, 2007, 365(1):54-61; Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, 3:2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10:111; Kabanov et al., *FEBS Lett.*, 1990, 259:327; Svinarchuk et al., *Biochimie*, 1993, 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36:3651; Shea et al., *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14:969), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36:3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277:923).

Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

V. Delivery of an RNAi Agent of the Disclosure

(425) The delivery of a RNAi agent of the disclosure to a cell e.g., a cell within a subject, such as a human subject (e.g., a subject in need thereof, such as a subject having a coronavirus-associated disorder, e.g., a subject having a coronavirus infection, e.g., a subject having Severe Acute Respiratory Syndrome 2 (SARS-CoV-2; COVID-19), Severe Acute Respiratory Syndrome (SARS-CoV), or Middle East Respiratory Syndrome (MERS-CoV)), can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an RNAi agent of the disclosure either in vitro or in vivo. In vivo delivery may also be performed directly by

administering a composition comprising an RNAi agent, e.g., a dsRNA, to a subject. Alternatively, in vivo delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the RNAi agent. These alternatives are discussed further below.

(426) In general, any method of delivering a nucleic acid molecule (in vitro or in vivo) can be adapted for use with a RNAi agent of the disclosure (see e.g., Akhtar S. and Julian R L., (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For in vivo delivery, factors to consider in order to deliver an RNAi agent include, for example, biological stability of the delivered agent, prevention of non-specific effects, and accumulation of the delivered agent in the target tissue. The non-specific effects of an RNAi agent can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the RNAi agent to be administered. Several studies have shown successful knockdown of gene products when an RNAi agent is administered locally. For example, pulmonary system delivery, e.g., inhalation, of a dsRNA, e.g., SOD1, has been shown to effectively knockdown gene and protein expression in lung tissue and that there is excellent uptake of the dsRNA by the bronchioles and alveoli of the lung. Intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, M J. et al., (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, S J. et al. (2003) *Mol. Vis.* 9:210-216) were also both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of a dsRNA in mice reduces tumor volume (Pille, J. et al. (2005) *Mol. Ther.* 11:267-274) and can prolong survival of tumor-bearing mice (Kim, W J. et al., (2006) *Mol. Ther.* 14:343-350; Li, S. et al., (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G. et al., (2004) *Nucleic Acids* 32:e49; Tan, P H. et al. (2005) *Gene Ther.* 12:59-66; Makimura, H. et al (2002) *BMC Neurosci.* 3:18; Shishkina, G T., et al. (2004) *Neuroscience* 129:521-528; Thakker, E R., et al. (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya, Y., et al. (2005) *J. Neurophysiol.* 93:594-602) and to the lungs by intranasal administration (Howard, K A. et al., (2006) *Mol. Ther.* 14:476-484; Zhang, X. et al., (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V. et al., (2005) *Nat. Med.* 11:50-55). For administering a RNAi agent systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-nucleases in vivo. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the RNAi agent to the target tissue and avoid undesirable off-target effects (e.g., without wishing to be bound by theory, use of GNAs as described herein has been identified to destabilize the seed region of a dsRNA, resulting in enhanced preference of such dsRNAs for on-target effectiveness, relative to off-target effects, as such off-target effects are significantly weakened by such seed region destabilization). RNAi agents can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, a RNAi agent directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J. et al., (2004) *Nature* 432:173-178). Conjugation of an RNAi agent to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, J O. et al., (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the RNAi agent can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of molecule RNAi agent (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an RNAi agent by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an RNAi agent, or induced to form a vesicle or micelle (see

e.g., Kim S H. et al., (2008) *Journal of Controlled Release* 129(2):107-116) that encases an RNAi agent. The formation of vesicles or micelles further prevents degradation of the RNAi agent when administered systemically. Methods for making and administering cationic-RNAi agent complexes are well within the abilities of one skilled in the art (see e.g., Sorensen, D R., et al. (2003) *J. Mol. Biol* 327:761-766; Verma, U N. et al., (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, A S et al. (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of RNAi agents include DOTAP (Sorensen, D R., et al (2003), supra; Verma, U N. et al., (2003), supra), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, T S. et al., (2006) *Nature* 441:111-114), cardiolipin (Chien, P Y. et al., (2005) *Cancer Gene Ther.* 12:321-328; Pal, A. et al., (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet M E. et al., (2008) *Pharm. Res.* August 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, D A. et al., (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H. et al., (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, a RNAi agent forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of RNAi agents and cyclodextrins can be found in U.S. Pat. No. 7,427,605, which is herein incorporated by reference in its entirety.

(427) Certain aspects of the instant disclosure relate to a method of reducing the expression of a coronavirus target gene in a cell, comprising contacting said cell with the double-stranded RNAi agent of the disclosure. In one embodiment, the cell is a hepatic cell, optionally a hepatocyte. In one embodiment, the cell is an extrahepatic cell, optionally a pulmonary system cell.

(428) Another aspect of the disclosure relates to a method of reducing the expression of a coronavirus target gene in a subject, comprising administering to the subject the double-stranded RNAi agent of the disclosure.

(429) Another aspect of the disclosure relates to a method of treating a subject having a coronavirus-associated disorder, comprising administering to the subject a therapeutically effective amount of the double-stranded RNAi agent of the disclosure, thereby treating the subject. Non-limiting examples of coronavirus-associated diseases include, for example, Severe Acute Respiratory Syndrome 2 (SARS-CoV-2; COVID-19), Severe Acute Respiratory Syndrome (SARS-CoV), or Middle East Respiratory Syndrome (MERS-CoV).

(430) For ease of exposition the formulations, compositions and methods in this section are discussed largely with regard to modified siRNA compounds. It may be understood, however, that these formulations, compositions and methods can be practiced with other siRNA compounds, e.g., unmodified siRNA compounds, and such practice is within the disclosure. A composition that includes a RNAi agent can be delivered to a subject by a variety of routes. Exemplary routes include pulmonary system, intravenous, intraventricular, topical, rectal, anal, vaginal, nasal, and ocular.

(431) In one embodiment, the double-stranded RNAi agent is administered by pulmonary system administration.

(432) In one embodiment, the double-stranded RNAi agent is administered by inhalation.

(433) In one embodiment, the double-stranded RNAi agent is administered by intranasal administration.

(434) In one embodiment, the double-stranded RNAi agent is administered subcutaneously.

(435) The RNAi agents of the disclosure can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically include one or more species of RNAi agent and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional

media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions. (436) The pharmaceutical compositions of the present disclosure may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be pulmonary system, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral, or parenteral. Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal, or intramuscular injection, or intrathecal or intraventricular administration.

(437) The route and site of administration may be chosen to enhance targeting. For example, to target muscle cells, intramuscular injection into the muscles of interest would be a logical choice. Lung cells might be targeted by administering the RNAi agent in aerosol form. The vascular endothelial cells could be targeted by coating a balloon catheter with the RNAi agent and mechanically introducing the RNA.

(438) Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids, and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves, and the like may also be useful.

(439) Compositions for oral administration include powders or granules, suspensions or solutions in water, syrups, elixirs or non-aqueous media, tablets, capsules, lozenges, or troches. In the case of tablets, carriers that can be used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, and lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc, are commonly used in tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. When aqueous suspensions are required for oral use, the nucleic acid compositions can be combined with emulsifying and suspending agents. If desired, certain sweetening or flavoring agents can be added.

(440) Compositions for intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents, and other suitable additives.

(441) Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents, and other suitable additives. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir. For intravenous use, the total concentration of solutes may be controlled to render the preparation isotonic.

(442) In one embodiment, the administration of the siRNA compound, e.g., a double-stranded siRNA compound, or ssiRNA compound, composition is parenteral, e.g., intravenous (e.g., as a bolus or as a diffusible infusion), intradermal, intraperitoneal, intramuscular, intrathecal, intraventricular, intracranial, subcutaneous, transmucosal, buccal, sublingual, endoscopic, rectal, oral, vaginal, topical, pulmonary system, intranasal, urethral, or ocular. Administration can be provided by the subject or by another person, e.g., a health care provider. The medication can be provided in measured doses or in a dispenser which delivers a metered dose. Selected modes of delivery are discussed in more detail below.

(443) Pulmonary System Administration

(444) In one embodiment, the double-stranded RNAi agent is administered by pulmonary system administration. The pulmonary system includes the upper pulmonary system and the lower pulmonary system. The upper pulmonary system includes the nose and the pharynx. The pharynx includes the nasopharynx, oropharynx, and laryngopharynx. The lower pulmonary system includes the larynx, trachea, carina, bronchi, bronchioles, and alveoli.

(445) Pulmonary system administration may be intranasal administration or oral inhalative administration. Such administration permits both systemic and local delivery of the double stranded RNAi agents of the invention.

(446) Intranasal administration may include instilling or insufflating a double stranded RNAi agent

into the nasal cavity with syringes or droppers by applying a few drops at a time or via atomization. Suitable dosage forms for intranasal administration include drops, powders, nebulized mists, and sprays.

(447) Oral inhalative administration may include use of device, e.g., a passive breath driven or active power driven single/-multiple dose dry powder inhaler (DPI), to deliver a double stranded RNAi agent to the pulmonary system. Suitable dosage forms for oral inhalative administration include powders and solutions. Suitable devices for oral inhalative administration include nebulizers, metered-dose inhalers, and dry powder inhalers. Dry powder inhalers are of the most popular devices used to deliver drugs, especially proteins to the lungs. Exemplary commercially available dry powder inhalers include Spinhaler (Fisons Pharmaceuticals, Rochester, NY) and Rotahaler (GSK, RTP, NC). Several types of nebulizers are available, namely jet nebulizers, ultrasonic nebulizers, vibrating mesh nebulizers. Jet nebulizers are driven by compressed air. Ultrasonic nebulizers use a piezoelectric transducer in order to create droplets from an open liquid reservoir. Vibrating mesh nebulizers use perforated membranes actuated by an annular piezoelement to vibrate in resonant bending mode. The holes in the membrane have a large cross-section size on the liquid supply side and a narrow cross-section size on the side from where the droplets emerge. Depending on the therapeutic application, the hole sizes and number of holes can be adjusted. Selection of a suitable device depends on parameters, such as nature of the drug and its formulation, the site of action, and pathophysiology of the lung. Aqueous suspensions and solutions are nebulized effectively. Aerosols based on mechanically generated vibration mesh technologies also have been used successfully to deliver proteins to lungs.

(448) The amount of RNAi agent for pulmonary system administration may vary from one target gene to another target gene and the appropriate amount that has to be applied may have to be determined individually for each target gene. Typically, this amount ranges from 10 μg to 2 mg, preferably 50 μg to 1500 μg , more preferably 100 μg to 1000 μg .

(449) Vector Encoded RNAi Agents of the Disclosure

(450) RNAi agents targeting the coronavirus genome can be expressed from transcription units inserted into DNA or RNA vectors (see, e.g., Couture, A, et al., *TIG.* (1996), 12:5-10; WO 00/22113, WO 00/22114, and U.S. Pat. No. 6,054,299). Expression is preferably sustained (months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, et al., (1995) *Proc. Natl. Acad. Sci. USA* 92:1292).

(451) The individual strand or strands of a RNAi agent can be transcribed from a promoter on an expression vector. Where two separate strands are to be expressed to generate, for example, a dsRNA, two separate expression vectors can be co-introduced (e.g., by transfection or infection) into a target cell. Alternatively, each individual strand of a dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as inverted repeat polynucleotides joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

(452) RNAi agent expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of a RNAi agent as described herein. Delivery of RNAi agent expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

(453) Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but

not limited to lentiviral vectors, moloney murine leukemia virus, etc.; (c) adeno-associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, e.g., vaccinia virus vectors or avipox, e.g. canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, e.g. EPV and EBV vectors. Constructs for the recombinant expression of a RNAi agent will generally require regulatory elements, e.g., promoters, enhancers, etc., to ensure the expression of the RNAi agent in target cells. Other aspects to consider for vectors and constructs are known in the art.

VI. Compositions of the Invention

(454) The present disclosure also provides compositions, including pharmaceutical compositions and formulations which include the RNAi agents of the disclosure.

(455) For example, in one embodiment, the present invention provides compositions comprising two or more, e.g., 2, 3, or 4, dsRNA agents, e.g., dsRNA agents comprising a sense strand and an antisense strand forming a double stranded region, wherein each of the sense strands or each of the antisense strands is a sense strand or an antisense strand independently selected from the group consisting of any of the sense strands and antisense strands in any one of Table 2-5. In one embodiment, each of the sense strands or each of the antisense strands is a sense strand or an antisense strand independently selected from the sense strand or antisense strand of a duplex selected from the group consisting of AD-1184137, AD-1184147, AD-1184150, AD-1184210, AD-1184270, AD-1184233, AD-1184271, AD-1184212, AD-1184228, AD-1184223, AD-1231490, AD-1231513, AD-1231485, AD-1231507, AD-1231471, AD-1231494, AD-1231496, and AD-1231497. In another embodiment, each of the sense strands or each of the antisense strands is a sense strand or an antisense strand of a duplex independently selected from the group consisting of AD-1184137, AD-1184147, AD-1184150, AD-1231490, AD-1231513, AD-1231485, AD-1231471, AD-1231496, and AD-1231497. In another embodiment, each of the sense strands and each of the antisense strands is a sense strand and an antisense strand of a duplex independently selected from the group consisting of AD-1184137 and AD-1184150. In one embodiment, the composition comprises a first dsRNA agent comprising the sense strand nucleotide sequence 5'-UAACAAUGUUGCUGUUUCAAC-3' (SEQ ID NO: 5) and an antisense strand comprising the nucleotide sequence 5'-GUUUGAAAAGCAACAUUGUUAGU-3' (SEQ ID NO: 6); and a second dsRNA agent comprising the sense strand nucleotide sequence 5'-ACUGUACAGUCUAAAUGUCA-3' (SEQ ID NO: 7) and an antisense strand comprising the nucleotide sequence 5'-UGACAUUUUAGACUGUACAGUGG-3' (SEQ ID NO: 8). In one embodiment, the sense strand of the first dsRNA agent comprises the sense strand nucleotide sequence 5'-usasaca(Ahd)UfgUfUfGfcuuuuaasasa-3' (SEQ ID NO: 9) and the antisense strand of the first dsRNA agent comprises the nucleotide sequence 5'-VPusUfsuugAfaaagcaaCfaUfuguuasgsu-3' (SEQ ID NO: 10); and the sense strand of the second dsRNA agent comprises the nucleotides sequence 5'-ascuugu(Ahd)CfaGfUfCfuaaauguscsa-3' (SEQ ID NO: 11) and the antisense strand of the second dsRNA agent comprises the nucleotide sequence 5'-VPusGfsacaUfuuuagacUfgUfacagusgsg-3' (SEQ ID NO: 12), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U; s is a phosphorothioate linkage; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; and VP is Vinyl-phosphonate.

(456) In another embodiment, provided herein are pharmaceutical compositions containing an RNAi agent, or a composition, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the RNAi agent or the composition are useful for treating a subject who would benefit from inhibiting or reducing the expression of a coronavirus genome,

e.g., a subject having a coronavirus-associated disorder, e.g., a subject having a coronavirus infection, e.g., a subject having Severe Acute Respiratory Syndrome 2 (SARS-CoV-2; COVID-19), Severe Acute Respiratory Syndrome (SARS-CoV), or Middle East Respiratory Syndrome (MERS-CoV). Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for direct delivery into the pulmonary system by intranasal administration or oral inhalative administration, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal or intranasal delivery. Another example is compositions that are formulated for systemic administration via parenteral delivery, e.g., by intravenous (IV), intramuscular (IM), or for subcutaneous (subQ) delivery.

(457) In some embodiments, the pharmaceutical compositions of the invention are pyrogen free or non-pyrogenic.

(458) The pharmaceutical compositions of the disclosure may be administered in dosages sufficient to inhibit expression of a coronavirus genome. In general, a suitable dose of an RNAi agent of the disclosure will be a flat dose in the range of about 0.001 to about 200.0 mg about once per month to about once per year, typically about once per quarter (i.e., about once every three months) to about once per year, generally a flat dose in the range of about 1 to 50 mg about once per month to about once per year, typically about once per quarter to about once per year.

(459) After an initial treatment regimen (e.g., loading dose), the treatments can be administered on a less frequent basis.

(460) The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments.

(461) Advances in mouse genetics have generated a number of mouse models for the study of various coronavirus-associated diseases that would benefit from reduction in the expression of coronavirus. Such models can be used for in vivo testing of RNAi agents, as well as for determining a therapeutically effective dose. Suitable mouse models are known in the art and include, for example, the mouse models described elsewhere herein.

(462) The pharmaceutical compositions of the present disclosure can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be topical (e.g., by a transdermal patch), pulmonary system administration by intranasal administration or oral inhalative administration, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; subdermal, e.g., via an implanted device; or intracranial, e.g., by intraparenchymal, intrathecal or intraventricular, administration.

(463) The RNAi agents can be delivered in a manner to target a particular tissue, such as the liver, the lung (e.g., bronchioles, alveoli, or bronchus of the lung), or both the liver and lung.

(464) Pharmaceutical compositions and formulations for topical administration can include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable. Coated condoms, gloves and the like can also be useful. Suitable topical formulations include those in which the RNAi agents featured in the disclosure are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Suitable lipids and liposomes include neutral (e.g., dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (e.g., dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g., dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine

DOTMA). RNAi agents featured in the disclosure can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, RNAi agents can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, monoolein, dilaurin, glyceryl 1-monocaprinate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C.sub.1-20 alkyl ester (e.g., isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in U.S. Pat. No. 6,747,014, which is incorporated herein by reference.

(465) A. RNAi Agent Formulations Comprising Membranous Molecular Assemblies

(466) A RNAi agent for use in the compositions and methods of the disclosure can be formulated for delivery in a membranous molecular assembly, e.g., a liposome or a micelle. As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, e.g., one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the RNAi agent composition. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the RNAi agent composition, although in some examples, it may. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the RNAi agent are delivered into the cell where the RNAi agent can specifically bind to a target RNA and can mediate RNAi. In some cases the liposomes are also specifically targeted, e.g., to direct the RNAi agent to particular cell types.

(467) A liposome containing an RNAi agent can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The RNAi agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the RNAi agent and condense around the RNAi agent to form a liposome. After condensation, the detergent is removed, e.g., by dialysis, to yield a liposomal preparation of RNAi agent.

(468) If necessary a carrier compound that assists in condensation can be added during the condensation reaction, e.g., by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (e.g., spermine or spermidine). pH can also adjusted to favor condensation.

(469) Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as structural components of the delivery vehicle, are further described in, e.g., WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Felgner, P. L. et al., (1987) *Proc. Nat. Acad. Sci. USA* 8:7413-7417; U.S. Pat. Nos. 4,897,355; 5,171,678; Bangham et al., (1965) *M. Mol. Biol.* 23:238; Olson et al., (1979) *Biochim. Biophys. Acta* 557:9; Szoka et al., (1978) *Proc. Nat. Acad. Sci.* 75: 4194; Mayhew et al., (1984) *Biochim. Biophys. Acta* 775:169; Kim et al., (1983) *Biochim. Biophys. Acta* 728:339; and Fukunaga et al., (1984) *Endocrinol.* 115:757. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, e.g., Mayer et al., (1986) *Biochim. Biophys. Acta* 858:161. Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew et al., (1984) *Biochim. Biophys. Acta* 775:169. These methods are readily adapted to packaging RNAi

agent preparations into liposomes.

(470) Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged nucleic acid molecules to form a stable complex. The positively charged nucleic acid/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al. (1987) *Biochem. Biophys. Res. Commun.*, 147:980-985).

(471) Liposomes, which are pH-sensitive or negatively charged, entrap nucleic acids rather than complex with them. Since both the nucleic acid and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some nucleic acid is entrapped within the aqueous interior of these liposomes. pH sensitive liposomes have been used to deliver nucleic acids encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al. (1992) *Journal of Controlled Release*, 19:269-274).

(472) One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid or phosphatidylcholine or cholesterol.

(473) Examples of other methods to introduce liposomes into cells in vitro and in vivo include U.S. Pat. Nos. 5,283,185; 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Felgner, (1994) *J. Biol. Chem.* 269:2550; Nabel, (1993) *Proc. Natl. Acad. Sci.* 90:11307; Nabel, (1992) *Human Gene Ther.* 3:649; Gershon, (1993) *Biochem.* 32:7143; and Strauss, (1992) *EMBO J.* 11:417.

(474) Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporine A into different layers of the skin (Hu et al., (1994) *S.T.P. Pharma. Sci.*, 4(6):466).

(475) Liposomes also include “sterically stabilized” liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G.sub.M1, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen et al., (1987) *FEBS Letters*, 223:42; Wu et al., (1993) *Cancer Research*, 53:3765).

(476) Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (*Ann. N.Y. Acad. Sci.*, (1987), 507:64) reported the ability of monosialoganglioside G.sub.M1, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (*Proc. Natl. Acad. Sci. U.S.A.*, (1988), 85:6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes

comprising (1) sphingomyelin and (2) the ganglioside G_{sub}.M1 or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim et al).

(477) In one embodiment, cationic liposomes are used. Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although not able to fuse as efficiently with the plasma membrane, are taken up by macrophages in vivo and can be used to deliver RNAi agents to macrophages.

(478) Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated RNAi agents in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

(479) A positively charged synthetic cationic lipid, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in delivery of RNAi agent (see, e.g., Felgner, P. L. et al., (1987) *Proc. Natl. Acad. Sci. USA* 8:7413-7417, and U.S. Pat. No. 4,897,355 for a description of DOTMA and its use with DNA).

(480) A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. LipofectinTM (Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis(oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") (Boehringer Mannheim, Indianapolis, Indiana) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

(481) Other reported cationic lipid compounds include those that have been conjugated to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoyleamide ("DOGS") (TransfectamTM, Promega, Madison, Wisconsin) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPES") (see, e.g., U.S. Pat. No. 5,171,678).

(482) Another cationic lipid conjugate includes derivatization of the lipid with cholesterol ("DC-Chol") which has been formulated into liposomes in combination with DOPE (See, Gao, X. and Huang, L., (1991) *Biochim. Biophys. Res. Commun.* 179:280). Lipopolylysine, made by conjugating polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. et al., (1991) *Biochim. Biophys. Acta* 1065:8). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions. Other commercially available cationic lipid products include DMRIE and DMRIE-HP (Vical, La Jolla, California) and Lipofectamine (DOSPA) (Life Technology, Inc., Gaithersburg, Maryland). Other cationic lipids suitable for the delivery of oligonucleotides are described in WO 98/39359 and WO 96/37194.

(483) Liposomal formulations are particularly suited for topical administration, liposomes present

several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer RNAi agent into the skin. In some implementations, liposomes are used for delivering RNAi agent to epidermal cells and also to enhance the penetration of RNAi agent into dermal tissues, e.g., into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, e.g., Weiner et al., (1992) *Journal of Drug Targeting*, vol. 2, 405-410 and du Plessis et al., (1992) *Antiviral Research*, 18:259-265; Mannino, R. J. and Fould-Fogerite, S., (1998) *Biotechniques* 6:682-690; Itani, T. et al., (1987) *Gene* 56:267-276; Nicolau, C. et al. (1987) *Meth. Enzymol.* 149:157-176; Straubinger, R. M. and Papahadjopoulos, D. (1983) *Meth. Enzymol.* 101:512-527; Wang, C. Y. and Huang, L., (1987) *Proc. Natl. Acad. Sci. USA* 84:7851-7855).

(484) Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver a drug into the dermis of mouse skin. Such formulations with RNAi agent are useful for treating a dermatological disorder.

(485) Liposomes that include RNAi agents can be made highly deformable. Such deformability can enable the liposomes to penetrate through pore that are smaller than the average radius of the liposome. For example, transfersomes are a type of deformable liposomes. Transfersomes can be made by adding surface edge activators, usually surfactants, to a standard liposomal composition. Transfersomes that include RNAi agent can be delivered, for example, subcutaneously by infection in order to deliver RNAi agent to keratinocytes in the skin. In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. In addition, due to the lipid properties, these transfersomes can be self-optimizing (adaptive to the shape of pores, e.g., in the skin), self-repairing, and can frequently reach their targets without fragmenting, and often self-loading.

(486) Other formulations amenable to the present disclosure are described in PCT publication No. WO 2008/042973.

(487) Transfersomes, yet another type of liposomes, are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g., they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

(488) Surfactants find wide application in formulations such as those described herein, particularly in emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

(489) If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general, their HLB values range from 2 to about 18 depending on their

structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

(490) If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

(491) If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

(492) If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

(493) The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

(494) The RNAi agent for use in the methods of the disclosure can also be provided as micellar formulations. "Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

(495) A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of the siRNA composition, an alkali metal C.sub.8 to C.sub.22 alkyl sulphate, and a micelle forming compounds. Exemplary micelle forming compounds include lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof. The micelle forming compounds may be added at the same time or after addition of the alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients but vigorous mixing in order to provide smaller size micelles.

(496) In one method a first micellar composition is prepared which contains the siRNA composition and at least the alkali metal alkyl sulphate. The first micellar composition is then mixed with at least three micelle forming compounds to form a mixed micellar composition. In another method, the micellar composition is prepared by mixing the siRNA composition, the alkali metal alkyl sulphate and at least one of the micelle forming compounds, followed by addition of the remaining micelle forming compounds, with vigorous mixing.

(497) Phenol or m-cresol may be added to the mixed micellar composition to stabilize the formulation and protect against bacterial growth. Alternatively, phenol or m-cresol may be added with the micelle forming ingredients. An isotonic agent such as glycerin may also be added after formation of the mixed micellar composition.

(498) For delivery of the micellar formulation as a spray, the formulation can be put into an aerosol dispenser and the dispenser is charged with a propellant. The propellant, which is under pressure, is in liquid form in the dispenser. The ratios of the ingredients are adjusted so that the aqueous and propellant phases become one, i.e., there is one phase. If there are two phases, it is necessary to

shake the dispenser prior to dispensing a portion of the contents, e.g., through a metered valve. The dispensed dose of pharmaceutical agent is propelled from the metered valve in a fine spray.

(499) Propellants may include hydrogen-containing chlorofluorocarbons, hydrogen-containing fluorocarbons, dimethyl ether and diethyl ether. In certain embodiments, HFA 134a (1,1,1,2 tetrafluoroethane) may be used.

(500) The specific concentrations of the essential ingredients can be determined by relatively straightforward experimentation. For absorption through the oral cavities, it is often desirable to increase, e.g., at least double or triple, the dosage for through injection or administration through the gastrointestinal tract.

(501) Lipid Particles

(502) RNAi agents, e.g., dsRNAs of in the disclosure may be fully encapsulated in a lipid formulation, e.g., a LNP, or other nucleic acid-lipid particle.

(503) As used herein, the term “LNP” refers to a stable nucleic acid-lipid particle. LNPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (e.g., a PEG-lipid conjugate). LNPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (e.g., sites physically separated from the administration site). LNPs include “pSPLP,” which include an encapsulated condensing agent-nucleic acid complex as set forth in WO 00/03683. The particles of the present disclosure typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid-lipid particles of the present disclosure are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, e.g., U.S. Pat. Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; United States Patent publication No. 2010/0324120 and WO 96/40964.

(504) In one embodiment, the lipid to drug ratio (mass/mass ratio) (e.g., lipid to dsRNA ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. Ranges intermediate to the above recited ranges are also contemplated to be part of the disclosure.

(505) Certain specific LNP formulations for delivery of RNAi agents have been described in the art, including, e.g., “LNP01” formulations as described in, e.g., WO 2008/042973, which is hereby incorporated by reference.

(506) Additional exemplary lipid-dsRNA formulations are identified in the table below.

(507) TABLE-US-00001 cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate

Ionizable/Cationic Lipid Lipid:siRNA ratio SNALP-1 1,2-Dilinolenyloxy-N,N-

DLinDMA/DPPC/Cholesterol/PEG- dimethylaminopropane (DLinDMA) cDMA

(57.1/7.1/34.4/1.4) lipid:siRNA~7:1 2-XTC 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-

XTC/DPPC/Cholesterol/PEG-cDMA dioxolane (XTC) 57.1/7.1/34.4/1.4 lipid:siRNA~7:1 LNP05

2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]- XTC/DSPC/Cholesterol/PEG-DMG dioxolane (XTC)

57.5/7.5/31.5/3.5 lipid:siRNA~6:1 LNP06 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-

XTC/DSPC/Cholesterol/PEG-DMG dioxolane (XTC) 57.5/7.5/31.5/3.5 lipid:siRNA~11:1 LNP07

2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]- XTC/DSPC/Cholesterol/PEG-DMG dioxolane (XTC)

60/7.5/31/1.5, lipid:siRNA~6:1 LNP08 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-

XTC/DSPC/Cholesterol/PEG-DMG dioxolane (XTC) 60/7.5/31/1.5, lipid:siRNA~11:1 LNP09 2,2-

Dilinoleyl-4-dimethylaminoethyl-[1,3]- XTC/DSPC/Cholesterol/PEG-DMG dioxolane (XTC)

50/10/38.5/1.5 Lipid:siRNA 10:1 LNP10 (3aR,5s,6aS)-N,N-dimethyl-2,2-

ALN100/DSPC/Cholesterol/PEG- di((9Z,12Z)-octadeca-9,12- DMG dienyl)tetrahydro-3aH-

50/10/38.5/1.5 cyclopenta[d][1,3]dioxol-5-amine Lipid:siRNA 10:1 (ALN100) LNP11

(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31- MC-3/DSPC/Cholesterol/PEG-DMG tetraen-19-yl 4-

(dimethylamino)butanoate 50/10/38.5/1.5 (MC3) Lipid:siRNA 10:1 LNP12 1,1'-(2-(4-(2-((2-

(bis(2- Tech G1/DSPC/Cholesterol/PEG- hydroxydodecyl)amino)ethyl) DMG (2- hydroxydodecyl)amino)ethyl)piperazin-1- 50/10/38.5/1.5 yl)ethylazanediyldidodecan-2-ol (Tech Lipid:siRNA 10:1 G1) LNP13 XTC XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1 LNP14 MC3 MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:siRNA: 11:1 LNP15 MC3 MC3/DSPC/Chol/PEG-DSG/GalNAc- PEG-DSG 50/10/35/4.5/0.5 Lipid:siRNA: 11:1 LNP16 MC3 MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 7:1 LNP17 MC3 MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1 LNP18 MC3 MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 12:1 LNP19 MC3 MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:siRNA: 8:1 LNP20 MC3 MC3/DSPC/Chol/PEG- DPG 50/10/38.5/1.5 Lipid:siRNA: 10:1 LNP21 C12-200 C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 7:1 LNP22 XTC XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1 DSPC: distearoylphosphatidylcholine DPPC: dipalmitoylphosphatidylcholine PEG-DMG: PEG-didimyristoyl glycerol (C14-PEG, or PEG-C14) (PEG with avg mol wt of 2000) PEG-DSG: PEG-distyryl glycerol (C18-PEG, or PEG-C18) (PEG with avg mol wt of 2000) PEG- cDMA: PEG-carbamoyl-1,2-dimyristyloxypropylamine (PEG with avg mol wt of 2000) SNALP (1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in WO 2009/127060, which is hereby incorporated by reference. XTC comprising formulations are described in WO 2010/088537, the entire contents of which are hereby incorporated herein by reference. MC3 comprising formulations are described, e.g., in United States Patent Publication No. 2010/0324120, the entire contents of which are hereby incorporated by reference. ALNY-100 comprising formulations are described in WO 2010/054406, the entire contents of which are hereby incorporated herein by reference. C12-200 comprising formulations are described in WO 2010/129709, the entire contents of which are hereby incorporated herein by reference.

(508) Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitables. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In some embodiments, oral formulations are those in which dsRNAs featured in the disclosure are administered in conjunction with one or more penetration enhancer surfactants and chelators. Suitable surfactants include fatty acids or esters or salts thereof, bile acids or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucolic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (e.g., sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the disclosure can be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (e.g., p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate),

poly(isobutylcyanoacrylate), poly(isohexylcyanoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Pat. No. 6,887,906, U.S. 2003/0027780, and U.S. Pat. No. 6,747,014, each of which is incorporated herein by reference.

(509) Compositions for pulmonary system delivery may include aqueous solutions, e.g., for intranasal or oral inhalative administration, suitable carriers composed of, e.g., lipids (liposomes, niosomes, microemulsions, lipidic micelles, solid lipid nanoparticles) or polymers (polymer micelles, dendrimers, polymeric nanoparticles, nonogels, nanocapsules), adjuvant, e.g., for oral inhalative administration. Aqueous compositions may be sterile and may optionally contain buffers, diluents, absorption enhancers and other suitable additives.

(510) Compositions and formulations for parenteral, intraparenchymal (into the brain), intrathecal, intraventricular or intrahepatic administration can include sterile aqueous solutions which can also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

(511) Pharmaceutical compositions of the present disclosure include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly preferred are formulations that target the brain when treating APP-associated diseases or disorders.

(512) The pharmaceutical formulations of the present disclosure, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

(513) The compositions of the present disclosure can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present disclosure can also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions can further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol or dextran. The suspension can also contain stabilizers.

(514) Additional Formulations

(515) i. Emulsions

(516) The compositions of the present disclosure can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μ m in diameter (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, L.V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi et al., in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion.

Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk

aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution in either aqueous phase, oily phase or itself as a separate phase.

Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise, a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

(517) Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion can be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see e.g., Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

(518) Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (see e.g., Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (see e.g., Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

(519) Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

(520) A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and

Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

(521) Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

(522) Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that can readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used can be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

(523) The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (see e.g., Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of ease of formulation, as well as efficacy from an absorption and bioavailability standpoint (see e.g., Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

(524) ii. Microemulsions

(525) In one embodiment of the present disclosure, the compositions of RNAi agents and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see e.g., Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically, microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: *Controlled Release of Drugs: Polymers and Aggregate Systems*, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used, and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa., 1985, p. 271).

(526) The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (see e.g., Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

(527) Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (M0310), hexaglycerol monooleate (P0310), hexaglycerol pentaoleate (P0500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (M0750), decaglycerol sequioleate (S0750), decaglycerol decaoleate (DA0750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions can, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase can typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase can include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

(528) Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (see e.g., U.S. Pat. Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides et al., *Pharmaceutical Research*, 1994, 11, 1385-1390; Ritschel, *Meth. Find. Exp. Clin. Pharmacol.*, 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (see e.g., U.S. Pat. Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides et al., *Pharmaceutical Research*, 1994, 11, 1385; Ho et al., *J. Pharm. Sci.*, 1996, 85, 138-143). Often microemulsions can form spontaneously when their components are brought together at ambient temperature. This can be particularly advantageous when formulating thermolabile drugs, peptides or RNAi agents. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present disclosure will facilitate the increased systemic absorption of RNAi agents and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of RNAi agents and nucleic acids.

(529) Microemulsions of the present disclosure can also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the RNAi agents and nucleic acids of the present disclosure. Penetration enhancers used in the microemulsions of the present disclosure can be classified as belonging to one of five broad categories—surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p. 92). Each of these classes has been discussed above.

(530) iii. Microparticles

(531) An RNAi agent of the disclosure may be incorporated into a particle, e.g., a microparticle. Microparticles can be produced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

(532) iv. Penetration Enhancers

(533) In one embodiment, the present disclosure employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly RNAi agents, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

(534) Penetration enhancers can be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (see e.g., Malmsten, M. *Surfactants and polymers in drug delivery*, Informa Health Care, New York, NY, 2002; Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p. 92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

(535) Surfactants (or “surface-active agents”) are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of RNAi agents through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (see e.g., Malmsten, M. *Surfactants and polymers in drug delivery*, Informa Health Care, New York, NY, 2002; Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p. 92); and perfluorochemical emulsions, such as FC-43. Takahashi et al., *J. Pharm. Pharmacol.*, 1988, 40, 252).

(536) Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C.sub.1-20 alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (see e.g., Touitou, E., et al. *Enhancement in Drug Delivery*, CRC Press, Danvers, MA, 2006; Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p. 92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33; El Hariri et al., *J. Pharm. Pharmacol.*, 1992, 44, 651-654).

(537) The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (see e.g., Malmsten, M. *Surfactants and polymers in drug delivery*, Informa Health Care, New York, NY, 2002; Brunton, Chapter 38 in: Goodman & Gilman's *The Pharmacological Basis of Therapeutics*, 9th Ed., Hardman et al. Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term “bile salts” includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucolic acid (sodium glucolate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether

(POE) (see e.g., Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto et al., J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita et al., J. Pharm. Sci., 1990, 79, 579-583).

(538) Chelating agents, as used in connection with the present disclosure, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of RNAi agents through the mucosa is enhanced. With regards to their use as penetration enhancers in the present disclosure, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315-339). Suitable chelating agents include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, lauric acid and N-amino acyl derivatives of beta-diketones (enamines) (see e.g., Katdare, A. et al., Excipient development for pharmaceutical, biotechnology, and drug delivery, CRC Press, Danvers, MA, 2006; Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur et al., J. Control Rel., 1990, 14, 43-51).

(539) As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of RNAi agents through the alimentary mucosa (see e.g., Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers includes, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., J. Pharm. Pharmacol., 1987, 39, 621-626).

(540) Agents that enhance uptake of RNAi agents at the cellular level can also be added to the pharmaceutical and other compositions of the present disclosure. For example, cationic lipids, such as lipofectin (Junichi et al, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (WO 97/30731), are also known to enhance the cellular uptake of dsRNAs.

(541) Other agents can be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

(542) vi. Excipients

(543) In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc.).

(544) Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral

administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present disclosure. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

(545) Formulations for topical administration of nucleic acids can include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions can also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

(546) Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

(547) vii. Other Components

(548) The compositions of the present disclosure can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present disclosure, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present disclosure. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

(549) Aqueous suspensions can contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol or dextran. The suspension can also contain stabilizers.

(550) In some embodiments, pharmaceutical compositions featured in the disclosure include (a) one or more RNAi agents and (b) one or more agents which function by a non-RNAi mechanism and which are useful in treating a coronavirus-associated disorder. Examples of such agents include, but are not limited to SSRIs, venlafaxine, bupropion, and atypical antipsychotics.

(551) Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds that exhibit high therapeutic indices are preferred.

(552) The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the disclosure lies generally within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the disclosure, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (e.g., achieving a decreased concentration of the polypeptide) that includes the IC₅₀ (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels

in plasma can be measured, for example, by high performance liquid chromatography.

(553) In addition to their administration, as discussed above, the RNAi agents featured in the disclosure can be administered in combination with other known agents effective in treatment of pathological processes mediated by nucleotide repeat expression. In any event, the administering physician can adjust the amount and timing of RNAi agent administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VII. Kits

(554) In certain aspects, the instant disclosure provides kits that include a suitable container containing a pharmaceutical formulation of a siRNA compound, e.g., a double-stranded siRNA compound, or siRNA compound, (e.g., a precursor, e.g., a larger siRNA compound which can be processed into a siRNA compound, or a DNA which encodes an siRNA compound, e.g., a double-stranded siRNA compound, or siRNA compound, or precursor thereof). In certain embodiments the individual components of the pharmaceutical formulation may be provided in one container.

Alternatively, it may be desirable to provide the components of the pharmaceutical formulation separately in two or more containers, e.g., one container for a siRNA compound preparation, and at least another for a carrier compound. The kit may be packaged in a number of different configurations such as one or more containers in a single box. The different components can be combined, e.g., according to instructions provided with the kit. The components can be combined according to a method described herein, e.g., to prepare and administer a pharmaceutical composition. The kit can also include a delivery device. For example, the kit can include a delivery device suitable for pulmonary system administration, e.g., a device suitable for oral inhalative administration including nebulizers, metered-dose inhalers, and dry powder inhalers.

VIII. Methods for Inhibiting Coronavirus Expression

(555) The present disclosure also provides methods of inhibiting expression of a coronavirus genome in a cell. The methods include contacting a cell with an RNAi agent, e.g., double stranded RNAi agent, a composition comprising a double stranded RNAi agent of the invention, or a pharmaceutical compositions comprising a double stranded RNAi agent of the invention in an amount effective to inhibit expression of a coronavirus genome in the cell, thereby inhibiting expression of coronavirus in the cell. In some embodiments, the methods include contacting a cell with two or more double stranded RNAi agents, as described herein, e.g., any two or more, e.g., 2, 3, or 4, of the dsRNA agents selected from the group of dsRNA agents in Tables 2-5. In certain embodiments of the methods including two or more double stranded RNAi agents, the two or more double stranded RNAi agents may be present in the same composition, in separate compositions, or any combination thereof. In some embodiments, the methods of the invention include contacting a cell with a composition comprising two or more, e.g., 2, 3, or 4, double stranded RNAi agents of the invention, e.g., any two or more of the dsRNA agents selected from the group of dsRNA agents in Tables 2-5. In certain embodiments of the disclosure, expression of a coronavirus genome is inhibited preferentially in the pulmonary system (e.g., lung, bronchial, alveoli) cells. In other embodiments of the disclosure, expression of a coronavirus genome is inhibited preferentially in the liver (e.g., hepatocytes). In certain embodiments of the disclosure, expression of a coronavirus genome is inhibited in the pulmonary system (e.g., lung, bronchial, alveoli) cells and in liver (e.g., hepatocytes) cells.

(556) Contacting of a cell with a RNAi agent, e.g., a double stranded RNAi agent, may be done in vitro or in vivo. Contacting a cell in vivo with the RNAi agent includes contacting a cell or group of cells within a subject, e.g., a human subject, with the RNAi agent. Combinations of in vitro and in vivo methods of contacting a cell are also possible.

(557) In some embodiments of the methods of the invention which include contacting a cell with two or more double stranded RNAi agents, as described herein, e.g., any two or more, e.g., 2, 3, or 4, of the dsRNA agents selected from the group of dsRNA agents in Tables 2-5, the cell may be contacted with a first agent (or a composition comprising a first agent) at a first time, a second

agent (or a composition comprising a second agent) at a second time, a third agent (or a composition comprising a third agent) at a third time, and a fourth agent (or a composition comprising a fourth agent) at a fourth time; or the cell may be contacted with all of the agents (or a composition comprising all of the agents) at the same time. Alternatively, the cell may be contacted with a first agent (or a composition comprising a first agent) at a first time and a second, third, and/or fourth agent (or a composition comprising a second, third, and/or fourth agent) at a second time. Other combinations of contacting the cell with two or more agents (or compositions comprising two or more dsRNA agents) of the invention are also contemplated.

(558) Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In some embodiments, the targeting ligand is a carbohydrate moiety, e.g., a GalNAc ligand, or any other ligand that directs the RNAi agent to a site of interest.

(559) The term “inhibiting,” as used herein, is used interchangeably with “reducing,” “silencing,” “downregulating,” “suppressing” and other similar terms, and includes any level of inhibition. In certain embodiments, a level of inhibition, e.g., for an RNAi agent of the instant disclosure, can be assessed in cell culture conditions, e.g., wherein cells in cell culture are transfected via LipofectamineTM-mediated transfection at a concentration in the vicinity of a cell of 10 nM or less, 1 nM or less, etc. Knockdown of a given RNAi agent can be determined via comparison of pre-treated levels in cell culture versus post-treated levels in cell culture, optionally also comparing against cells treated in parallel with a scrambled or other form of control RNAi agent. Knockdown in cell culture of, e.g., preferably 50% or more, can thereby be identified as indicative of “inhibiting” or “reducing”, “downregulating” or “suppressing”, etc. having occurred. It is expressly contemplated that assessment of targeted mRNA or encoded protein levels (and therefore an extent of “inhibiting”, etc. caused by a RNAi agent of the disclosure) can also be assessed in in vivo systems for the RNAi agents of the instant disclosure, under properly controlled conditions as described in the art.

(560) The phrase “inhibiting expression of a coronavirus genome” or “inhibiting expression of coronavirus,” as used herein, includes inhibition of expression of any coronavirus genome as well as variants or mutants of a coronavirus genome that encode a coronavirus protein. Thus, the coronavirus genome may be a wild-type coronavirus genome, a mutant coronavirus genome, or a transgenic coronavirus genome in the context of a genetically manipulated cell, group of cells, or organism.

(561) “Inhibiting expression of a coronavirus genome” includes any level of inhibition of a coronavirus genome, e.g., at least partial suppression of the expression of a coronavirus genome, such as an inhibition by at least 20%. In certain embodiments, inhibition is by at least 30%, at least 40%, preferably at least 50%, at least about 60%, at least 70%, at least about 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%; or to below the level of detection of the assay method. In a preferred method, inhibition is measured at a 10 nM concentration of the siRNA using the luciferase assay provided in Example 1.

(562) The expression of a coronavirus genome may be assessed based on the level of any variable associated with coronavirus genome expression, e.g., coronavirus RNA level or coronavirus protein level or coronavirus replication.

(563) Inhibition may be assessed by a decrease in an absolute or relative level of one or more of these variables compared with a control level. The control level may be any type of control level that is utilized in the art, e.g., a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, e.g., buffer only control or inactive agent control).

(564) In some embodiments of the methods of the disclosure, expression of a coronavirus genome is inhibited by at least 20%, 30%, 40%, preferably at least 50%, 60%, 70%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In certain embodiments, the methods include a

clinically relevant inhibition of expression of coronavirus, e.g. as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of a coronavirus genome.

(565) Inhibition of the expression of a coronavirus genome may be manifested by a reduction of the amount of genome expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which a coronavirus genome is transcribed and which has or have been treated (e.g., by contacting the cell or cells with a RNAi agent of the disclosure, or by administering a RNAi agent of the disclosure to a subject in which the cells are or were present) such that the expression of a coronavirus genome is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s) not treated with a RNAi agent or not treated with a RNAi agent targeted to the genome of interest). The degree of inhibition may be expressed in terms of:

$$(566) \frac{(\text{genome in control cells}) - (\text{genome in treated cells})}{(\text{genome in control cells})} 100\%$$

(567) In other embodiments, inhibition of the expression of a coronavirus genome may be assessed in terms of a reduction of a parameter that is functionally linked to a coronavirus genome expression, e.g., coronavirus protein expression. Coronavirus genome silencing may be determined in any cell expressing a coronavirus genome, either endogenous or heterologous from an expression construct, and by any assay known in the art.

(568) Inhibition of the expression of a coronavirus protein may be manifested by a reduction in the level of the coronavirus protein that is expressed by a cell or group of cells (e.g., the level of protein expressed in a sample derived from a subject). As explained above, for the assessment of genome suppression, the inhibition of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

(569) A control cell or group of cells that may be used to assess the inhibition of the expression of a coronavirus genome includes a cell or group of cells that has not yet been contacted with an RNAi agent of the disclosure. For example, the control cell or group of cells may be derived from an individual subject (e.g., a human or animal subject) prior to treatment of the subject with an RNAi agent.

(570) The level of coronavirus genome that is expressed by a cell or group of cells may be determined using any method known in the art for assessing RNA expression. In one embodiment, the level of expression of coronavirus in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, e.g., RNA of the coronavirus genome. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy™ RNA preparation kits (Qiagen®) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays, northern blotting, in situ hybridization, and microarray analysis. Circulating coronavirus genome may be detected using methods the described in WO2012/177906, the entire contents of which are hereby incorporated herein by reference.

(571) In some embodiments, the level of expression of coronavirus is determined using a nucleic acid probe. The term “probe”, as used herein, refers to any molecule that is capable of selectively binding to a specific coronavirus nucleic acid or protein, or fragment thereof. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

(572) Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of RNA levels involves contacting the isolated RNA with a nucleic acid molecule (probe) that can hybridize to coronavirus RNA. In one embodiment, the

RNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated RNA on an agarose gel and transferring the RNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the RNA is contacted with the probe(s), for example, in an Affymetrix® gene chip array. A skilled artisan can readily adapt known RNA detection methods for use in determining the level of coronavirus RNA.

(573) An alternative method for determining the level of expression of coronavirus in a sample involves the process of nucleic acid amplification or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, e.g., by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the disclosure, the level of expression of coronavirus is determined by quantitative fluorogenic RT-PCR (i.e., the TaqMan™ System), by a Dual-Glo® Luciferase assay, or by other art-recognized method for measurement of coronavirus genome expression or protein level.

(574) The expression level of coronavirus RNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of coronavirus expression level may also comprise using nucleic acid probes in solution.

(575) In some embodiments, the level of RNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of this PCR method is described and exemplified in the Examples presented herein. Such methods can also be used for the detection of coronavirus nucleic acids.

(576) The level of coronavirus protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like. Such assays can also be used for the detection of proteins indicative of the presence or replication of coronavirus proteins.

(577) In some embodiments, the efficacy of the methods of the disclosure in the treatment of a coronavirus-related disease is assessed by a decrease in coronavirus genome level (e.g, by assessment of a coronavirus level, e.g., in the lung, by biopsy, or otherwise).

(578) In some embodiments, the efficacy of the methods of the disclosure in the treatment of a coronavirus-related disease is assessed by a decrease in coronavirus genome level (e.g, by assessment of a liver sample for coronavirus level, by biopsy, or otherwise).

(579) In some embodiments of the methods of the disclosure, the RNAi agent is administered to a subject such that the RNAi agent is delivered to a specific site within the subject. The inhibition of expression of a coronavirus genome may be assessed using measurements of the level or change in the level of coronavirus genome or coronavirus protein in a sample derived from a specific site within the subject, e.g., lung and/or liver cells. In certain embodiments, the methods include a

clinically relevant inhibition of expression of coronavirus, e.g. as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of coronavirus. (580) As used herein, the terms detecting or determining a level of an analyte are understood to mean performing the steps to determine if a material, e.g., protein, RNA, is present. As used herein, methods of detecting or determining include detection or determination of an analyte level that is below the level of detection for the method used.

IX. Methods of Treating or Preventing Coronavirus-Associated Diseases

(581) The present disclosure also provides methods of using an RNAi agent of the disclosure, two or more, e.g., 2, 3, or 4, double stranded RNAi agents of the disclosure (e.g., each agent independently targeting a portion of a coronavirus genome), a composition (such as a pharmaceutical composition) containing a RNAi agent of the disclosure, two or more, e.g., 2, 3, or 4, compositions (such as pharmaceutical compositions), each independently comprising a double stranded RNAi agent of the invention, or a composition comprising two or more, e.g., 2, 3, or 4, double stranded RNAi agents of the disclosure to reduce or inhibit coronavirus expression in a cell. The methods include contacting the cell with a dsRNA of the disclosure, a composition of the disclosure, or a pharmaceutical composition of the disclosure and maintaining the cell for a time sufficient to obtain degradation of the RNA transcripts of a coronavirus genome, thereby inhibiting expression of the coronavirus genome in the cell. Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of coronavirus may be determined by determining the RNA expression level of a coronavirus genome using methods routine to one of ordinary skill in the art, e.g., northern blotting, qRT-PCR; by determining the protein level of a coronavirus protein using methods routine to one of ordinary skill in the art, such as western blotting, immunological techniques.

(582) In the methods of the disclosure the cell may be contacted in vitro or in vivo, i.e., the cell may be within a subject.

(583) A cell suitable for treatment using the methods of the disclosure may be any cell that expresses a coronavirus genome. A cell suitable for use in the methods of the disclosure may be a mammalian cell, e.g., a primate cell (such as a human cell or a non-human primate cell, e.g., a monkey cell or a chimpanzee cell), a non-primate cell (such as a rat cell, or a mouse cell. In one embodiment, the cell is a human cell, e.g., a human lung cell. In one embodiment, the cell is a human cell, e.g., a human liver cell. In one embodiment, the cell is a human cell, e.g., a human lung cell and a human liver cell.

(584) Coronavirus genome expression is inhibited in the cell by at least about 30, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or about 100%, i.e., to below the level of detection. In preferred embodiments, coronavirus expression is inhibited by at least 50%.

(585) The in vivo methods of the disclosure may include administering to a subject a composition containing a RNAi agent, where the RNAi agent includes a nucleotide sequence that is complementary to at least a part of target coronavirus sequence, e.g., an RNA transcript of the coronavirus genome, of the coronavirus to be treated. In some embodiments, the subject is administered two or more, e.g., 2, 3, or 4, compositions, each independently comprising an RNAi agent of the invention. The compositions may be the same or different. In other embodiments, the subject is administered a composition comprising two or more, e.g., 2, 3, or 4, dsRNA agents, each independently targeting a portion of the coronavirus genome.

(586) When the organism to be treated is a mammal such as a human, the composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (e.g., intraventricular, intraparenchymal, and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection. In certain embodiments, the compositions are administered

by pulmonary system delivery, e.g., inhalation or intranasal delivery.

(587) In some embodiments, the administration is via a depot injection. A depot injection may release the RNAi agent in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, e.g., a desired inhibition of coronavirus, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

(588) In one embodiment, the double-stranded RNAi agent is administered by pulmonary system administration, e.g., intranasal administration or oral inhalative administration. Pulmonary system administration may be via a syringe, a dropper, atomization, or use of device, e.g., a passive breath driven or active power driven single/-multiple dose dry powder inhaler (DPI) device.

(589) The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

(590) In one aspect, the present disclosure also provides methods for inhibiting the expression of a coronavirus genome in a mammal. The methods include administering to the mammal a dsRNA that targets a coronavirus genome in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the RNA transcript of the coronavirus genome, thereby inhibiting expression of the coronavirus genome in the cell. In some embodiments, the dsRNA is present in a composition, such as a pharmaceutical composition. In some embodiments, the mammal is administered two or more, e.g., 2, 3, or 4, dsRNA agents of the invention. In some embodiments, each dsRNA agent administered to the subject is independently present in a composition. In other embodiments, the mammal is administered a composition comprising two or more, e.g., 2, 3, or 4, dsRNAs of the invention.

(591) Reduction in genome expression can be assessed by any methods known in the art and by methods, e.g. qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known in the art and by methods, e.g. ELISA, described herein. In one embodiment, a lung biopsy sample serves as the tissue material for monitoring the reduction in coronavirus genome or protein expression (or of a proxy therefore).

(592) The present disclosure further provides methods of treatment of a subject in need thereof. The treatment methods of the disclosure include administering an RNAi agent of the disclosure to a subject, e.g., a subject that would benefit from inhibition of coronavirus expression, in a therapeutically effective amount of a RNAi agent targeting a coronavirus genome or a pharmaceutical composition comprising a RNAi agent targeting a coronavirus genome. In some embodiments, the subject is administered a therapeutically effective amount of two or more, e.g., 2, 3, or 4, dsRNA agents of the invention. In some embodiments, each dsRNA agent administered to the subject is independently present in a composition. In other embodiments, the subject is administered a composition comprising two or more, e.g., 2, 3, or 4, dsRNAs of the invention.

(593) In addition, the present disclosure provides methods of preventing, treating or inhibiting the progression of a coronavirus-associated disease or disorder, such as severe acute respiratory syndrome (SARS), the Middle East respiratory syndrome (MERS), and severe acute respiratory syndrome-2 (SARS-2). The methods include administering to the subject a therapeutically effective amount of any of the RNAi agent, e.g., dsRNA agents, or the pharmaceutical composition provided herein, thereby preventing, treating, or inhibiting the progression of the coronavirus-associated disease or disorder in the subject. In some embodiments, the mammal is administered a therapeutically effective amount of two or more, e.g., 2, 3, or 4, dsRNA agents of the invention. In some embodiments, each dsRNA agent administered to the subject is independently present in a composition. In other embodiments, the mammal is administered a composition comprising two or more, e.g., 2, 3, or 4, dsRNAs of the invention.

(594) In some embodiments of the methods of the invention which include administering two or

more double stranded RNAi agents, as described herein, e.g., any two or more, e.g., 2, 3, or 4, of the dsRNA agents selected from the group of dsRNA agents in Tables 2-5, the subject may be administered a first agent (or a composition comprising a first agent) at a first time, a second agent (or a composition comprising a second agent) at a second time, a third agent (or a compositions comprising a third agent) at a third time, and a fourth agent (or a composition comprising a fourth agent) at a fourth time; or the subject may be administered all of the agents (or a composition comprising all of the agents at the same time, Alternatively, the subject may be administered a first agent (or a composition comprising a first agent) at a first time and a second, third, and/or fourth agent (or a composition comprising a second, third and/or fourth agent) at a second time. Other combinations of contacting the cell with two or more agents of the invention are also contemplated.

(595) An RNAi agent of the disclosure may be administered as a “free RNAi agent.” A free RNAi agent is administered in the absence of a pharmaceutical composition. The naked RNAi agent may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the RNAi agent can be adjusted such that it is suitable for administering to a subject.

(596) Alternatively, an RNAi agent of the disclosure may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

(597) Subjects that would benefit from a reduction or inhibition of coronavirus genome expression are those having a coronavirus-associated disease, e.g., subjects of an age greater than 60 years and/or subjects who are immunocompromised.

(598) The disclosure further provides methods for the use of a RNAi agent or a pharmaceutical composition thereof, e.g., for treating a subject that would benefit from reduction or inhibition of coronavirus expression, e.g., a subject having a coronavirus-associated disorder, in combination with other pharmaceuticals or other therapeutic methods, e.g., with known pharmaceuticals or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders. For example, in certain embodiments, an RNAi agent targeting coronavirus is administered in combination with, e.g., an agent useful in treating a coronavirus-associated disorder as described elsewhere herein or as otherwise known in the art. For example, additional agents and treatments suitable for treating a subject that would benefit from reduction in coronavirus expression, e.g., a subject having a coronavirus-associated disorder, may include agents currently used to treat symptoms of coronavirus. The RNAi agent and additional therapeutic agents may be administered at the same time or in the same combination, e.g., via pulmonary system administration, or the additional therapeutic agent can be administered as part of a separate composition or at separate times or by another method known in the art or described herein.

(599) Exemplary additional therapeutics and treatments include, for example, an antiviral agent, an immune stimulator, a therapeutic vaccine, a viral entry inhibitor, and a combination of any of the foregoing.

(600) In one embodiment, the method includes administering a composition featured herein such that expression of the target coronavirus genome is decreased, for at least one month. In preferred embodiments, expression is decreased for at least 2 months, or 6 months.

(601) Preferably, the RNAi agents useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target coronavirus genome. Compositions and methods for inhibiting the expression of these genes using RNAi agents can be prepared and performed as described herein.

(602) Administration of the dsRNA according to the methods of the disclosure may result in a reduction of the severity, signs, symptoms, or markers of such diseases or disorders in a patient with a coronavirus-associated disorder. By “reduction” in this context is meant a statistically significant or clinically significant decrease in such level. The reduction can be, for example, at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%,

85%, 90%, 95%, or about 100%.

(603) Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of a RNAi agent targeting coronavirus or pharmaceutical composition thereof, “effective against” a coronavirus-associated disorder indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as an improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating coronavirus-associated disorders and the related causes.

(604) A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50%, or more can be indicative of effective treatment. Efficacy for a given RNAi agent drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

(605) Alternatively, the efficacy can be measured by a reduction in the severity of disease as determined by one skilled in the art of diagnosis based on a clinically accepted disease severity grading scale. Any positive change resulting in e.g., lessening of severity of disease measured using the appropriate scale, represents adequate treatment using a RNAi agent or RNAi agent formulation as described herein.

(606) Subjects can be administered a therapeutic amount of dsRNA, such as about 0.01 mg/kg to about 200 mg/kg.

(607) The RNAi agent can be administered via the pulmonary system over a period of time, on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. Administration of the RNAi agent can reduce coronavirus genome levels, e.g., in a cell, tissue, blood, lung sample or other compartment of the patient by at least 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or at least about 99% or more. In a preferred embodiment, administration of the RNAi agent can reduce coronavirus genome levels, e.g., in a cell, tissue, blood, pulmonary system sample or other compartment of the patient by at least 50%.

(608) Before administration of a full dose of the RNAi agent, patients can be administered a smaller dose, such as a 5% infusion reaction, and monitored for adverse effects, such as an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (e.g., TNF-alpha or INF-alpha) levels.

(609) Alternatively, the RNAi agent can be administered by pulmonary system administration or subcutaneously, i.e., by subcutaneous injection. One or more injections may be used to deliver the desired, e.g., monthly dose of RNAi agent to a subject. The injections may be repeated over a period of time. The administration may be repeated on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regime may include administration of a therapeutic amount of RNAi agent on a regular basis, such as monthly or extending to once a quarter, twice per year, once per year. In certain embodiments, the RNAi agent is administered about once per month to about once per

quarter (i.e., about once every three months).

(610) Unless otherwise defined, 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 invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the RNAi agents and methods featured in the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

(611) An informal Sequence Listing is filed herewith and forms part of the specification as filed.

(612) This invention is further illustrated by the following examples which should not be construed as limiting. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are hereby incorporated herein by reference.

EXAMPLES

Example 1. iRNA Synthesis

(613) Source of Reagents

(614) Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

(615) siRNA Design

(616) The selection of siRNA designs targeting coronavirus was driven by two primary factors: a) potency and b), the desire to employ siRNA with near-perfect matches with greater than 90% fractional coverage of the known coronaviruses sequences, e.g., SARS-CoV, MERS-CoV and SARS-CoV-2.

(617) A detailed list of a set of the unmodified siRNA sense and antisense strand sequences targeting SARS-CoV-2 is shown in Table 2 and 4.

(618) A detailed list of a set of the modified siRNA sense and antisense strand sequences targeting SARS-CoV-2 is shown in Tables 3 and 5.

(619) siRNA Synthesis

(620) siRNAs were synthesized and annealed using routine methods known in the art. Briefly, siRNA sequences were synthesized on a 1 μ mol scale using a Mermade 192 synthesizer (BioAutomation) with phosphoramidite chemistry on solid supports. The solid support was controlled pore glass (500-1000 Å) loaded with a custom GalNAc ligand (3'-GalNAc conjugates), universal solid support (AM Chemicals), or the first nucleotide of interest. Ancillary synthesis reagents and standard 2-cyanoethyl phosphoramidite monomers (2'-deoxy-2'-fluoro, 2'-O-methyl, RNA, DNA) were obtained from Thermo-Fisher (Milwaukee, WI), Hongene (China), or Chemgenes (Wilmington, MA, USA). Additional phosphoramidite monomers were procured from commercial suppliers, prepared in-house, or procured using custom synthesis from various CMOs. Phosphoramidites were prepared at a concentration of 100 mM in either acetonitrile or 9:1 acetonitrile:DMF and were coupled using 5-Ethylthio-1H-tetrazole (ETT, 0.25 M in acetonitrile) with a reaction time of 400 s. Phosphorothioate linkages were generated using a 100 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (9:1 v/v). Oxidation time was 5 minutes. All sequences were synthesized with final removal of the DMT group ("DMT-Off").

(621) Upon completion of the solid phase synthesis, solid-supported oligoribonucleotides were treated with 300 μ L of Methylamine (40% aqueous) at room temperature in 96 well plates for approximately 2 hours to afford cleavage from the solid support and subsequent removal of all additional base-labile protecting groups. For sequences containing any natural ribonucleotide

linkages (2'-OH) protected with a tert-butyl silyl (TBDMS) group, a second deprotection step was performed using TEA.3HF (triethylamine trihydrofluoride). To each oligonucleotide solution in aqueous methylamine was added 200 μ L of dimethyl sulfoxide (DMSO) and 300 μ L TEA.3HF and the solution was incubated for approximately 30 mins at 60° C. After incubation, the plate was allowed to come to room temperature and crude oligonucleotides were precipitated by the addition of 1 mL of 9:1 acetone:ethanol or 1:1 ethanol:isopropanol. The plates were then centrifuged at 4° C. for 45 mins and the supernatant carefully decanted with the aid of a multichannel pipette. The oligonucleotide pellet was resuspended in 20 mM NaOAc and subsequently desalted using a HiTrap size exclusion column (5 mL, GE Healthcare) on an Agilent LC system equipped with an autosampler, UV detector, conductivity meter, and fraction collector. Desalted samples were collected in 96 well plates and then analyzed by LC-MS and UV spectrometry to confirm identity and quantify the amount of material, respectively.

(622) Duplexing of single strands was performed on a Tecan liquid handling robot. Sense and antisense single strands were combined in an equimolar ratio to a final concentration of 10 μ M in 1 \times PBS in 96 well plates, the plate sealed, incubated at 100° C. for 10 minutes, and subsequently allowed to return slowly to room temperature over a period of 2-3 hours. The concentration and identity of each duplex was confirmed and then subsequently utilized for in vitro screening assays.

Example 2. In Vitro Screening of siRNA Duplexes

(623) SARS-CoV-2 Expression Plasmid Construction

(624) Dual-Glo® Luciferase constructs generated in the psiCHECK-2 vector which was obtained from Promega (Catalog No. C8021 (Madison, WI)). The SARS-CoV-2 vector sequences were synthesized and incorporated into the psiCHECK2 vector by Blue Heron Biotech (Bothell, WA). The final constructs are referred to CV-concat-02 and CV-concat-21 (see, FIG. 2). The nucleotide sequence of SARS-CoV-2 incorporated into the psiCHECK-2 vector to generate CV-concat-02 is provided in SEQ ID NO:3 and the nucleotide sequence of SARS-CoV-2 incorporated into the psiCHECK-2 vector to generate CV-concat-21 is provided in SEQ ID NO:4,

(625) Cell Culture and Transfections

(626) Cos7 cells (ATCC, Manassas, VA) were grown to near confluence at 37° C. in an atmosphere of 5% CO₂ in DMEM (ATCC) supplemented with 10% FBS, before being released from the plate by trypsinization. Cos7 cells were co-transfected with psiCHECK2-CV-concat-02 or psiCHECK2-CV-concat-21 plasmids and siRNA in 384-well plates at a density of 5 \times 10⁴ cells per well using Lipofectamine™ 2000 transfection reagent (Catalog No. 11668019, Invitrogen (Carlsbad, CA)). For each well of a 384 well plate, 0.1 μ L of Lipofectamine was added to 50 ng of plasmid vector in 5 μ L of Opti-MEM and allowed to complex at room temperature for 15 minutes. The mixture was then added to the cells which were resuspended in 40 μ L of fresh complete media. Cells were incubated for 48 hours before luciferase is measured.

(627) Single dose experiments are performed at 1 nM or 10 nM final duplex concentration.

(628) Dual-Glo® Luciferase Assay (Promega, Cat No. E2980)

(629) Forty-eight hours after the siRNAs were transfected, Firefly (transfection control) and *Renilla* (fused to SARS-CoV-2 target sequence) luciferase were measured. First, media was removed from cells. Then Firefly luciferase activity was measured by adding 20 μ L of Dual-Glo® Luciferase Reagent equal to the culture medium volume to each well and mix. The mixture was incubated at room temperature for 30 minutes before luminescence (500 nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal. *Renilla* luciferase activity was measured by adding 20 μ L of room temperature of Dual-Glo® Stop & Glo® Reagent to each well and the plates are incubated for 10-15 minutes before luminescence was again measured to determine the *Renilla* luciferase signal. The Dual-Glo® Stop & Glo® Reagent, quench the firefly luciferase signal and sustain luminescence for the *Renilla* luciferase reaction. siRNA activity was determined by normalizing the *Renilla* (SARS-CoV-2) signal to the Firefly (control) signal within each well. The magnitude of siRNA activity was then assessed relative to cells that were

transfected with the same vector but are not treated with siRNA or are treated with a non-targeting siRNA. All transfections are done at n=4 or greater.

(630) The results of the single dose screens of the duplexes in Tables 2 and 3 are provided in Table 6 and the results of the single dose screens of the duplexes in Tables 4 and 5 are provided in Table 7.

(631) TABLE-US-00002 TABLE 1 Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds. Abbreviation Nucleotide(s) A Adenosine-3'-phosphate Ab beta-L-adenosine--phosphate Abs beta-L-adenosine-3'-phosphorothioate Af 2'-fluoroadenosine-3'-phosphate Afs 2'-fluoroadenosine-3'-phosphorothioate As adenosine-3'-phosphorothioate C cytidine-3'-phosphate Cb beta-L-cytidine--phosphate Cbs beta-L-cytidine-3'-phosphorothioate Cf 2'-fluorocytidine-3'-phosphate Cfs 2'-fluorocytidine-3'-phosphorothioate Cs cytidine-3'-phosphorothioate G guanosine-3'-phosphate Gb beta-L-guanosine--phosphate Gbs beta-L-guanosine--phosphorothioate Gf 2'-fluoroguanosine-3'-phosphate Gfs 2'-fluoroguanosine-3'-phosphorothioate Gs guanosine-3'-phosphorothioate T 5'-methyluridine-3'-phosphate Tf 2'-fluoro-5-methyluridine-3'-phosphate Tfs 2'-fluoro-5-methyluridine-3'-phosphorothioate Ts 5-methyluridine-3'-phosphorothioate U Uridine-3'-phosphate Uf 2'-fluorouridine-3'-phosphate Ufs 2'-fluorouridine-3'-phosphorothioate Us uridine-3'-phosphorothioate N any nucleotide, modified or unmodified a 2'-O-methyladenosine-3'-phosphate as 2'-O-methyladenosine-3'-phosphorothioate c 2'-O-methylcytidine-3'-phosphate cs 2'-O-methylcytidine-3'-phosphorothioate g 2'-O-methylguanosine-3'-phosphate gs 2'-O-methylguanosine-3'-phosphorothioate t 2'-O-methyl-5-methyluridine-3'-phosphate ts 2'-O-methyl-5-methyluridine-3'-phosphorothioate u 2'-O-methyluridine-3'-phosphate us 2'-O-methyluridine-3'-phosphorothioate s phosphorothioate linkage L96 N-[tris(GalNAc-alkyl)-amidodecanoyl]-4-hydroxyprolinol(Hyp-(GalNAc-alkyl)3)  Y34 2-hydroxymethyl-tetrahydrofuran-4-methoxy-3-phosphate (abasic 2'-OMe furanose) Y44 inverted abasic DNA (2-hydroxymethyl-tetrahydrofuran-5-phosphate) L10 N-(cholesterylcarboxamidocaproyl)-4-hydroxyprolinol (Hyp-C6-Chol) (Agn) Adenosine-glycol nucleic acid (GNA) (Cgn) Cytidine-glycol nucleic acid (GNA) (Ggn) Guanosine-glycol nucleic acid (GNA) (Tgn) Thymidine-glycol nucleic acid (GNA) S-Isomer P Phosphate VP Vinyl-phosphonate dA -deoxyadenosine--phosphate dAs -deoxyadenosine--phosphorothioate dC -deoxycytidine--phosphate dCs -deoxycytidine--phosphorothioate dG -deoxyguanosine--phosphate dGs -deoxyguanosine--phosphorothioate dT -deoxythymidine--phosphate dTs -deoxythymidine--phosphorothioate dU -deoxyuridine dUs -deoxyuridine--phosphorothioate (C2p) cytidine--phosphate (G2p) guanosine--phosphate (U2p) uridine--phosphate (A2p) adenosine--phosphate (Ahd) 2'-O-hexadecyl-adenosine-3'-phosphate (Ahds) 2'-O-hexadecyl-adenosine-3'-phosphorothioate (Chd) 2'-O-hexadecyl-cytidine-3'-phosphate (Chds) 2'-O-hexadecyl-cytidine-3'-phosphorothioate (Ghd) 2'-O-hexadecyl-guanosine-3'-phosphate (Ghds) 2'-O-hexadecyl-guanosine-3'-phosphorothioate (Uhd) 2'-O-hexadecyl-uridine-3'-phosphate (Uhds) 2'-O-hexadecyl-uridine-3'-phosphorothioate

(632) TABLE-US-00003 TABLE 2 Unmodified Sense and Antisense Strand
Coronavirus dsRNA Sequences Strand of the Viral RNA SEQ SEQ Targeted Duplex Sense
Strand ID Antisense Strand ID Target RNA mRNA mRNA Targets Targets Targets by the
Name Sequence 5' to 3' NO: Sequence 5' to 3' NO Accession Version start end
SARS2? SARS? MERS? Agent AD- AUCUGUUCUCUAAACGAACU 17

AAAGUUCGUUUUAGAGAACAGAU 372 BetaCoV/Wuhan-Hu- 55 77 TRUE TRUE FALSE +
1183935 U U 1/2019|EPI_ISL_402125 AD- UCUGUUCUCUAAACGAACUU 18
AAAGUUCGUUUUAGAGAACAGAU 373 BetaCoV/Wuhan-Hu- 56 78 TRUE TRUE FALSE +
1183936 U C 1/2019|EPI_ISL_402125 AD- GUUCUCUAAACGAACUUUAA 19
UUUAAAGUUCGUUUUAGAGAACAA 374 BetaCoV/Wuhan-Hu- 59 81 TRUE TRUE FALSE +
1183937 A G 1/2019|EPI_ISL_402125 AD- CUGCUUACGGUUUCGUCCGU 20
CACGGACGAAACCGUAAGCAGC 375 BetaCoV/Wuhan-Hu- 188 210 TRUE TRUE FALSE +
1183938 G C 1/2019|EPI_ISL_402125 AD- GGUGUGACCGAAAGGUAAGA 21
AUCUUACCUUUCGGUCACACCC 376 BetaCoV/Wuhan-Hu- 245 267 TRUE TRUE FALSE +
1183939 U G 1/2019|EPI_ISL_402125 AD- CCGAAAGGUAAGAUGGAGAG 22
GCUCUCCAUCUUACCUUUCGGU 377 BetaCoV/Wuhan-Hu- 252 274 TRUE TRUE FALSE +
1183940 C C 1/2019|EPI_ISL_402125 AD- AAACACACGUCCAACUCAGU 23
AACUGAGUUGGACGUGUGUUUU 378 BetaCoV/Wuhan-Hu- 295 317 TRUE TRUE FALSE +
1183941 U C 1/2019|EPI_ISL_402125 AD- ACACACGUCCAACUCAGUUU 24
CAAACUGAGUUGGACGUGUGUU 379 BetaCoV/Wuhan-Hu- 297 319 TRUE TRUE FALSE +
1183942 G U 1/2019|EPI_ISL_402125 AD- GUCCAACUCAGUUUGCCUGU 25
AACAGGCAAACUGAGUUGGACG 380 BetaCoV/Wuhan-Hu- 303 325 TRUE TRUE FALSE +
1183943 U U 1/2019|EPI_ISL_402125 AD- CUUCUUCGUAAGAACGGUAA 26
AUUACCGUUCUUACGAAGAAGA 381 BetaCoV/Wuhan-Hu- 627 649 TRUE TRUE FALSE +
1183944 U A 1/2019|EPI_ISL_402125 AD- UUCUUCGUAAGAACGGUAAU 27
UAUUACCGUUCUUACGAAGAAG 382 BetaCoV/Wuhan-Hu- 628 650 TRUE TRUE FALSE +
1183945 A A 1/2019|EPI_ISL_402125 AD- UCUUCGUAAGAACGGUAAUA 28
UUAUUACCGUUCUUACGAAGAA 383 BetaCoV/Wuhan-Hu- 629 651 TRUE TRUE FALSE +
1183946 A G 1/2019|EPI_ISL_402125 AD- UUCCUUAACUUCUCUUCAA 29
CUUGAAGAGAAGUUUAAGGAA 384 BetaCoV/Wuhan-Hu- 2156 2178 TRUE FALSE FALSE
- 1183947 G GG 1/2019|EPI_ISL_402125 AD- UCACCUAAAUUCAAGGCUUU 30
UAAAGCCUUGAAUUUAGGUGAA 385 BetaCoV/Wuhan-Hu- 2365 2387 TRUE FALSE
FALSE - 1183948 A A 1/2019|EPI_ISL_402125 AD- CUCUGGAUUUAACACACUUU 31
GAAAGUGUGUUAUAAUCCAGAGA 386 BetaCoV/Wuhan-Hu- 2418 2440 TRUE FALSE
FALSE - 1183949 C A 1/2019|EPI_ISL_402125 AD- CUUCUCAUUAAGUACUUUAU 32
GAUAAAGUACUUAUUGAGAAG 387 BetaCoV/Wuhan-Hu- 2813 2835 TRUE FALSE FALSE
- 1183950 C UG 1/2019|EPI_ISL_402125 AD- ACUCUUCUUCUUCACAAUCA 33
GUGAUUGUGAAGAAGAAGAGU 388 BetaCoV/Wuhan-Hu- 3066 3088 TRUE FALSE FALSE
- 1183951 C UU 1/2019|EPI_ISL_402125 AD- UUAUUUAAAACUUCUGACA 34
UUGUCAGUAAGUUUUAUAAUAC 389 BetaCoV/Wuhan-Hu- 3353 3375 TRUE TRUE FALSE
+ 1183952 A C 1/2019|EPI_ISL_402125 AD- UAUCUACACAAACUCUUAAA 35
CUUUAAGAGUUUGUGUAGAUAC 390 BetaCoV/Wuhan-Hu- 3747 3769 TRUE FALSE
FALSE - 1183953 G U 1/2019|EPI_ISL_402125 AD- AUCUUGUUUUCUCUGUUCAA 36
GUUGAACAGAGAAAACAAGAUG 391 BetaCoV/Wuhan-Hu- 3929 3951 TRUE FALSE
FALSE - 1183954 C A 1/2019|EPI_ISL_402125 AD- CACUUUUAUCACCUCUCUUA 37
UUAAGAGAGGUGAUAAAAGUG 392 BetaCoV/Wuhan-Hu- 4848 4870 TRUE FALSE FALSE
- 118395 A UA 1/2019|EPI_ISL_402125 AD- UCUCUGAAGAAGUAGUGGAA 38
UUUCCACUACUUCUUCAGAGAC 393 BetaCoV/Wuhan-Hu- 6403 6425 TRUE TRUE FALSE
+ 1183956 A U 1/2019|EPI_ISL_402125 AD- UCUGAAGAAGUAGUGGAAAA 39
AUUUUCCACUACUUCUUCAGAG 394 BetaCoV/Wuhan-Hu- 6405 6427 TRUE TRUE FALSE
+ 1183957 U A 1/2019|EPI_ISL_402125 AD- CUGAAGAAGUAGUGGAAAAU 40
GAUUUCCACUACUUCUUCAGA 395 BetaCoV/Wuhan-Hu- 6406 6428 TRUE TRUE FALSE
+ 1183958 C G 1/2019|EPI_ISL_402125 AD- AGUGGAAAUCCUACCAUAC 41
UGUAUGGUAGGAUUUCCACUA 396 BetaCoV/Wuhan-Hu- 6416 6438 TRUE TRUE FALSE
+ 1183959 A C 1/2019|EPI_ISL_402125 AD- GUGGAAAUCCUACCAUACA 42

CUGUAUAGGAUUUAGGUAUUUACCACU 397 BetaCoV/Wuhan-Hu- 6417 6439 TRUE TRUE FALSE
+ 1183960 G A 1/2019|EPL_ISL_402125 AD- GCUCUUCUAAACCUCAUAAA 43
UUUUAUGAGGUUUAGAAGAGC 398 BetaCoV/Wuhan-Hu- 9454 9476 TRUE FALSE FALSE
- 1183961 A UU 1/2019|EPL_ISL_402125 AD- AUGGUUCACCAUCUGGUGUU 44
AACACCAGAUGGUGAACCAUU 399 BetaCoV/Wuhan-Hu- 10408 10430 TRUE TRUE
FALSE + 1183962 U G 1/2019|EPL_ISL_402125 AD- CAUGUGGUAGUGUUGGUUUU 45
UAAAACCAACACUACCACAUGA 400 BetaCoV/Wuhan-Hu- 10483 10505 TRUE TRUE
FALSE + 1183963 A A 1/2019|EPL_ISL_402125 AD- CAGUCAUAAUCUAUGUUAAA 46
UUUUAACAUAGAUUAUGACUGU 401 BetaCoV/Wuhan-Hu- 10501 10523 TRUE FALSE
FALSE - 1183964 A G 1/2019|EPL_ISL_402125 AD- UUUUGAUGUUGUAGACAAU 47
CAUUGUCUAAACAACAUCAAAAG 402 BetaCoV/Wuhan-Hu- 10931 10953 TRUE TRUE
FALSE + 1183965 G G 1/2019|EPL_ISL_402125 AD- AUGGUAUUGCUUUAGAUCAA 48
CUUGAUCUAAAGCAUUACCAUA 403 BetaCoV/Wuhan-Hu- 11431 11453 TRUE TRUE
FALSE + 1183966 G A 1/2019|EPL_ISL_402125 AD- GCUAGAUUCCCUAAGAGUGA 49
AUCACUCUUAGGGAAUCUAGCC 404 BetaCoV/Wuhan-Hu- 12843 12865 TRUE TRUE
FALSE + 1183967 U C 1/2019|EPL_ISL_402125 AD- ACAACAUCUUAACACAAUUA 50
CUAAUUGUGUUAAGAUGUUGU 405 BetaCoV/Wuhan-Hu- 13140 13162 TRUE FALSE
FALSE - 1183968 G GU 1/2019|EPL_ISL_402125 AD- UAAGAUGUUGUGUACACACA 51
GUGUGUGUACACAACAUCUUA 406 BetaCoV/Wuhan-Hu- 13148 13170 TRUE TRUE
FALSE + 1183969 C C 1/2019|EPL_ISL_402125 AD- AGAUGUUGUGUACACACACU 52
CAGUGUGUGUACACAACAUCUU 407 BetaCoV/Wuhan-Hu- 13150 13172 TRUE TRUE
FALSE + 1183970 G A 1/2019|EPL_ISL_402125 AD- GUUGUGUACACACACUGGUA 53
GUACCAGUGUGUGUACACAACA 408 BetaCoV/Wuhan-Hu- 13154 13176 TRUE TRUE
FALSE + 118397 C U 1/2019|EPL_ISL_402125 AD- CAGGCACUAGUACUGAUGUC 54
CGACAUCAGUACUAGUGCCUGU 409 BetaCoV/Wuhan-Hu- 13509 13531 TRUE TRUE
FALSE + 1183972 G G 1/2019|EPL_ISL_402125 AD- CCGUCUAUUCUAAACUUAAA 55
CUUUAAGUUUAGAAUAGACGGU 410 BetaCoV/Wuhan-Hu- 13743 13765 TRUE FALSE
FALSE - 1183973 G G 1/2019|EPL_ISL_402125 AD- UUUUAAAUAUUGGGAUCAGA 56
GUCUGAUCCCAAUAUUUAAAAU 411 BetaCoV/Wuhan-Hu- 14296 14318 TRUE FALSE
TRUE + 1183974 C A 1/2019|EPL_ISL_402125 AD- UUUAAAUAUUGGGAUCAGAC 57
UGUCUGAUCCCAAUAUUUAAAA 412 BetaCoV/Wuhan-Hu- 14297 14319 TRUE FALSE
TRUE + 1183975 A U 1/2019|EPL_ISL_402125 AD- CACCUACAAGUUUUGGACCA 58
GUGGUCCAAAACUUGUAGGUGG 413 BetaCoV/Wuhan-Hu- 14403 14425 TRUE TRUE
FALSE + 1183976 C G 1/2019|EPL_ISL_402125 AD- AAUCAGGAUGUAAACUUA 59
AUGUAAGUUUACAUCUGAUUA 414 BetaCoV/Wuhan-Hu- 14504 14526 TRUE TRUE
FALSE + 1183977 U U 1/2019|EPL_ISL_402125 AD- AGGAUGUAAACUACAUA 60
AGCUAUGUAAGUUUACAUCUG 415 BetaCoV/Wuhan-Hu- 14508 14530 TRUE TRUE
FALSE + 1183978 U A 1/2019|EPL_ISL_402125 AD- ACAAUGUUGCUIIUCAAACU 61
CAGUUUGAAAAGCAACAUGUU 416 BetaCoV/Wuhan-Hu- 14646 14668 TRUE TRUE
FALSE + 1183979 G A 1/2019|EPL_ISL_402125 AD- UGUUGCUUUUCAACUGUCA 62
UUGACAGUUUGAAAAGCAACA 417 BetaCoV/Wuhan-Hu- 14650 14672 TRUE TRUE
FALSE + 1183980 A U 1/2019|EPL_ISL_402125 AD- UUGCUUUUCAACUGUCAAA 63
GUUUGACAGUUUGAAAAGCAAC 418 BetaCoV/Wuhan-Hu- 14652 14674 TRUE TRUE
FALSE + 1183981 C A 1/2019|EPL_ISL_402125 AD- GCUUUUCAACUGUCAAAACC 64
GGGUUUGACAGUUUGAAAAGCA 419 BetaCoV/Wuhan-Hu- 14654 14676 TRUE TRUE
FALSE + 1183982 C A 1/2019|EPL_ISL_402125 AD- UAUGACUUUGCUGUGUCUAA 65
CUUAGACACAGCAAAGUCAUAG 420 BetaCoV/Wuhan-Hu- 14696 14718 TRUE TRUE
FALSE + 1183983 G A 1/2019|EPL_ISL_402125 AD- GUUUCUUAAGGAAGGAAGU 66
AACUUCUUCUUAAGGAAGGAAG 421 BetaCoV/Wuhan-Hu- 14718 14740 TRUE TRUE
FALSE + 1183984 U C 1/2019|EPL ISL 402125 AD- UUUCUUAAGGAAGGAAGU 67

GAACUUCUCCUUAUAAAGAAAC 422 BetaCoV/Wuhan-Hu- 14719 14741 TRUE TRUE
FALSE + 1183985 C C 1/2019|EPL_ISL_402125 AD- AGGAAGGAAGUUCUGUUGAA 68
AUUCAACAGAACUUCUUCU 423 BetaCoV/Wuhan-Hu- 14727 14749 TRUE TRUE
FALSE + 1183986 U A 1/2019|EPL_ISL_402125 AD- AACACUUCUUCUUGCUCAG 69
CCUGAGCAAAGAAGAAGUGUUU 424 BetaCoV/Wuhan-Hu- 14751 14773 TRUE TRUE
FALSE + 1183987 G U 1/2019|EPL_ISL_402125 AD- ACUUCUUCUUGCUCAGGAU 70
CAUCCUGAGCAAAGAAGAAGUG 425 BetaCoV/Wuhan-Hu- 14754 14776 TRUE TRUE
FALSE + 1183988 G U 1/2019|EPL_ISL_402125 AD- AAUGUGUGAUAUCAGACAAC 71
AGUUGUCUGAUAUCACACAUUG 426 BetaCoV/Wuhan-Hu- 14824 14846 TRUE TRUE
FALSE + 1183989 U U 1/2019|EPL_ISL_402125 AD- CCAUUUAAUAAAUGGGGUAA 72
CUUACCCCAUUUAUUAAAUGGA 427 BetaCoV/Wuhan-Hu- 14951 14973 TRUE TRUE
FALSE + 1183990 G A 1/2019|EPL_ISL_402125 AD- CAAUGAGUUAUGAGGAUCAA 73
CUUGAUCCUCAUAACUCAUUGA 428 BetaCoV/Wuhan-Hu- 14991 15013 TRUE TRUE
FALSE + 1183991 G A 1/2019|EPL_ISL_402125 AD- UGCAAAGAAUAGAGCUCGCA 74
GUGCGAGCUCUAUUCUUGCAC 429 BetaCoV/Wuhan-Hu- 15085 15107 TRUE TRUE
TRUE + 1183992 C U 1/2019|EPL_ISL_402125 AD- GCAAAGAAUAGAGCUCGCAC 75
GGUGCGAGCUCUAUUCUUGCA 430 BetaCoV/Wuhan-Hu- 15086 15108 TRUE TRUE
TRUE + 1183993 C C 1/2019|EPL_ISL_402125 AD- CAAAGAAUAGAGCUCGCACC 76
CGGUGCGAGCUCUAUUCUUGC 431 BetaCoV/Wuhan-Hu- 15087 15109 TRUE TRUE
TRUE + 1183994 G A 1/2019|EPL_ISL_402125 AD- AAAGAAUAGAGCUCGCACCG 77
ACGGUGCGAGCUCUAUUCUUG 432 BetaCoV/Wuhan-Hu- 15088 15110 TRUE TRUE
TRUE + 1183995 U C 1/2019|EPL_ISL_402125 AD- UGUCUCUAUCUGUAGUACUA 78
AUAGUACUACAGAUAGAGACAC 433 BetaCoV/Wuhan-Hu- 15115 15137 TRUE TRUE
FALSE + 1183996 U C 1/2019|EPL_ISL_402125 AD- CUCUAUCUGUAGUACUAUGA 79
GUCAUAGUACUACAGAUAGAGA 434 BetaCoV/Wuhan-Hu- 15118 15140 TRUE TRUE
FALSE + 1183997 C C 1/2019|EPL_ISL_402125 AD- UCACCUUAUGGGUUGGGGAUU 80
UAAUCCCAACCCAUAAGGUGAG 435 BetaCoV/Wuhan-Hu- 15274 15296 TRUE TRUE
TRUE + 1183998 A G 1/2019|EPL_ISL_402125 AD- CACCUUAUGGGUUGGGGAUUA 81
AUAUCCCAACCCAUAAGGUGA 436 BetaCoV/Wuhan-Hu- 15275 15297 TRUE TRUE
TRUE + 1183999 U G 1/2019|EPL_ISL_402125 AD- ACCUUAUGGGUUGGGGAUUAU 82
GAUAAUCCCAACCCAUAAGGUG 437 BetaCoV/Wuhan-Hu- 15276 15298 TRUE TRUE
TRUE + 1184000 C A 1/2019|EPL_ISL_402125 AD- CCUUAUGGGUUGGGGAUUAUC 83
GGAUAAUCCCAACCCAUAAGGU 438 BetaCoV/Wuhan-Hu- 15277 15299 TRUE TRUE
TRUE + 1184001 C G 1/2019|EPL_ISL_402125 AD-2 ACUUGUUCUUGCUCGCAAAC 84
UGUUUGCGAGCAAGAACAAGUG 439 BetaCoV/Wuhan-Hu- 15343 15365 TRUE TRUE
FALSE + 1184002 A A 1/2019|EPL_ISL_402125 AD- CUUGUUCUUGCUCGCAAACA 85
AUGUUUGCGAGCAAGAACAAGU 440 BetaCoV/Wuhan-Hu- 15344 15366 TRUE TRUE
FALSE + 1184003 U G 1/2019|EPL_ISL_402125 AD- CACAACUGCUUAUGCUAAUA 86
CUAUUAGCAUAAGCAGUUGUGG 441 BetaCoV/Wuhan-Hu- 15493 15515 TRUE TRUE
FALSE + 1184004 G C 1/2019|EPL_ISL_402125 AD- UGAGUGUCUCUAUAGAAUA 87
CUAUUUCUAUAGAGACACUCAU 442 BetaCoV/Wuhan-Hu- 15622 15644 TRUE TRUE
FALSE + 1184005 G A 1/2019|EPL_ISL_402125 AD- UGGACUGAGACUGACCUUAC 88
AGUAAGGUCAGUCUCAGUCCAA 443 BetaCoV/Wuhan-Hu- 15836 15858 TRUE TRUE
FALSE + 1184006 U C 1/2019|EPL_ISL_402125 AD- UUACCCAGAUCCAUCAAGAA 89
AUUCUUGAUGGAUCUGGGUAAG 444 BetaCoV/Wuhan-Hu- 15928 15950 TRUE TRUE
FALSE + 1184007 U G 1/2019|EPL_ISL_402125 AD- GAUGGUACACUUAUGAUUGA 90
UUCAAUCAUAAGUGUACCAUCU 445 BetaCoV/Wuhan-Hu- 15989 16011 TRUE TRUE
FALSE + 1184008 A G 1/2019|EPL_ISL_402125 AD- AAACAUCCUAAUCAGGAGUA 91
AUACUCCUGAUUAGGAUGUUUA 446 BetaCoV/Wuhan-Hu- 16049 16071 TRUE TRUE
FALSE + 1184009 U G 1/2019|EPL_ISL_402125 AD- AACAUCCUAAUCAGGAGUAU 92

CAUACUCUGAUUUGUUU 447 BetaCoV/Wuhan-Hu- 16050 16072 TRUE TRUE
FALSE + 1184010 G A 1/2019|EPL_ISL_402125 AD- AGGAGUAUGCUGAUGUCUUU 93
GAAAGACAUCAGCAUACUCCUG 448 BetaCoV/Wuhan-Hu- 16062 16084 TRUE TRUE
FALSE + 1184011 C A 1/2019|EPL_ISL_402125 AD- CCUGAGUUUUAUGAGGCUAU 94
CAUAGCCUCAUAAAACUCAGGU 449 BetaCoV/Wuhan-Hu- 16190 16212 TRUE TRUE
FALSE + 1184012 G U 1/2019|EPL_ISL_402125 AD- GCAGCAGAAACGCUCAAAGC 95
AGCUUUGAGCGUUUCUGCUGCA 450 BetaCoV/Wuhan-Hu- 16634 16656 TRUE TRUE
FALSE + 1184013 U A 1/2019|EPL_ISL_402125 AD- CCUGGUACUGGUAAGAGUCA 96
AUGACUCUUACCAGUACCAGGU 451 BetaCoV/Wuhan-Hu- 17084 17106 TRUE TRUE
FALSE + 1184014 U G 1/2019|EPL_ISL_402125 AD- UAUAGAUAUAAUGUAGUAGAA 97
AUUCUACUACAUAUUAUCUAUAG 452 BetaCoV/Wuhan-Hu- 17212 17234 TRUE TRUE
FALSE + 1184015 U G 1/2019|EPL_ISL_402125 AD- CUGCUGAAAUUGUUGACACU 98
CAGUGUCAACAAUUUCAGCAGG 453 BetaCoV/Wuhan-Hu- 17568 17590 TRUE TRUE
FALSE + 1184016 G A 1/2019|EPL_ISL_402125 AD- GCUGAAAUUGUUGACACUGU 99
CACAGUGUCAACAAUUUCAGCA 454 BetaCoV/Wuhan-Hu- 17570 17592 TRUE TRUE
FALSE + 1184017 G G 1/2019|EPL_ISL_402125 AD- UGAAAUUGUUGACACUGUGA 100
CUCACAGUGUCAACAAUUUCAG 455 BetaCoV/Wuhan-Hu- 17572 17594 TRUE TRUE
FALSE + 1184018 G C 1/2019|EPL_ISL_402125 AD- AAUUGUUGACACUGUGAGU 101
CACUCACAGUGUCAACAAUUUC 456 BetaCoV/Wuhan-Hu- 17574 17596 TRUE TRUE
FALSE + 1184019 G A 1/2019|EPL_ISL_402125 AD- UGUUGACACUGUGAGUGCUU 102
AAAGCACUCACAGUGUCAACAA 457 BetaCoV/Wuhan-Hu- 17578 17600 TRUE TRUE
FALSE + 1184020 U U 1/2019|EPL_ISL_402125 AD- GCAUGAUGUUUCAUCUGCAA 103
AUUGCAGAUGAAACAUAUGCG 458 BetaCoV/Wuhan-Hu- 17677 17699 TRUE TRUE
FALSE + 1184021 U U 1/2019|EPL_ISL_402125 AD- UUCACCUUAUAAUUCACAGA 104
UUCUGUGAAUUAUAAGGUGAA 459 BetaCoV/Wuhan-Hu- 17770 17792 TRUE TRUE TRUE
+ 1184022 A AU 1/2019|EPL_ISL_402125 AD- UUCACCUUAUAAUUCACAGA 104
UUCUGUGAAUUAUAAGGUGAA 459 BetaCoV/Wuhan-Hu- 17770 17792 TRUE TRUE TRUE
+ 1184022 A AU 1/2019|EPL_ISL_402125 AD- UCACCUUAUAAUUCACAGAA 105
AUUCUGUGAAUUAUAAGGUGA 460 BetaCoV/Wuhan-Hu- 17771 17793 TRUE TRUE TRUE
+ 1184023 U AA 1/2019|EPL_ISL_402125 AD- ACCUUAUAAUUCACAGAAUG 106
GCAUUCUGUGAAUUAUAAGGUG 461 BetaCoV/Wuhan-Hu- 17773 17795 TRUE TRUE
TRUE + 1184024 C A 1/2019|EPL_ISL_402125 AD- CCUUAUAAUUCACAGAAUGC 107
AGCAUUCUGUGAAUUAUAAGGU 462 BetaCoV/Wuhan-Hu- 17774 17796 TRUE FALSE
TRUE + 1184025 U G 1/2019|EPL_ISL_402125 AD- CCUUAUAAUUCACAGAAUGC 107
AGCAUUCUGUGAAUUAUAAGGU 462 BetaCoV/Wuhan-Hu- 17774 17796 TRUE FALSE
TRUE + 1184025 U G 1/2019|EPL_ISL_402125 AD- UAUAUUCACAGAAUGCUGU 108
UACAGCAUUCUGUGAAUUAUAA 463 BetaCoV/Wuhan-Hu- 17777 17799 TRUE FALSE
TRUE + 1184026 A G 1/2019|EPL_ISL_402125 AD- AACUGUUGAUUCAUCACAGG 109
CCCUGUGAUGAAUCAACAGUUU 464 BetaCoV/Wuhan-Hu- 17827 17849 TRUE TRUE
FALSE + 1184027 G G 1/2019|EPL_ISL_402125 AD- GAAUAUGACUAUGUCAUAUU 110
GAAUAUGACAUAAGUCAUAUUCU 465 BetaCoV/Wuhan-Hu- 17852 17874 TRUE TRUE
FALSE + 1184028 C G 1/2019|EPL_ISL_402125 AD- CUUGUCAUAAAGGUCUCUAU 111
GAUAGAGACCUUUAUGACAAGU 466 BetaCoV/Wuhan-Hu- 17968 17990 TRUE FALSE
FALSE - 1184029 C U 1/2019|EPL_ISL_402125 AD- CUCAUCUCUAUGAUGGGUUU 112
AAAACCCAUCAUAGAGAUGAGU 467 BetaCoV/Wuhan-Hu- 18197 18219 TRUE TRUE
FALSE + 1184030 U C 1/2019|EPL_ISL_402125 AD- AGGUCCUAUUUUCACAAAAU 113
UAUUUUGUGAAAAUAGGACCUG 468 BetaCoV/Wuhan-Hu- 18628 18650 TRUE FALSE
FALSE - 1184031 A A 1/2019|EPL_ISL_402125 AD- UUUACAAACAAUUUGAUACU 114
AAGUAUCAAUUGUUUGUAAAC 469 BetaCoV/Wuhan-Hu- 19566 19588 TRUE TRUE
FALSE + 1184032 U C 1/2019|EPL_ISL_402125 AD- AUAUACUCAACUGUGUCAAU 115

UUAUGACAGUUAUUAU 470 BetaCoV/Wuhan-Hu- 20794 20816 TRUE TRUE
FALSE + 1184033 A U 1/2019|EPL_ISL_402125 AD- UAGUACUUUCUUUUGAACUU 116
GAAGUUCAAAAGAAAGUACUAC 471 BetaCoV/Wuhan-Hu- 23092 23114 TRUE TRUE
FALSE + 1184034 C U 1/2019|EPL_ISL_402125 AD- UGGUAAACACUAAUAGUAAAA 117
AUUUUACUAUAGUGUUUACCAC 472 BetaCoV/Wuhan-Hu- 23712 23734 TRUE FALSE
FALSE - 1184035 U A 1/2019|EPL_ISL_402125 AD- CAAUACCAUUAACCUAUAA 118
CUUAUAGGUUUAUUGGUUAUUG 473 BetaCoV/Wuhan-Hu- 24270 24292 TRUE FALSE
FALSE - 1184036 G GA 1/2019|EPL_ISL_402125 AD- AAUGUUCUCUAUGAGAACCA 119
UUGGUUCUCAUAGAGAACAUUC 474 BetaCoV/Wuhan-Hu- 24300 24322 TRUE TRUE
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GUAGCAGCAAGAUUAGCAGAAG 475 BetaCoV/Wuhan-Hu- 24620 24642 TRUE TRUE
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UAGUAGCAGCAAGAUUAGCAGA 476 BetaCoV/Wuhan-Hu- 24622 24644 TRUE TRUE
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CAACAUCUGGUGAUGUAUGAUU 477 BetaCoV/Wuhan-Hu- 25033 25055 TRUE TRUE
FALSE + 1184040 G C 1/2019|EPL_ISL_402125 AD- GUCAAAUAUACAUAACACAUA 123
UUAUGUGUAAUGUAAUUGAC 478 BetaCoV/Wuhan-Hu- 25362 25384 TRUE TRUE
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AGGCACGCUAGUAGUCGUCGUC 479 BetaCoV/Wuhan-Hu- 26192 26214 TRUE TRUE
FALSE + 1184042 U G 1/2019|EPL_ISL_402125 AD- CGACGACUACUAGCGUGCCU 125
AAGGCACGCUAGUAGUCGUCGU 480 BetaCoV/Wuhan-Hu- 26193 26215 TRUE TRUE
FALSE + 1184043 U C 1/2019|EPL_ISL_402125 AD- ACGACUACUAGCGUGCCUUU 126
CAAAGGCACGCUAGUAGUCGUC 481 BetaCoV/Wuhan-Hu- 26195 26217 TRUE TRUE
FALSE + 1184044 G G 1/2019|EPL_ISL_402125 AD- CGACUACUAGCGUGCCUUUG 127
ACAAAGGCACGCUAGUAGUCGU 482 BetaCoV/Wuhan-Hu- 26196 26218 TRUE TRUE
FALSE + 1184045 U C 1/2019|EPL_ISL_402125 AD- GAGUACGAACUUAUGUACUC 128
UGAGUACAUAAGUUCGUACUCA 483 BetaCoV/Wuhan-Hu- 26231 26253 TRUE TRUE
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GAAUGAGUACAUAAGUUCGUAC 484 BetaCoV/Wuhan-Hu- 26234 26256 TRUE TRUE
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CUCUUCCGAAACGAAUGAGUAC 485 BetaCoV/Wuhan-Hu- 26246 26268 TRUE TRUE
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AAAGAAGUACGCUAUUAACUAU 486 BetaCoV/Wuhan-Hu- 26280 26302 TRUE TRUE
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AGAAUACCACGAAAGCAAGAAA 487 BetaCoV/Wuhan-Hu- 26301 26323 TRUE TRUE
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AACUAGCAAGAAUACCACGAAA 488 BetaCoV/Wuhan-Hu- 26309 26331 TRUE TRUE
FALSE + 1184051 U G 1/2019|EPL_ISL_402125 AD- UAGCCAUCCUUACUGCGCUU 134
GAAGCGCAGUAAGGAUGGCUAG 489 BetaCoV/Wuhan-Hu- 26334 26356 TRUE TRUE
FALSE + 1184052 C U 1/2019|EPL_ISL_402125 AD- AGCCAUCCUUACUGCGCUUC 135
CGAAGCGCAGUAAGGAUGGCUA 490 BetaCoV/Wuhan-Hu- 26335 26357 TRUE TRUE
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UCGAAGCGCAGUAAGGAUGGCU 491 BetaCoV/Wuhan-Hu- 26336 26358 TRUE TRUE
FALSE + 1184054 A A 1/2019|EPL_ISL_402125 AD- CCAUCCUUACUGCGCUUCGA 137
AUCGAAGCGCAGUAAGGAUGGC 492 BetaCoV/Wuhan-Hu- 26337 26359 TRUE TRUE
FALSE + 1184055 U U 1/2019|EPL_ISL_402125 AD- ACUGCGCUUCGAUUGUGUGC 138
CGCACACAAUCGAAGCGCAGUA 493 BetaCoV/Wuhan-Hu- 26345 26367 TRUE TRUE
FALSE + 1184056 G A 1/2019|EPL_ISL_402125 AD- UGCGCUUCGAUUGUGUGCGU 139
UACGCACACAAUCGAAGCGCAG 494 BetaCoV/Wuhan-Hu- 26347 26369 TRUE TRUE
FALSE + 1184057 A U 1/2019|EPL_ISL_402125 AD- CGCUUCGAUUGUGUGCGUAC 140

AGUACGCACACAAUCGAAGCG 495 BetaCoV/Wuhan-Hu- 26349 26371 TRUE TRUE
FALSE + 1184058 U A 1/2019|EPL_ISL_402125 AD- GCUUCGAUUGUGUGCGUACU 141
CAGUACGCACACAAUCGAAGCG 496 BetaCoV/Wuhan-Hu- 26350 26372 TRUE TRUE
FALSE + 1184059 G C 1/2019|EPL_ISL_402125 AD- GUUCCUGAUCUUCUGGUCUA 142
UUAGACCAGAAGAUCAGGAACU 497 BetaCoV/Wuhan-Hu- 26450 26472 TRUE TRUE
FALSE + 1184060 A C 1/2019|EPL_ISL_402125 AD- UUCCUGAUCUUCUGGUCUAA 143
UUUAGACCAGAAGAUCAGGAAC 498 BetaCoV/Wuhan-Hu- 26451 26473 TRUE TRUE
FALSE + 1184061 A U 1/2019|EPL_ISL_402125 AD- CCUGAUCUUCUGGUCUAAAC 144
CGUUUAGACCAGAAGAUCAGGA 499 BetaCoV/Wuhan-Hu- 26453 26475 TRUE TRUE
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UAGUUCGUUUAGACCAGAAGAU 500 BetaCoV/Wuhan-Hu- 26458 26480 TRUE TRUE
FALSE + 1184063 A C 1/2019|EPL_ISL_402125 AD- CUUCUGGUCUAAACGAACUA 146
UUAGUUCGUUUAGACCAGAAGA 501 BetaCoV/Wuhan-Hu- 26459 26481 TRUE TRUE
FALSE + 1184064 A U 1/2019|EPL_ISL_402125 AD- GGAACCUAGUAAUAGGUUUC 147
GGAAACCUAUUACUAGGUUCCA 502 BetaCoV/Wuhan-Hu- 26579 26601 TRUE TRUE
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GAAAGCGUUCGUGAUGUAGCAA 503 BetaCoV/Wuhan-Hu- 27030 27052 TRUE TRUE
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AAGAAAGCGUUCGUGAUGUAGC 505 BetaCoV/Wuhan-Hu- 27032 27054 TRUE TRUE
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CAAUUUGUAAUAAGAAAGCGUU 506 BetaCoV/Wuhan-Hu- 27043 27065 TRUE TRUE
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UCUGUUGUCACUACUGUACAA 507 BetaCoV/Wuhan-Hu- 27180 27202 TRUE TRUE
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AUGAAACAUCUGUUGUCACUUA 508 BetaCoV/Wuhan-Hu- 27188 27210 TRUE TRUE
FALSE + 1184071 U C 1/2019|EPL_ISL_402125 AD- CUACUCUAAUAUACCAUUUA 154
CUAAAUGGUAAUAUAGAGUAG 509 BetaCoV/Wuhan-Hu- 28021 28043 TRUE FALSE
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AUUAUUGGGUAAACCUUGGGGC 510 BetaCoV/Wuhan-Hu- 28395 28417 TRUE TRUE
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AAGACGCAGUAUUAUUGGGUAA 511 BetaCoV/Wuhan-Hu- 28405 28427 TRUE TRUE
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GUAGUAGCCAAUUGGUCAUCU 512 BetaCoV/Wuhan-Hu- 28512 28534 TRUE TRUE
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CUUGAGGAAGUUGUAGCACGAU 514 BetaCoV/Wuhan-Hu- 28741 28763 TRUE TRUE
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CCUUGAGGAAGUUGUAGCACGA 515 BetaCoV/Wuhan-Hu- 28742 28764 TRUE TRUE
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UCCCUUGAGGAAGUUGUAGCAC 516 BetaCoV/Wuhan-Hu- 28744 28766 TRUE TRUE
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UUGUCCCUUGAGGAAGUUGUAG 517 BetaCoV/Wuhan-Hu- 28747 28769 TRUE TRUE
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GUUGUCCCUUGAGGAAGUUGUA 518 BetaCoV/Wuhan-Hu- 28748 28770 TRUE TRUE
FALSE + 1184081 C G 1/2019|EPL_ISL_402125 AD- ACUCCUCAAGGAACAACA 164
AAUGUUGUCCCUUGAGGAAGUU 519 BetaCoV/Wuhan-Hu- 28751 28773 TRUE TRUE
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FALSE + 1184084 A G 1/2019|EPL_ISL_402125 AD- AAGGAACAACAUGCCAAA 167
CUUUUGGCAAUGUUGUCCUUG 522 BetaCoV/Wuhan-Hu- 28759 28781 TRUE TRUE
FALSE + 1184085 G A 1/2019|EPL_ISL_402125 AD- ACAUUGCCAAAAGGCUUCUA 168
GUAGAAGCCUUUUGGCAAUGUU 523 BetaCoV/Wuhan-Hu- 28767 28789 TRUE TRUE
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CUCAAGCUGGUUCAAUCUGUCA 524 BetaCoV/Wuhan-Hu- 28944 28966 TRUE TRUE
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AGAUUUCUUAGUGACAGUUUGG 525 BetaCoV/Wuhan-Hu- 29001 29023 TRUE TRUE
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UCCCUUGGGUUUGUUCUGGACC 526 BetaCoV/Wuhan-Hu- 29104 29126 TRUE TRUE
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AUUCUGCACAAGAGUAGACUAU 530 BetaCoV/Wuhan-Hu- 29597 29619 TRUE TRUE
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CAUUCUGCACAAGAGUAGACUA 531 BetaCoV/Wuhan-Hu- 29598 29620 TRUE TRUE
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UUCAUUCUGCACAAGAGUAGAC 532 BetaCoV/Wuhan-Hu- 29600 29622 TRUE TRUE
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AGAAUUCAUUCUGCACAAGAGU 533 BetaCoV/Wuhan-Hu- 29604 29626 TRUE TRUE
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AUGUGAGAUUAAAGUUAACUAC 534 BetaCoV/Wuhan-Hu- 29650 29672 TRUE TRUE
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AGAUUGCUAUGUGAGAUUAAA 536 BetaCoV/Wuhan-Hu- 29658 29680 TRUE TRUE
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AAGAUUGCUAUGUGAGAUUAA 537 BetaCoV/Wuhan-Hu- 29659 29681 TRUE TRUE
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GUGGCUCUUUCAAGUCCUCCCU 538 BetaCoV/Wuhan-Hu- 29699 29721 TRUE TRUE
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UCGUUUAGAGAACAGAUUCUACA 539 BetaCoV/Wuhan-Hu- 51 73 TRUE TRUE FALSE +
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GUUCGUUUAGAGAACAGAUUA 540 BetaCoV/Wuhan-Hu- 53 75 TRUE TRUE FALSE +
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UUUUAAAGUUCGUUUAGAGAAC 541 BetaCoV/Wuhan-Hu- 60 82 TRUE TRUE FALSE +
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AUUUUAAAGUUCGUUUAGAGA 542 BetaCoV/Wuhan-Hu- 61 83 TRUE TRUE FALSE +
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1184110 U U 1/2019|EPL_ISL_402125 AD- UGCUUACGGUUUCGUCCGUG 193
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CAACACGGACGAAACCGUAAGC 550 BetaCoV/Wuhan-Hu- 191 213 TRUE TRUE FALSE +
1184113 G A 1/2019|EPL_ISL_402125 AD- UUACGGUUUCGUCCGUGUUG 196
GCAACACGGACGAAACCGUAAG 551 BetaCoV/Wuhan-Hu- 192 214 TRUE TRUE FALSE +
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CCAUCUUAACCUUUCGGUCACAC 552 BetaCoV/Wuhan-Hu- 247 269 TRUE TRUE FALSE +
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UACCGUUCUUAACGAAGAAGAAC 561 BetaCoV/Wuhan-Hu- 625 647 TRUE TRUE FALSE +
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UUACCGUUCUUAACGAAGAAGAA 562 BetaCoV/Wuhan-Hu- 626 648 TRUE TRUE FALSE +
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GAAGAAGAGCAAGAAGAAGAU 563 BetaCoV/Wuhan-Hu- 3179 3201 TRUE FALSE FALSE
- 1184126 C UG 1/2019|EPL_ISL_402125 AD- GGUUAUUUAAAACUUACUGA 209
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+ 1184127 C C 1/2019|EPL_ISL_402125 AD- UCUACACAAACUCUUAAGA 210
UUCUUUAAGAGUUUGUGUAGA 565 BetaCoV/Wuhan-Hu- 3745 3767 TRUE FALSE FALSE
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+ 1184130 G G 1/2019|EPL_ISL_402125 AD- ACACUCUUAACAGUAUUCUU 213
AAAGAAUACUGUUAAGAGUGUC 568 BetaCoV/Wuhan-Hu- 6862 6884 TRUE FALSE
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CCAUAACAUAAGUUGUACAUAU 569 BetaCoV/Wuhan-Hu- 7529 7551 TRUE TRUE FALSE
+ 1184132 G C 1/2019|EPL_ISL_402125 AD- UGUACAACUAUUGUUAUGG 215

ACCAUUAACAAUAGUUGUUGUACA 570 BetaCoV/Wuhan-Hu- 7530 7552 TRUE TRUE FALSE
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+ 1184134 A A 1/2019|EPL_ISL_402125 AD- UCAUGUGGUAGUGUUGGUUU 217
AAAACCAACACUACCACAUGAA 572 BetaCoV/Wuhan-Hu- 10482 10504 TRUE TRUE
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GACAUUUUAGACUGUACAGUGG 573 BetaCoV/Wuhan-Hu- 11831 11853 TRUE TRUE
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UGACAUUUUAGACUGUACAGUG 8 BetaCoV/Wuhan-Hu- 11832 11854 TRUE TRUE FALSE
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UACCAUACCUCUAUUUAGGUUG 576 BetaCoV/Wuhan-Hu- 12969 12991 TRUE TRUE
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AAUCCUUUAGGAUUUGGAUGAU 577 BetaCoV/Wuhan-Hu- 13268 13290 TRUE TRUE
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GCACUACACCGCAAACCCGUU 579 BetaCoV/Wuhan-Hu- 13465 13487 TRUE TRUE
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CUCUUAACUACAAAGUAAGAAU 581 BetaCoV/Wuhan-Hu- 13639 13661 TRUE TRUE
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UUGAAAUAAUCAUCAUCAAC 583 BetaCoV/Wuhan-Hu- 13891 13913 TRUE TRUE
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AAA AUUACCGGGUUUGACAGUU 587 BetaCoV/Wuhan-Hu- 14663 14685 TRUE TRUE
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CAACAGAACUCCUCCUUA 590 BetaCoV/Wuhan-Hu- 14724 14746 TRUE TRUE
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FALSE + 1184157 A AA 1/2019|EPL_ISL_402125 AD- GGUAAGGCUAGACUUUAUA 238

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FALSE + 1184160 A U 1/2019|EPL_ISL_402125 AD- UAGCUGGUGUCUCUAUCUGU 241
UACAGAUAGAGACACCAGCUAC 596 BetaCoV/Wuhan-Hu- 15108 15130 TRUE TRUE
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FALSE + 1184163 A A 1/2019|EPL_ISL_402125 AD- UUGUUCUUGCUCGCAAACAU 244
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CCUGGUUUAACAUAUAGUGAAC 600 BetaCoV/Wuhan-Hu- 15451 15473 TRUE TRUE
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ACUAUUAGCAUAAGCAGUUGUG 601 BetaCoV/Wuhan-Hu- 15494 15516 TRUE TRUE
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CACUAUUAGCAUAAGCAGUUGU 602 BetaCoV/Wuhan-Hu- 15495 15517 TRUE TRUE
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FALSE + 1184168 U U 1/2019|EPL_ISL_402125 AD- ACUGCUUAUGCUAUAGUGU 249
AACACUAUUAGCAUAAGCAGUU 604 BetaCoV/Wuhan-Hu- 15497 15519 TRUE TRUE
FALSE + 1184169 U G 1/2019|EPL_ISL_402125 AD- CACUCAUAAAGUCUGUGUUG 250
ACAACACAGACUUUAUGAGUGU 605 BetaCoV/Wuhan-Hu- 15609 15631 TRUE FALSE
FALSE - 1184170 U C 1/2019|EPL_ISL_402125 AD- GAGUGUCUCUAUAGAAAUAG 251
UCUAUUUCUAUAGAGACACUCA 606 BetaCoV/Wuhan-Hu- 15623 15645 TRUE TRUE
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UUUAGUAAGGUCAGUCUCAGUC 607 BetaCoV/Wuhan-Hu- 15839 15861 TRUE TRUE
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CUGUUUAACUAGCAUUGUAUGU 608 BetaCoV/Wuhan-Hu- 15884 15906 TRUE TRUE
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AAUCAUAAGUGUACCAUCUGUU 609 BetaCoV/Wuhan-Hu- 15986 16008 TRUE TRUE
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GCUAGAAAACUCAUCUGAGAU 613 BetaCoV/Wuhan-Hu- 17006 17028 TRUE TRUE
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UCACUUUGAAUUUAUCAAACA 621 BetaCoV/Wuhan-Hu- 17259 17281 TRUE TRUE
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AAAUUAUCUGGUUCUAGUGUGC 622 BetaCoV/Wuhan-Hu- 17479 17501 TRUE TRUE
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GAAUAUUCUGGUUCUAGUGUG 623 BetaCoV/Wuhan-Hu- 17480 17502 TRUE TRUE
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ACUCACAGUGUCAACAAUUUCA 624 BetaCoV/Wuhan-Hu- 17573 17595 TRUE TRUE
FALSE + 1184189 U G 1/2019|EPL_ISL_402125 AD- CCCUGCUUGGAGAAAAGCUG 270
ACAGCUUUUCUCCAAGCAGGGU 625 BetaCoV/Wuhan-Hu- 17743 17765 TRUE TRUE
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CAGCAUUCUGUGAAUUAUAAGG 626 BetaCoV/Wuhan-Hu- 17775 17797 TRUE FALSE
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CAGCAUUCUGUGAAUUAUAAGG 626 BetaCoV/Wuhan-Hu- 17775 17797 TRUE FALSE
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UGAAUAUGACAUAGUCAUAUUC 628 BetaCoV/Wuhan-Hu- 17853 17875 TRUE TRUE
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GUGAAUAUGACAUAGUCAUAUU 629 BetaCoV/Wuhan-Hu- 17854 17876 TRUE TRUE
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AGUGAAUAUGACAUAGUCAUAU 630 BetaCoV/Wuhan-Hu- 17855 17877 TRUE TRUE
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CCCAUCAUAGAGAUGAGUCUUC 631 BetaCoV/Wuhan-Hu- 18193 18215 TRUE TRUE
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ACCCAUCAUAGAGAUGAGUCUU 632 BetaCoV/Wuhan-Hu- 18194 18216 TRUE TRUE
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GCCACAUUUUCUAAACUCUGAA 633 BetaCoV/Wuhan-Hu- 19615 19637 TRUE TRUE
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UAUUUAUAGAAAGUUGAUGGU 634 BetaCoV/Wuhan-Hu- 20152 20174 TRUE FALSE
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UCGAACAUAUCGUUUAUGGAGA 635 BetaCoV/Wuhan-Hu- 20315 20337 TRUE FALSE
FALSE - 1184200 A U 1/2019|EPL_ISL_402125 AD- CAUAGGAAUAAAUCUUCUA 281
UUAGAAGAUUUUAUUCCUAUGG 636 BetaCoV/Wuhan-Hu- 20413 20435 TRUE FALSE
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GCACAAGUUUGUAAUAUUAGGA 637 BetaCoV/Wuhan-Hu- 22548 22570 TRUE TRUE
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GAGCUAAGUUGUUUAACAAGCG 638 BetaCoV/Wuhan-Hu- 24443 24465 TRUE TRUE
FALSE + 1184203 C U 1/2019|EPL_ISL_402125 AD- CUUGUUAACAACUAGCUC 284
GGAGCUAAGUUGUUUAACAAGC 639 BetaCoV/Wuhan-Hu- 24444 24466 TRUE TRUE
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UGACAUUUUAGUAGCAGCAAGA 640 BetaCoV/Wuhan-Hu- 24630 24652 TRUE TRUE
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CUCAUAAACAAAUCCAUAAGUU 641 BetaCoV/Wuhan-Hu- 25387 25409 TRUE TRUE
FALSE + 1184206 G C 1/2019|EPL_ISL_402125 AD- CGACGACGACUACUAGCGUG 287

GCACGCUAGUCGUCGG 642 BetaCoV/Wuhan-Hu- 26190 26212 TRUE TRUE
FALSE + 1184207 C U 1/2019|EPL_ISL_402125 AD- GACGACGACUACUAGCGUGC 288
GGCACGCUAGUAGUCGUCGUCG 643 BetaCoV/Wuhan-Hu- 26191 26213 TRUE TRUE
FALSE + 1184208 C G 1/2019|EPL_ISL_402125 AD- AUGAGUACGAACUUAUGUAC 289
AGUACAUAAGUUCGUACUCAUC 644 BetaCoV/Wuhan-Hu- 26229 26251 TRUE TRUE
FALSE + 1184209 U A 1/2019|EPL_ISL_402125 AD- CGAACUUAUGUACUCAUUCG 290
ACGAAUGAGUACAUAAGUUCGU 645 BetaCoV/Wuhan-Hu- 26236 26258 TRUE TRUE
FALSE + 1184210 U A 1/2019|EPL_ISL_402125 AD- GAACUUAUGUACUCAUUCGU 291
AACGAAUGAGUACAUAAGUUCG 646 BetaCoV/Wuhan-Hu- 26237 26259 TRUE TRUE
FALSE + 1184211 U U 1/2019|EPL_ISL_402125 AD- AACUUAUGUACUCAUUCGUU 292
AAACGAAUGAGUACAUAAGUUC 647 BetaCoV/Wuhan-Hu- 26238 26260 TRUE TRUE
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GAAACGAAUGAGUACAUAAGUU 648 BetaCoV/Wuhan-Hu- 26239 26261 TRUE TRUE
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UAUUAACUAUUAACGUACCUGU 655 BetaCoV/Wuhan-Hu- 26268 26290 TRUE TRUE
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CUAUUAACUAUUAACGUACCUG 656 BetaCoV/Wuhan-Hu- 26269 26291 TRUE TRUE
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GAAGUACGCUAUUAACUAUUA 657 BetaCoV/Wuhan-Hu- 26277 26299 TRUE TRUE
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AAGAAUACCACGAAAGCAAGAA 658 BetaCoV/Wuhan-Hu- 26302 26324 TRUE TRUE
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CAAGAAUACCACGAAAGCAAGA 659 BetaCoV/Wuhan-Hu- 26303 26325 TRUE TRUE
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GCAAGAAUACCACGAAAGCAAG 660 BetaCoV/Wuhan-Hu- 26304 26326 TRUE TRUE
FALSE + 1184225 C A 1/2019|EPL_ISL_402125 AD- UGCUUUCGUGGUAUUCUUGC 306
AGCAAGAAUACCACGAAAGCAA 661 BetaCoV/Wuhan-Hu- 26305 26327 TRUE TRUE
FALSE + 1184226 U G 1/2019|EPL_ISL_402125 AD- GCUUUCGUGGUAUUCUUGCU 307
UAGCAAGAAUACCACGAAAGCA 662 BetaCoV/Wuhan-Hu- 26306 26328 TRUE TRUE
FALSE + 1184227 A A 1/2019|EPL_ISL_402125 AD- UUUCGUGGUAUUCUUGCUAG 308
ACUAGCAAGAAUACCACGAAAG 663 BetaCoV/Wuhan-Hu- 26308 26330 TRUE TRUE
FALSE + 1184228 U C 1/2019|EPL_ISL_402125 AD- CUGCGCUUCGAUUGUGUGCG 309
ACGCACACAAUCGAAGCGCAGU 664 BetaCoV/Wuhan-Hu- 26346 26368 TRUE TRUE
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CACUUAACAAUAUUGCAGC 667 BetaCoV/Wuhan-Hu- 26369 26392 TRUE TRUE
FALSE + 1184232 G U 1/2019|EPL_ISL_402125 AD- GCUGCAAUAUUGUUAACGUG 313
UCACGUUAACAAUAUUGCAGCA 668 BetaCoV/Wuhan-Hu- 26370 26392 TRUE TRUE
FALSE + 1184233 A G 1/2019|EPL_ISL_402125 AD- CUGCAAUAUUGUUAACGUGA 314
CUCACGUUAACAAUAUUGCAGC 669 BetaCoV/Wuhan-Hu- 26371 26393 TRUE TRUE
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GACCAGAAGAUCAAGGAACUCUA 670 BetaCoV/Wuhan-Hu- 26447 26469 TRUE TRUE
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UCGUUUAGACCAGAAGAUCAGG 671 BetaCoV/Wuhan-Hu- 26454 26476 TRUE TRUE
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GUUCGUUUAGACCAGAAGAUC 672 BetaCoV/Wuhan-Hu- 26456 26478 TRUE TRUE
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ACCUAUUACUAGGUUCCAUGU 674 BetaCoV/Wuhan-Hu- 26575 26597 TRUE TRUE
FALSE + 1184239 U U 1/2019|EPL_ISL_402125 AD- AAUGGAACCUAGUAAUAGGU 320
AACCUAUUACUAGGUUCCAUG 675 BetaCoV/Wuhan-Hu- 26576 26598 TRUE TRUE
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AAACCUAUUACUAGGUUCCA 676 BetaCoV/Wuhan-Hu- 26577 26599 TRUE TRUE
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AUAGGAAACCUAUUACUAGGU 678 BetaCoV/Wuhan-Hu- 26582 26604 TRUE TRUE
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UAAGAAAGCGUUCGUGAUGUAG 679 BetaCoV/Wuhan-Hu- 27033 27055 TRUE TRUE
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UUUGUAAUAAGAAAGCGUUCGU 686 BetaCoV/Wuhan-Hu- 27040 27062 TRUE TRUE
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GAAACAUCUGUUGUCACUUA 687 BetaCoV/Wuhan-Hu- 27186 27208 TRUE TRUE
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AAGGAUAGCAGAAAGGCUAAA 688 BetaCoV/Wuhan-Hu- 27793 27815 TRUE TRUE
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CAAGGAUAGCAGAAAGGCUAA 689 BetaCoV/Wuhan-Hu- 27794 27816 TRUE TRUE
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ACAAGGAUAGCAGAAAGGCUA 690 BetaCoV/Wuhan-Hu- 27795 27817 TRUE TRUE
FALSE + 1184255 U A 1/2019|EPL_ISL_402125 AD- CCUUCUGCUAUUCCUUGUU 336
AAACAAGGAUAGCAGAAAGGC 691 BetaCoV/Wuhan-Hu- 27797 27819 TRUE TRUE
FALSE + 1184256 U U 1/2019|EPL_ISL_402125 AD- CUUUCUGCUAUUCCUUGUUU 337

UUAACAAGCAUAGCAGAAAG 692 BetaCoV/Wuhan-Hu- 27798 27820 TRUE TRUE
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UAAAACAAGGAAUAGCAGAAAG 693 BetaCoV/Wuhan-Hu- 27799 27821 TRUE TRUE
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UUAACAAGGAAUAGCAGAAA 694 BetaCoV/Wuhan-Hu- 27800 27822 TRUE TRUE
FALSE + 1184259 A G 1/2019|EPL_ISL_402125 AD- UCUGCUAUUCCUUGUUUUUAA 340
AUUAAAACAAGGAAUAGCAGAA 695 BetaCoV/Wuhan-Hu- 27801 27823 TRUE TRUE
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AAUUAAAACAAGGAAUAGCAGA 696 BetaCoV/Wuhan-Hu- 27802 27824 TRUE TRUE
FALSE + 1184261 U A 1/2019|EPL_ISL_402125 AD- CCAAGGUUUACCCAAUAAUA 342
GUAUUAUUGGGUAAACCUUGGG 697 BetaCoV/Wuhan-Hu- 28397 28419 TRUE TRUE
FALSE + 1184262 C G 1/2019|EPL_ISL_402125 AD- AAGGUUUACCCAAUAAUACU 343
CAGUAUUAUUGGGUAAACCUUG 698 BetaCoV/Wuhan-Hu- 28399 28421 TRUE TRUE
FALSE + 1184263 G G 1/2019|EPL_ISL_402125 AD- AGGUUUACCCAAUAAUACUG 344
GCAGUAUUAUUGGGUAAACCUU 699 BetaCoV/Wuhan-Hu- 28400 28422 TRUE TRUE
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GCCAAUUUGGUCAUCUGGACUG 701 BetaCoV/Wuhan-Hu- 28506 28528 TRUE TRUE
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AGCCAAUUUGGUCAUCUGGACU 702 BetaCoV/Wuhan-Hu- 28507 28529 TRUE TRUE
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GCGUAGAAGCCUUUUGGCAAUG 703 BetaCoV/Wuhan-Hu- 28769 28791 TRUE TRUE
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UGCGUAGAAGCCUUUUGGCAAU 704 BetaCoV/Wuhan-Hu- 28770 28792 TRUE TRUE
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GCAGCAGAUUUCUAGUGACAG 707 BetaCoV/Wuhan-Hu- 29006 29028 TRUE TRUE
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GCAGGAAGAAGAGUCACAGUUU 708 BetaCoV/Wuhan-Hu- 29441 29463 TRUE TRUE
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GAGAAUUCAUUCUGCACAAGAG 709 BetaCoV/Wuhan-Hu- 29605 29627 TRUE TRUE
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CGAGAAUUCAUUCUGCACAAGA 710 BetaCoV/Wuhan-Hu- 29606 29628 TRUE TRUE
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ACGAGAAUUCAUUCUGCACAAG 711 BetaCoV/Wuhan-Hu- 29607 29629 TRUE TRUE
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UACGAGAAUUCAUUCUGCACA 712 BetaCoV/Wuhan-Hu- 29608 29630 TRUE TRUE
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UUACGAGAAUUCAUUCUGCACA 713 BetaCoV/Wuhan-Hu- 29609 29631 TRUE TRUE
FALSE + 1184278 A A 1/2019|EPL_ISL_402125 AD- UGCAGAAUGAAUUCUCGUAA 359
GUUACGAGAAUUCAUUCUGCAC 714 BetaCoV/Wuhan-Hu- 29610 29632 TRUE TRUE
FALSE + 1184279 C A 1/2019|EPL_ISL_402125 AD- GCAGAAUGAAUUCUCGUAAAC 360
AGUUACGAGAAUUCAUUCUGCA 715 BetaCoV/Wuhan-Hu- 29611 29633 TRUE TRUE
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UAGUUACGAGAAUUCAUUCUGC 716 BetaCoV/Wuhan-Hu- 29612 29634 TRUE TRUE
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AAAGAUUGCUAUGUGAUA 717 BetaCoV/Wuhan-Hu- 29660 29682 TRUE TRUE
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 AUUAAAGAUUGCUAUGUGAGA 718 BetaCoV/Wuhan-Hu- 29663 29685 TRUE TRUE
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 UGAUUAAGAUUGCUAUGUGA 720 BetaCoV/Wuhan-Hu- 29665 29687 TRUE TRUE
 FALSE + 1184285 A GA 1/2019|EPL_ISL_402125 AD- CACAUAGCAAUCUUUAAUCA 366
 CUGAUUAAGAUUGCUAUGUGA 721 BetaCoV/Wuhan-Hu- 29666 29688 TRUE TRUE
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 UAAUUUUACACAUUAGGGCUCU 722 BetaCoV/Wuhan-Hu- 29796 29818 TRUE TRUE
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 AAUUAUUUUACACAUUAGGGC 724 BetaCoV/Wuhan-Hu- 29799 29821 TRUE TRUE
 FALSE + 1184289 U U 1/2019|EPL_ISL_402125 AD- CCUAAUGUGUAAAAUUAUU 370
 AAUUAUUUUACACAUUAGGG 725 BetaCoV/Wuhan-Hu- 29800 29822 TRUE TRUE
 FALSE + 1184290 U C 1/2019|EPL_ISL_402125 AD- CCAUGUGAUUUUAAUAGCU 371
 AAGCUAUUAAAUCACAUGGGG 726 BetaCoV/Wuhan-Hu- 29834 29856 TRUE TRUE
 FALSE + 1184291 U A 1/2019|EPL_ISL_402125

(633) TABLE-US-00004 TABLE 3 Modified Sense and Antisense Strand
 Coronavirus dsRNA Sequences Strand of the Viral RNA Duplex Sense Strand
 Sequence Antisense Strand Sequence mRNA Target Sequence Targeted by Name 5'
 to 3' SEQ ID NO: 5' to 3' SEQ ID NO: 5' to 3' SEQ ID NO: the Agent AD-
 asuscug(Uhd)UfcUfCfUfaaacgaa 727 VPusAfsugdTc(G2p)uuuagaGfaAfcag 1082
 AGATCTGTTCTCTAAACGAA 1437 + 1183935 csusa auscsu CTT AD-
 uscsugu(Uhd)CfuCfUfAfaacgaac 728 VPusAfsagdTu(C2p)guuuagAfgAfaca 1083
 GATCTGTTCTCTAAACGAAC 1438 + 1183936 ususa gasusc TTT AD-
 gsusucu(Chd)UfaAfAfCfgaacuuu 729 VPusUfsuadAa(G2p)uucguuUfaGfaga 1084
 CTGTTCTCTAAACGAAC TTT 1439 + 1183937 asasa acsasg AAA AD-
 csusgcu(Uhd)AfcGfGfUfuucgucc 730 VPusAfsagdGa(C2p)gaaaccGfuAfagca 1085
 GGCTGCTTACGGTTTCGTCC 1440 + 1183938 gsusa gscsc GTG AD-
 gsgsugu(Ghd)AfcCfGfAfaagguaa 731 VPusUfscudTa(C2p)cuuucgGfuCfagca 1086
 CGGGTGTGACCGAAAGGTA 1441 + 1183939 gsasa cscsg AGAT AD-
 cscsgaa(Ahd)GfgUfAfAfgauggag 732 VPusCfsucdTc(C2p)aucuuaCfcUfuucg 1087
 GACCGAAAGGTAAGATGGA 1442 + 1183940 asgsa gsusc GAGC AD-
 asasaca(Chd)AfcGfUfCfcaacucag 733 VPusAfsucdGa(G2p)uuggacGfuGfugu 1088
 GAAAACACACGTCCAAC TCA 1443 + 1183941 susa uususc GTT AD-
 ascsaca(Chd)GfuCfCfAfacucaguu 734 VPusAfsaadCu(G2p)aguuggAfcGfugu 1089
 AAACACACGTCCAAC TCA 1444 + 1183942 susa gususu TTG AD-
 gsuscca(Ahd)CfuCfAfGfuugccu 735 VPusAfsadGg(C2p)aaacugAfgUfugg 1090
 ACGTCCAAC TCA 1445 + 1183943 gsusa acsgsu GTT AD-
 csusucu(Uhd)CfgUfAfAfgaacggu 736 VPusUfsuadCc(G2p)uucuuCfGfaga 1091
 TTCTTCTTCGTAAGAACGGT 1446 + 1183944 asasa agsasa AAT AD-
 ususcuu(Chd)GfuAfAfGfaacgua 737 VPusAfsuudAc(C2p)guucuuAfcGfaag 1092
 TCTTCTTCGTAAGAACGGT 1447 + 1183945 asusa aasgsa ATA AD-
 uscsuuc(Ghd)UfaAfGfAfacgguaa 738 VPusUfsaudTa(C2p)cguucuUfaCfgaag 1093
 CTTCTTCGTAAGAACGGT 1448 + 1183946 usasa asasg TAA AD-
 ususccu(Uhd)AfaAfCfUfucucuuc 739 VPusUfsugdAa(G2p)agaaguUfuAfagg 1094
 CCTTCCTTAAACTTCTTCTTCA 1449 - 1183947 asasa aasgs AG AD-

acc(Uhd)AfaUfUfAfaaggu 740 VPusAfsaadGc(C2p)uugaauUfuAfggu 1095
TTTCACCTAAATTCAAGGCT 1450 – 1183948 ususa gasasa TTA AD-
csuscug(Ghd)AfuUfUfAfacacacu 741 VPusAfsaadGu(G2p)uguuaaAfuCfcag 1096
TTCTCTGGATTAAACACACTT 1451 – 1183949 ususa agsasa TC AD-
csusucu(Chd)AfuUfAfAfguacuuu 742 VPusAfsuadAa(G2p)uacuuaAfuGfaga 1097
CACTTCTCATTAAGTACTTTA 1452 – 1183950 asusa agsusg TC AD-
ascsucu(Uhd)CfuUfCfUfucacaau 743 VPusUfsgadTu(G2p)ugaagaAfgAfaga 1098
AAACTCTTCTTCTTCACAATC 1453 – 1183951 csasa gususu AC AD-
ususauu(Uhd)AfaAfAfCfuuacuga 744 VPusUfsgudCa(G2p)uaaguuUfuAfaau 1099
GGTTATTTAAACTTACTGA 1454 + 1183952 csasa aascsc CAA AD-
usasucu(Ahd)CfaCfAfAfacucuuu 745 VPusUfsuudAa(G2p)aguugUfgUfaga 1100
AGTATCTACACAACTCTTA 1455 – 1183953 asasa uascsu AAG AD-
asuscuu(Ghd)UfuUfUfCfucuguuc 746 VPusUfsugdAa(C2p)agagaaAfaCfaaga 1101
TCATCTTGTTTTCTCTGTTCA 1456 – 1183954 asasa usgsa AC AD-
csascuu(Uhd)UfaUfCfAfccucucu 747 VPusUfsaadGa(G2p)aggugaUfaAfaag 1102
TACTTTTATCACCTCTCTT 1457 – 1183955 usasa ugsusa AA AD-
uscucu(Ghd)AfaGfAfAfguagugg 748 VPusUfsuccCa(C2p)uacuucUfuCfaga 1103
AGTCTCTGAAGAAGTAGTGG 1458 + 1183956 asasa gascsu AAA AD-
uscuga(Ahd)GfaAfGfUfaguggaa 749 VPusUfsuudTc(C2p)acuacuUfcUfucag 1104
TCTCTGAAGAAGTAGTGGA 1459 + 1183957 asasa asgsa AAT AD-
csusgaa(Ghd)AfaGfUfAfguggaaa 750 VPusAfsuudTu(C2p)cacuacUfuCfuua 1105
CTCTGAAGAAGTAGTGGA 1460 + 1183958 asusa gsasg ATC AD-
asgsugg(Ahd)AfaAfUfCfcuaccu 751 VPusGfsuadTg(G2p)uaggauUfuUfcca 1106
GTAGTGGAATCCTACCAT 1461 + 1183959 ascsa cusasc ACA AD-
gsusgga(Ahd)AfaUfCfCfuaccuua 752 VPusUfsgudAu(G2p)guaggaUfuUfucc 1107
TAGTGGAATCCTACCAT 1462 + 1183960 csasa acsusa CAG AD-
gscsucu(Uhd)CfuAfAfAfccucaua 753 VPusUfsuudAu(G2p)agguuuAfgAfaga 1108
AAGCTCTTCTAAACCTCATA 1463 – 1183961 asasa gcsusu AAA AD-
asusggu(Uhd)CfaCfCfAfucuggug 754 VPusAfsacdAc(C2p)agauggUfgAfacc 1109
CAATGGTTCACCATCTGGTG 1464 + 1183962 ususa aususg TTT AD-
csasugu(Ghd)GfuAfGfUfguuggu 755 VPusAfsaadAc(C2p)aacacuAfcCfacau 1110
TTCATGTGGTAGTGTGGTTT 1465 + 1183963 uususa gsasa TA AD-
csasguc(Ahd)UfaAfUfCfuauguua 756 VPusUfsuudAa(C2p)auagauUfaUfgac 1111
CACAGTCATAATCTATGTTA 1466 – 1183964 asasa ugsusg AAA AD-
ususuug(Ahd)UfgUfUfGfuuagaca 757 VPusAfsuudGu(C2p)uaacaaCfaUfcaaa 1112
CCTTTTGATGTTGTTAGACA 1467 + 1183965 asusa asgsg ATG AD-
asusggu(Ahd)AfuGfCfUfuuagauc 758 VPusUfsugdAu(C2p)uaaagcAfuUfacc 1113
TTATGGTAATGCTTTAGATC 1468 + 1183966 asasa ausasa AAG AD-
gscsuag(Ahd)UfuCfCfCfuaagagu 759 VPusUfscadCu(C2p)uuagggAfaUfcua 1114
GGGCTAGATTCCCTAAGAGT 1469 + 1183967 gsasa gcscsc GAT AD-
ascsaac(Ahd)UfcUfUfAfacacaauu 760 VPusUfsaadTu(G2p)uguuaaGfaUfguu 1115
ACACAACATCTTAACACAAT 1470 – 1183968 sasa gusgsu TAG AD-
usasaga(Uhd)GfuUfGfUfguacaca 761 VPusUfsgudGu(G2p)uacacaAfcAfucu 1116
GTTAAGATGTTGTGTACACA 1471 + 1183969 csasa uasasc CAC AD-
asgsaug(Uhd)UfgUfGfUfacacaca 762 VPusAfsugdGu(G2p)uguacaCfaAfcau 1117
TAAGATGTTGTGTACACACA 1472 + 1183970 csusa cususa CTG AD-
gsusugu(Ghd)UfaCfAfCfacacugg 763 VPusUfsacdCa(G2p)ugugugUfaCfaca 1118
ATGTTGTGTACACACTGG 1473 + 1183971 usasa acsasu TAC AD-
csasggc(Ahd)CfuAfGfUfacugaug 764 VPusGfsacdAu(C2p)aguacuAfgUfgcc 1119
CACAGGCACTAGTACTGATG 1474 + 1183972 uscsa ugsusg TCG AD-

cscsguc(Uhd)AfuUfUfUfaaaucua 765 VPusUfscudAa(G2p)uuuagaAfuAfgac 1120
CACCGTCTATTCTAAACTTA 1475 – 1183973 asasa ggsusg AAG AD-
ususuaa(Ahd)AfuAfUfUfgggauca 766 VPusUfscudGa(Tgn)cccaauAfuUfuaaa 1121
TATTTTAAATATTGGGATCA 1476 + 1183974 gsasa asusa GAC AD-
ususuaa(Ahd)UfaUfUfGfggaucag 767 VPusGfsucdTg(Agn)ucccaaUfaUfuuaa 1122
ATTTTAAATATTGGGATCAG 1477 + 1183975 ascsa asasu ACA AD-
csasccu(Ahd)CfaAfGfUfuuggac 768 VPusUfsggdTc(C2p)aaaacuUfgUfagg 1123
CCCACCTACAAGTTTTGGAC 1478 + 1183976 csasa ugsgsg CAC AD-
asasuca(Ghd)GfaUfGfUfaaacua 769 VPusUfsgudAa(G2p)uuuacaUfcCfuga 1124
ATAATCAGGATGTAAACTTA 1479 + 1183977 csasa uusas CAT AD-
asgsgau(Ghd)UfaAfAfCfuuaaua 770 VPusGfscudAu(G2p)uaaguuUfaCfauc 1125
TCAGGATGTAAACTTACATA 1480 + 1183978 gscsa cusgsa GCT AD-
ascsaau(Ghd)UfuGfCfUfuuucaaa 771 VPusAfsudTu(G2p)aaaagcAfaCfauu 1126
TAACAATGTTGCTTTTCAAA 1481 + 1183979 csusa gususa CTG AD-
usgsuug(Chd)UfuUfUfCfaaacugu 772 VPusUfsgadCa(G2p)uuugaaAfaGfcaa 1127
AATGTTGCTTTTCAAACACTGT 1482 + 1183980 csasa casusu CAA AD-
ususgcu(Uhd)UfuCfAfAfacuguca 773 VPusUfsuudGa(C2p)aguugAfaAfagc 1128
TGTTGCTTTTCAAACACTGTCA 1483 + 1183981 asasa aascsa AAC AD-
gscsuuu(Uhd)CfaAfAfCfugucuaa 774 VPusGfsgudTu(G2p)acaguuUfgAfaaa 1129
TTGCTTTTCAAACACTGTCAAA 1484 + 1183982 cscsa gcsasa CCC AD-
usasuga(Chd)UfuUfGfCfugugucu 775 VPusUfsuadGa(C2p)acagcaAfaGfucau 1130
TCTATGACTTTGCTGTGTCTA 1485 + 1183983 asasa asgsa AG AD-
gsusuuc(Uhd)UfuAfAfGfgaaggaa 776 VPusAfsudTc(C2p)uuccuuAfaAfgaaa 1131
GGGTTTCTTTAAGGAAGGAA 1486 + 1183984 gsusa cscsc GTT AD-
ususucu(Uhd)UfaAfGfGfaaggaag 777 VPusAfsadTu(C2p)cuuccuUfaAfagaa 1132
GGTTTCTTTAAGGAAGGAAG 1487 + 1183985 ususa ascsc TTC AD-
asgsgaa(Ghd)GfaAfGfUfucuguug 778 VPusUfsudAa(C2p)agaacuUfcCfuucc 1133
TAAGGAAGGAAGTTCTGTTG 1488 + 1183986 asasa ususa AAT AD-
asascac(Uhd)UfcUfUfCfuugguc 779 VPusCfsugdAg(C2p)aaagaaGfaAfgug 1134
AAAACACTTCTTCTTTGCTC 1489 + 1183987 asgsa uususu AGG AD-
ascsuuc(Uhd)UfcUfUfUfgcucagg 780 VPusAfsudCu(G2p)agcaaaGfaAfgaa 1135
ACACTTCTTCTTTGCTCAGG 1490 + 1183988 asusa gusgsu ATG AD-
asasugu(Ghd)UfgAfUfAfucagaca 781 VPusGfsuudGu(C2p)ugauauCfaCfaca 1136
ACAATGTGTGATATCAGACA 1491 + 1183989 ascsa uusgsu ACT AD-
cscsaau(Uhd)AfaUfAfAfauggggu 782 VPusUfsuadCc(C2p)cauuuaUfuAfaau 1137
TTCCATTTAATAAATGGGGT 1492 + 1183990 asasa ggsasa AAG AD-
csasaug(Ahd)GfuUfAfUfgaggau 783 VPusUfsugdAu(C2p)cucauaAfcUfcu 1138
TTCAATGAGTTATGAGGATC 1493 + 1183991 asasa ugsasa AAG AD-
usgscaa(Ahd)GfaAfUfAfgagcucg 784 VPusUfsgcdGa(G2p)cucuauUfcUfuug 1139
AGTGCAAAGAATAGAGCTC 1494 + 1183992 csasa cascsu GCAC AD-
gscsaaa(Ghd)AfaUfAfGfagcucgc 785 VPusGfsugdCg(Agn)gcucuaUfuCfuuu 1140
GTGCAAAGAATAGAGCTCGC 1495 + 1183993 ascsa gcsasc ACC AD-
csasaag(Ahd)AfuAfGfAfgcucgca 786 VPusGfsgudGc(G2p)agcucuAfuUfcuu 1141
TGCAAAGAATAGAGCTCGCA 1496 + 1183994 cscsa ugscsa CCG AD-
asasaga(Ahd)UfaGfAfGfucgcac 787 VPusCfsggdTg(C2p)gagcucUfaUfucu 1142
GCAAAGAATAGAGCTCGCA 1497 + 1183995 csgsa uusgsc CCGT AD-
usgsucu(Chd)UfaUfCfUfguaguac 788 VPusUfsagdTa(C2p)uacagaUfaGfagac 1143
GGTGTCTCTATCTGTAGTAC 1498 + 1183996 usasa ascsc TAT AD-
csuscua(Uhd)CfuGfUfAfguacuau 789 VPusUfscadTa(G2p)uacuacAfgAfuaga 1144
GTCTCTATCTGTAGTACTAT 1499 + 1183997 gsasa gsasc GAC AD-

uscac(Uhd)UfaUfGfGuuggga 790 VPusAfsaudCc(C2p)aacccaUfaAfggug 1145
CCTCACCTTATGGGTTGGGA 1500 + 1183998 ususa asgsg TTA AD-
csasccu(Uhd)AfuGfGfGfuugggau 791 VPusUfsaadTc(C2p)caacccAfuAfaggu 1146
CTCACCTTATGGGTTGGGAT 1501 + 1183999 usasa gsasg TAT AD-
ascscuu(Ahd)UfgGfGfUfugggauu 792 VPusAfsuadAu(C2p)ccaaccCfaUfaagg 1147
TCACCTTATGGGTTGGGATT 1502 + 1184000 asusa usgsa ATC AD-
cscsuua(Uhd)GfgGfUfUfgggauua 793 VPusGfsaudAa(Tgn)cccaacCfcAfuaag 1148
CACCTTATGGGTTGGGATTA 1503 + 1184001 uscsa gsusg TCC AD-
ascsuug(Uhd)UfcUfUfGfcugcaa 794 VPusGfsuudTg(C2p)gagcaaGfaAfcaag 1149
TCACTTGTCTCTTGCTCGCAA 1504 + 1184002 ascsa usgsa ACA AD-
csusugu(Uhd)CfuUfGfCfucgcaaa 795 VPusUfsgudTu(G2p)cgagcaAfgAfaca 1150
CACTTGTCTCTTGCTCGCAAA 1505 + 1184003 csasa agsusg CAT AD-
csasca(Chd)UfgCfUfUfaugcuua 796 VPusUfsaudTa(G2p)cauaagCfaGfuug 1151
GCCACAACCTGCTTATGCTAA 1506 + 1184004 usasa ugsgsc TAG AD-
usgsagu(Ghd)UfcUfCfUfauagaaa 797 VPusUfsaudTu(C2p)uauagaGfaCfacuc 1152
TATGAGTGTCTCTATAGAAA 1507 + 1184005 usasa asusa TAG AD-
usgsgac(Uhd)GfaGfAfCfugaccuu 798 VPusGfsuadAg(G2p)ucagucUfcAfguc 1153
GTTGGACTGAGACTGACCTT 1508 + 1184006 ascsa casasc ACT AD-
ususacc(Chd)AfgAfUfCfcaucaag 799 VPusUfsucdTu(G2p)auggauCfuGfggu 1154
CCTTACCCAGATCCATCAAG 1509 + 1184007 asasa aasgsg AAT AD-
gsasugg(Uhd)AfcAfCfUfuaugauu 800 VPusUfscadAu(C2p)auaaguGfuAfcca 1155
CAGATGGTACACTTATGATT 1510 + 1184008 gsasa ucsusg GAA AD-
asasaca(Uhd)CfcUfAfAfucaggag 801 VPusUfsacdTc(C2p)ugauuaGfgAfugu 1156
CTAAACATCCTAATCAGGAG 1511 + 1184009 usasa uusasg TAT AD-
asascau(Chd)CfuAfAfUfcaggagu 802 VPusAfsuadCu(C2p)cugauuAfgGfaug 1157
TAAACATCCTAATCAGGAGT 1512 + 1184010 asusa uususa ATG AD-
asgsgag(Uhd)AfuGfCfUfgaugucu 803 VPusAfsaadGa(C2p)aucagcAfuAfcucc 1158
TCAGGAGTATGCTGATGTCT 1513 + 1184011 ususa usgsa TTC AD-
cscsuga(Ghd)UfuUfUfAfugaggcu 804 VPusAfsuadGc(C2p)ucauaaAfaCfucag 1159
AACCTGAGTTTTATGAGGCT 1514 + 1184012 asusa gsusu ATG AD-
gscsagc(Ahd)GfaAfAfCfGcucaaa 805 VPusGfscudTu(G2p)agcguuUfcUfgcu 1160
TTGCAGCAGAAACGCTCAAA 1515 + 1184013 gscsa gcsasa GCT AD-
cscsugg(Uhd)AfcUfGfGfuaagagu 806 VPusUfsgadCu(C2p)uuaccaGfuAfcca 1161
CACCTGGTACTGGTAAGAGT 1516 + 1184014 csasa ggsusg CAT AD-
usasuag(Ahd)UfaAfAfUfuguaguag 807 VPusUfsucdTa(C2p)uacauuUfaUfcuau 1162
CCTATAGATAAATGTAGTAG 1517 + 1184015 asasa asgsg AAT AD-
csusgcu(Ghd)AfaAfUfUfguugaca 808 VPusAfsgudGu(C2p)aacaauUfuCfagc 1163
TCCTGCTGAAATTGTTGACA 1518 + 1184016 csusa agsgsa CTG AD-
gscsuga(Ahd)AfuUfGfUfugacacu 809 VPusAfscadGu(G2p)ucaacaAfuUfuca 1164
CTGCTGAAATTGTTGACACT 1519 + 1184017 gsusa gcsasg GTG AD-
usgsaaa(Uhd)UfgUfUfGfacacugu 810 VPusUfscadCa(G2p)ugucacCfaAfuuu 1165
GCTGAAATTGTTGACACTGT 1520 + 1184018 gsasa casgsc GAG AD-
asasauu(Ghd)UfuGfAfCfaguguga 811 VPusAfscudCa(C2p)agugucAfaCfaau 1166
TGAAATTGTTGACACTGTGA 1521 + 1184019 gsusa uuscsa GTG AD-
usgsuug(Ahd)CfaCfUfGfugagugc 812 VPusAfsagdCa(C2p)ucacagUfgUfcaac 1167
ATTGTTGACACTGTGAGTGC 1522 + 1184020 ususa asasu TTT AD-
gscsaug(Ahd)UfgUfUfUfcaucugc 813 VPusUfsugdCa(G2p)augaaaCfaUfcau 1168
ACGCATGATGTTTCATCTGC 1523 + 1184021 asasa gcsgsu AAT AD-
ususcac(Chd)UfuAfUfAfauucaca 814 VPusUfscudGu(G2p)aaauauAfaGfgug 1169
ATTTACCTTATAATTCACA 1524 + 1184022 gsasa aasasu GAA AD-

usascac(Chd)UfuUfUfAfuucacaca 814 VPusUfscudGu(G2p)aaauuauAfaGfgug 1169
ATTTACCTTATAATTCACA 1524 + 1184022 gsasa aasasu GAA AD-
uscsacc(Uhd)UfaUfAfAfuucacag 815 VPusUfsucdTg(Tgn)gaauuaUfaAfggu 1170
TTTCACCTTATAATTCACAG 1525 + 1184023 asasa gasasa AAT AD-
ascscuu(Ahd)UfaAfUfUfcacagaa 816 VPusCfsaudTc(Tgn)gugaauUfaUfaagg 1171
TCACCTTATAATTCACAGAA 1526 + 1184024 usgsa usgsa TGC AD-
cscsuua(Uhd)AfaUfUfCfacagaau 817 VPusGfscadTu(C2p)ugugaaUfuAfuaa 1172
CACCTTATAATTCACAGAAT 1527 + 1184025 gscsa ggsusg GCT AD-
cscsuua(Uhd)AfaUfUfCfacagaau 817 VPusGfscadTu(C2p)ugugaaUfuAfuaa 1172
CACCTTATAATTCACAGAAT 1527 + 1184025 gscsa ggsusg GCT AD-
usasuaa(Uhd)UfcAfCfAfgaaugcu 818 VPusAfsadGc(Agn)uucuguGfaAfuua 1173
CTTATAATTCACAGAATGCT 1528 + 1184026 gsusa uasasg GTA AD-
asascug(Uhd)UfgAfUfUfcaucaca 819 VPusCfscudGu(G2p)augaauCfaAfcag 1174
CAAACCTGTTGATTCATCACA 1529 + 1184027 gsgsa uususg GGG AD-
gsasuaa(Uhd)GfaCfUfAfugucaua 820 VPusAfsaudAu(G2p)acauagUfcAfuaa 1175
CAGAATATGACTATGTCATA 1530 + 1184028 ususa ucsusg TTC AD-
csusugu(Chd)AfuAfAfAfggucucu 821 VPusAfsuadGa(G2p)accuuuAfuGfaca 1176
AACTTGTCTATAAAGGTCTCT 1531 – 1184029 asusa agsusu ATC AD-
csuscau(Chd)UfcUfAfUfgauggu 822 VPusAfsaadCc(C2p)aucauaGfaGfauga 1177
GACTCATCTCTATGATGGGT 1532 + 1184030 ususa gsusc TTT AD-
asgsguc(Chd)UfaUfUfUfucacaaa 823 VPusAfsuudTu(G2p)ugaaaaUfaGfgac 1178
TCAGGTCCTATTTTCACAAA 1533 – 1184031 asusa cusgsa ATA AD-
ususuaa(Chd)AfaCfAfAfuuguaa 824 VPusAfsagdAu(C2p)aaauugUfuUfgua 1179
GGTTTACAAACAATTTGATA 1534 + 1184032 csusa aascsc CTT AD-
asusuaa(Chd)UfcAfAfCfugugua 825 VPusAfsuudGa(C2p)acaguuGfaGfuau 1180
AAATATACTCAACTGTGTCA 1535 + 1184033 asusa aususu ATA AD-
usasgua(Chd)UfuUfCfUfuugaac 826 VPusAfsagdTu(C2p)aaaagaAfaGfuacu 1181
AGTAGTACTTTCTTTTGAAC 1536 + 1184034 ususa ascsu TC AD-
usgsgua(Ahd)CfaCfUfAfauaguaa 827 VPusUfsuudTa(C2p)uauuagUfgUfuac 1182
TGTGGTAACACTAATAGTAA 1537 – 1184035 asasa cascsa AAT AD-
csasuaa(Chd)CfaUfUfAfaaccuaa 828 VPusUfsuadTa(G2p)guuuuaUfgGfuau 1183
TCCAATACCATTAAACCTAT 1538 – 1184036 sasa ugsgsa AAG AD-
asasugu(Uhd)CfuCfUfAfugagaac 829 VPusUfsggdTu(C2p)ucauagAfgAfaca 1184
AGAATGTTCTCTATGAGAAC 1539 + 1184037 csasa uuscsu CAA AD-
ususcug(Chd)UfaAfUfCfuugcugc 830 VPusUfsagdCa(G2p)caagauUfaGfcaga 1185
GCTTCTGCTAATCTTGCTGCT 1540 + 1184038 usasa asgsc AC AD-
csusgcu(Ahd)AfuCfUfUfgcugcua 831 VPusAfsagdAg(C2p)agcaagAfuUfagc 1186
TTCTGCTAATCTTGCTGCTAC 1541 + 1184039 csusa agsasa TA AD-
asuscau(Ahd)CfaUfCfAfcagaug 832 VPusAfsadAu(C2p)uggugaUfgUfaug 1187
GAATCATACATCACCAGATG 1542 + 1184040 ususa aususc TTG AD-
gsuscaa(Ahd)UfuAfCfAfuacaca 833 VPusUfsaudGu(G2p)uaauguAfaUfuug 1188
GAGTCAAATTACATTACACA 1543 + 1184041 usasa acsusc TAA AD-
ascsgac(Ghd)AfcUfAfCfuagcug 834 VPusGfsgcdAc(G2p)cuaguaGfuCfugc 1189
CGACGACGACTACTAGCGTG 1544 + 1184042 cscsa guscsG CCT AD-
csgsacg(Ahd)CfuAfCfUfagcugc 835 VPusAfsagdCa(C2p)gcuaguAfgUfcgu 1190
GACGACGACTACTAGCGTGC 1545 + 1184043 csusa cgsusc CTT AD-
ascsgac(Uhd)AfcUfAfGfcgugccu 836 VPusAfsaadGg(C2p)acgcuaGfuAfguc 1191
CGACGACTACTAGCGTGCT 1546 + 1184044 ususa guscsG TTG AD-
csgsacu(Ahd)CfuAfGfCfugccuu 837 VPusCfsaadAg(G2p)cacgcuAfgUfagu 1192
GACGACTACTAGCGTGCTT 1547 + 1184045 usgsa cgsusc TGT AD-

gsasgua(Chd)GfaCfUfuauguac 838 VPusGfsagdTa(C2p)auaaguUfcGfuacu 1193
ATGAGTACGAACCTTATGTAC 1548 + 1184046 uscsa csasu TCA AD-
usascga(Ahd)CfuUfAfUfuguacuca 839 VPusAfsaudGa(G2p)uacauaAfgUfucg 1194
AGTACGAACCTTATGTACTCA 1549 + 1184047 ususa uascsu TTC AD-
usascuc(Ahd)UfuCfGfUfuucggaa 840 VPusUfscudTc(C2p)gaaacgAfaUfgagu 1195
TGTACTCATTCGTTTCGGAA 1550 + 1184048 gsasa ascsa GAG AD-
usasguu(Ahd)AfuAfGfCfuguacuuc 841 VPusAfsagdAa(G2p)uacgcuAfuUfaac 1196
AATAGTTAATAGCGTACTTC 1551 + 1184049 ususa uasusu TTT AD-
ususcuu(Ghd)CfuUfUfCfugugua 842 VPusGfsaadTa(C2p)cacgaaAfgCfaaga 1197
TTTTCTTGCTTTTCGTGGTATT 1552 + 1184050 uscsa asasa CT AD-
ususcgu(Ghd)GfuAfUfUfcuugcua 843 VPusAfsudAg(C2p)aagaauAfcCfacga 1198
CTTTCGTGGTATTCTTGCTAG 1553 + 1184051 gsusa asasg TT AD-
usasgcc(Ahd)UfcCfUfUfacugcgc 844 VPusAfsagdCg(C2p)aguaagGfaUfggc 1199
ACTAGCCATCCTTACTGCGC 1554 + 1184052 ususa uasgsu TTC AD-
asgscca(Uhd)CfcUfUfAfcugcgc 845 VPusGfsaadGc(G2p)caguaaGfgAfgg 1200
CTAGCCATCCTTACTGCGCT 1555 + 1184053 uscsa cusasg TCG AD-
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TAGCCATCCTTACTGCGCTT 1556 + 1184054 csgsa gcsusa CGA AD-
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AGCCATCCTTACTGCGCTTC 1557 + 1184055 gsasa ggscsu GAT AD-
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TACTGCGCTTCGATTGTGT 1558 + 1184056 gscsa usasa GCG AD-
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GCGCTTCGATTGTGTGCGTA 1561 + 1184059 csusa gcsusc CTG AD-
gsusucc(Uhd)GfaUfCfUfucguc 852 VPusUfsagdAc(C2p)agaagaUfcAfgga 1207
GAGTTCCTGATCTTCTGGTCT 1562 + 1184060 usasa acsusc AA AD-
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GGCCCCAAGGTTTACCCAAT 1575 + 1184073 sasa gsgscs AAT AD-
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CAGATGACCAAATTGGCTAC 1577 + 1184075 usasa ucsusg TAC AD-
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CGTGCTACAACCTCCTCAAG 1581 + 1184079 gsasa cscsg GAA AD-
usascaa(Chd)UfuCfCfUfcaaggaac 872 VPusUfsugdTc(C2p)uugaggAfaGfuug 1227
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CAACTTCCTCAAGGAACAAC 1584 + 1184082 susa gususg ATT AD-
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CTGGTGTCTCTATCTGTAGT 1664 + 1184162 ascsa g ACT AD-
uscsucu(Ahd)UfcUfGfUfaguacua 953 VPusCfsauaGfuacuacaGfaUfagagascs 1308
TGTCTCTATCTGTAGTACTAT 1665 + 1184163 usgsa a GA AD-
ususguu(Chd)UfuGfCfUfcgcaaac 954 VPusAfsuguUfugcgagcAfaGfaacaasgs 1309
ACTTGTTCTTGCTCGCAAAC 1666 + 1184164 asusa u ATA AD-
ususcac(Uhd)AfuAfUfGfuuaaacc 955 VPusCfsuggUfuuaacauAfuAfgugaascs 1310
GGTTCACTATATGTTAAACC 1667 + 1184165 asgsa c AGG AD-
ascsaac(Uhd)GfcUfUfAfugcuauu 956 VPusCfsuauUfagcauaaGfcAfguugusgs 1311
CCACAACGTGTTATGCTAAT 1668 + 1184166 asgsa g AGT AD-
csasacu(Ghd)CfuUfAfUfgcuaua 957 VPusAfscuaUfuagcauaAfgCfaguugsus 1312
CACAACGTGTTATGCTAATA 1669 + 1184167 gsusa g GTG AD-
asascug(Chd)UfuAfUfGfcuaauag 958 VPusCfsacuAfuuaagcaUfaGfcaguusgs 1313
ACAACGTGTTATGCTAATAG 1670 + 1184168 usgsa u TGT AD-
ascsgc(Uhd)UfaUfGfCfuauuagu 959 VPusAfscacUfaauagcaUfaAfgcagusus 1314
CAACTGCTTATGCTAATAGT 1671 + 1184169 gsusa g GTT AD-
csascuc(Ahd)UfaAfAfGfucugugu 960 VPusCfsaacAfcagacuuUfaUfgagugsus 1315
GACACTCATAAAGTCTGTGT 1672 – 1184170 usgsa c TGT AD-

gsasgug(Uhd)CfuUfAfuaagaaa 961 VPusUfsuauUfucuaugAfgAfcacucsas 1316
ATGAGTGTCTCTATAGAAAT 1673 + 1184171 asgsa u AGA AD-
ascsuga(Ghd)AfcUfGfAfcuuacu 962 VPusUfsuagUfaaggucaGfuCfucaguscs 1317
GGACTGAGACTGACCTTACT 1674 + 1184172 asasa c AAA AD-
csasuac(Ahd)AfuGfCfUfaguuaaa 963 VPusUfsguuUfaacuagcAfuUfguaugsus 1318
AACATACAATGCTAGTTAAA 1675 + 1184173 csasa u CAG AD-
ascsaga(Uhd)GfgUfAfCfacuuau 964 VPusAfsucaUfaaguguaCfcAfucugusus 1319
AAACAGATGGTACACTTATG 1676 + 1184174 asusa u ATT AD-
csasgau(Ghd)GfuAfCfAfcuuau 965 VPusAfsaucAfuaaguguAfcCfaucugusus 1320
AACAGATGGTACACTTATGA 1677 + 1184175 ususa u TTG AD-
asgsgag(Ahd)GfuAfCfAfcuuuuga 966 VPusUfsuucAfaagguguAfcUfcuccusas 1321
ATAGGAGAGTACACCTTTGA 1678 + 1184176 asasa u AAA AD-
usasucu(Chd)AfgAfUfGfaguuuuc 967 VPusUfsagaAfaacucauUfcUfgagausas 1322
AATATCTCAGATGAGTTTTC 1679 + 1184177 usasa u TAG AD-
asuscuc(Ahd)GfaUfGfAfguuuuc 968 VPusCfsuagAfaaacucaUfcUfgagausas 1323
ATATCTCAGATGAGTTTCT 1680 + 1184178 asgsa u AGC AD-
asusguu(Ghd)CfaAfAfUfaucaaaa 969 VPusCfsuuuUfgauuuuUfgCfaacausus 1324
CAATGTTGCAAATTATCAAA 1681 + 1184179 asgsa g AGG AD-
gsusugc(Ahd)AfaUfUfAfucaaaaag 970 VPusAfsccuUfuugauaaUfuUfgcaacsas 1325
ATGTTGCAAATTATCAAAAAG 1682 + 1184180 gsusa u GTT AD-
gsusacu(Ghd)GfuAfAfGfagucauu 971 VPusAfsaaaUfgacucuuAfcCfaguacscs 1326
TGGTACTGGTAAGAGTCATT 1683 + 1184181 ususa a TTG AD-
usascug(Ghd)UfaAfGfAfgucauuu 972 VPusCfsaaaAfugacucuUfaCfcaguascs 1327
GGTACTGGTAAGAGTCATTT 1684 + 1184182 usgsa c TGC AD-
gsusaga(Ghd)UfgUfUfUfugauaaa 973 VPusAfsauuUfaucaaaaCfaCfucuaacsasc 1328
GTGTAGAGTGTTTTGATAAA 1685 + 1184183 ususa TTC AD-
asgsagu(Ghd)UfuUfUfGfauuuuu 974 VPusUfsgaaUfuuaucuaAfaCfacucusas 1329
GTAGAGTGTTTTGATAAATT 1686 + 1184184 csasa c CAA AD-
gsusguu(Uhd)UfgAfUfAfaauucua 975 VPusCfsuuuGfaauuuuauCfaAfaacacsus 1330
GAGTGTTTTGATAAATTCAA 1687 + 1184185 asgsa c AGT AD-
gsusuuu(Ghd)AfuAfAfAfuucuaag 976 VPusCfsacuUfugaauuuAfuCfaaaacsas 1331
GTGTTTTGATAAATTCAAAG 1688 + 1184186 usgsa c TGA AD-
csascac(Uhd)AfgAfAfCfcagaaua 977 VPusAfsauaUfucugguuCfuAfgugugsc 1332
GGCACACTAGAACCAGAAT 1689 + 1184187 ususa sc ATTT AD-
ascsacu(Ahd)GfaAfCfCfagaauau 978 VPusAfsauuAfuucugguUfcUfagugusg 1333
GCACACTAGAACCAGAATAT 1690 + 1184188 ususa Sc TTC AD-
gsasaau(Uhd)GfuUfGfAfcacugug 979 VPusCfsucaCfagugucaAfcAfauuucsas 1334
CTGAAATTGTTGACACTGTG 1691 + 1184189 asgsa g AGT AD-
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AACCCTGCTTGGAGAAAAGC 1692 + 1184190 usgsa u TGT AD-
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ususaua(Ahd)UfuCfAfCfagaau 982 VPusCfsagcAfuucugugAfaUfuauaasgs 1337
CCTTATAATTCACAGAATGC 1694 + 1184192 usgsa g TGT AD-
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AGAATATGACTATGTCATAT 1695 + 1184193 uscsa u TCA AD-
asusaug(Ahd)CfuAfUfGfucuuuu 984 VPusUfsgaaUfaugacauAfgUfcuuuus 1339
GAATATGACTATGTCATATT 1696 + 1184194 csasa c CAC AD-

usasuga(Chd)UfaUfGfUfcuauuc 985 VPusGfugaAfuauugacaUfaGfucauasus 1340
AATATGACTATGTCATATTC 1697 + 1184195 ascsa u ACT AD-
asasgac(Uhd)CfaUfCfUfcuauugau 986 VPusCfscacUfcuauagagaUfgAfgucuuuscs 1341
AGAAGACTCATCTCTATGAT 1698 + 1184196 gsgsa u GGG AD-
asgsacu(Chd)AfuCfUfCfuaugaug 987 VPusCfscacUfcuauagagaUfgAfgucuuuscs 1342
GAAGACTCATCTCTATGATG 1699 + 1184197 gsgsa c GGT AD-
uscsaga(Ghd)UfuUfAfGfaaaugu 988 VPusCfscacAfuuuucuaAfaCfucugasas 1343
CTTCAGAGTTTAGAAAATGT 1700 + 1184198 gsgsa g GGC AD-
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ACACCATCAACTTTCTTATA 1701 – 1184199 asusa u ATA AD-
csuscca(Uhd)AfaAfCfGfauauguu 990 VPusCfsgaaCfauaucguUfuAfuggagsas 1345
ATCTCCATAAACGATATGTT 1702 – 1184200 csgsa u CGA AD-
csasuag(Ghd)AfaUfAfAfaaucuuc 991 VPusUfsagaAfgauuuuaUfuCfcuauugsgs 1346
TCCATAGGAATAAAATCTTC 1703 – 1184201 usasa a TAA AD-
cscsuua(Uhd)AfuUfAfCfaaacuug 992 VPusCfsacaAfguuuguaAfuAfuuaaggsas 1347
TTCCTAATATTACAACTTG 1704 + 1184202 usgsa a TGC AD-
gscsuug(Uhd)UfaAfAfCfaacuuag 993 VPusAfsagcUfaaguuguUfuAfcaagcsgs 1348
ACGCTTGTTAAACAACCTTAG 1705 + 1184203 csusa u CTC AD-
csusugu(Uhd)AfaAfCfAfacuuagc 994 VPusGfsagcUfaaguuguUfuAfcaagcsgs 1349
CGCTTGTTAAACAACCTTAGC 1706 + 1184204 uscsa g TCC AD-
csusugc(Uhd)GfcUfAfCfuuaaauug 995 VPusGfsacaUfuuuaguaGfcAfgcaagsas 1350
ATCTTGCTGCTACTAAAATG 1707 + 1184205 uscsa u TCA AD-
ascsuua(Uhd)GfgAfUfUfuguuuau 996 VPusUfscacAfaacaaauCfcAfuuaagusus 1351
GAACTTATGGATTTGTTTAT 1708 + 1184206 gsasa c GAG AD-
csgsacg(Ahd)CfgAfCfUfacuagcg 997 VPusCfsacgCfuaguaguCfgUfcgucgsgs 1352
ACCGACGACGACTACTAGCG 1709 + 1184207 usgsa u TGC + AD-
gsascga(Chd)GfaCfUfAfcuagcgu 998 VPusGfscacGfcuaguagUfcGfucgucsgs 1353
CCGACGACGACTACTAGCGT 1710 1184208 gscsa g GCC AD-
asusgag(Uhd)AfcGfAfAfcuuaugu 999 VPusGfsuacAfuuauguGfuAfcucauscs 1354
TGATGAGTACGAACCTTATGT 1711 + 1184209 ascsa a ACT AD-
csgsaac(Uhd)UfaUfGfUfacuauu 1000 VPusCfsgaaUfgaguacaUfaAfguucgsus 1355
TACGAACCTTATGTACTCATT 1712 + 1184210 csgsa a CGT AD-
gsasacu(Uhd)AfuGfUfAfcuauuc 1001 VPusAfsagaAfuaguaguAfuAfguucgsus 1356
ACGAACCTTATGTACTCATT 1713 + 1184211 gsusa u GTT AD-
asascuu(Ahd)UfgUfAfCfucuuug 1002 VPusAfsagcAfaugaguaCfaUfaaguuscs 1357
CGAACCTTATGTACTCATTG 1714 + 1184212 ususa g TTT AD-
ascsuua(Uhd)GfuAfCfUfcuauucgu 1003 VPusAfsaacGfaaugaguAfcAfuuaagusus 1358
GAACTTATGTACTCATTG 1715 + 1184213 ususa c TTC AD-
csusuau(Ghd)UfaCfUfCfauucguu 1004 VPusGfsaaaCfgaugagUfaCfauaagsus 1359
AACTTATGTACTCATTGTTT 1716 + 1184214 uscsa u CG AD-
ususaug(Uhd)AfcUfCfAfuucguuu 1005 VPusCfsgaaAfcgaauagaGfuAfcuuaasgs 1360
ACTTATGTACTCATTGTTT 1717 + 1184215 csgsa u GG AD-
usasugu(Ahd)CfuCfAfUfcguuuuc 1006 VPusCfscgaAfacgaugAfgUfacuauasas 1361
CTTATGTACTCATTGTTT 1718 + 1184216 gsgsa g GA AD-
asusgua(Chd)UfcAfUfUfcguuuug 1007 VPusUfscgaAfaacgaauGfaGfuacausas 1362
TTATGTACTCATTGTTT 1719 + 1184217 gsasa a AA AD-
usgsuac(Uhd)CfaUfUfCfguuuug 1008 VPusUfsuccGfaaacgaUfgAfguacausas 1363
TATGTACTCATTGTTT 1720 + 1184218 asasa a AAG AD-
gsusacu(Chd)AfuUfCfGfuuuugga 1009 VPusCfsuucCfgaacgaAfuGfaguacsas 1364
ATGTACTCATTGTTT 1721 + 1184219 asgsa u AGA AD-

csasggu(Ahd)CfgUfAfAfaguuaa 1010 VPusAfsuuaAfcuauuaaCfgUfaccugsus 1365
GACAGGTACGTTAATAGTTA 1722 + 1184220 asusa c ATA AD-
asgsgua(Chd)GfuUfAfAfaguuaa 1011 VPusUfsauuAfacuauuaAfcGfuaccusgs 1366
ACAGGTACGTTAATAGTTAA 1723 + 1184221 usasa u TAG AD-
usasaua(Ghd)UfuAfAfUfagcguac 1012 VPusAfsaguAfcgcuauuAfaCfuauuasas 1367
GTTAATAGTTAATAGCGTAC 1724 + 1184222 ususa c TTC AD-
uscsuug(Chd)UfuUfCfGfugguauu 1013 VPusAfsгааUfaccacgaAfaGfcaagasasa 1368
TTTCTTGCTTTTCGTGGTATTTC 1725 + 1184223 csusa TT AD- csusugc(Uhd)UfuCfGfUfgguauuc
1014 VPusAfsagaAfuaccacgAfaAfgcaagsas 1369 TTCTTGCTTTTCGTGGTATTCT 1726 +
1184224 ususa a TG AD- usugcu(Uhd)UfcGfUfGfguauucu 1015
VPusCfsaagAfauccacGfaAfagcaasgsa 1370 TCTTGCTTTTCGTGGTATTCTT 1727 + 1184225
usgsa GC AD- usgscuu(Uhd)CfgUfGfGfuauucuu 1016 VPusGfscaaGfaauaccaCfgAfaagcasag
1371 CTTGCTTTTCGTGGTATTCTTG 1728 + 1184226 gscsa CT AD-
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TTGCTTTTCGTGGTATTCTTGC 1729 + 1184227 csusa a TA AD-
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GCTTTTCGTGGTATTCTTGCTA 1730 + 1184228 uasgsa GT AD-
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TACTGCGCTTCGATTGTGTG 1731 + 1184229 csgsa a CGT AD-
gsusacu(Ghd)CfuGfCfAfaauugu 1020 VPusUfsaacAfaauuugcAfgCfaguacsgs 1375
GCGTACTGCTGCAATATTGT 1732 + 1184230 usasa c TAA AD-
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TACTGCTGCAATATTGTTAA 1733 + 1184231 csgsa a CGT AD-
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TGCTGCAATATTGTTAACGT 1736 + 1184234 gsasa a GAG AD-
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CTAGAGTTCCTGATCTTCTG 1737 + 1184235 gsusa g GTC AD-
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TCCTGATCTTCTGGTCTAAA 1738 + 1184236 csgsa a CGA AD-
gsasucu(Uhd)CfuGfGfUfcuaaacg 1027 VPusUfsucgUfuuagaccAfgAfagaucsas 1382
CTGATCTTCTGGTCTAAACG 1739 + 1184237 asasa g AAC AD-
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csasaug(Ghd)AfaCfCfUfaguaua 1029 VPusCfsсuaUfuacuaggUfuCfcauugsus 1384
AACAATGGAACCTAGTAATA 1741 + 1184239 gsgsa u GGT AD-
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ACAATGGAACCTAGTAATAG 1742 + 1184240 gsusa u GTT AD-
asusgga(Ahd)CfcUfAfGfuauuagg 1031 VPusAfsaccUfauuacuaGfgUfuccausus 1386
CAATGGAACCTAGTAATAGG 1743 + 1184241 ususa g TTT AD-
asascu(Ahd)GfuAfAfUfagguuuc 1032 VPusAfsгgaAfacuauuAfcUfagguuscs 1387
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ascscua(Ghd)UfaAfUfAfgguuucc 1033 VPusUfsaggAfaaccuauUfaCfuaggusus 1388
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ascsauc(Ahd)CfgAfAfCfcguuucu 1035 VPusUfsaagAfaagcguuCfgUfgaugusas 1390

CTACATCACGAACGCTTTCT 1747 + 1184245 usasa g TAT AD-
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 asuscac(Ghd)AfaCfGfCfuuuucua 1037 VPusAfsauaAfgaaagcgUfuCfugaugs 1392
 ACATCACGAACGCTTTCTTA 1749 + 1184247 ususa u TTA AD-
 uscsacg(Ahd)AfcGfCfUfuucuuau 1038 VPusUfsauaAfgaaagcGfuUfcgugasus 1393
 CATCACGAACGCTTTCTTAT 1750 + 1184248 usasa g TAC AD-
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 AGCCTTTCTGCTATTCCCTTGT 1758 + 1184256 ususa u TT AD-
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 asgsucc(Ahd)GfaUfGfAfccaaaau 1056 VPusCfscaaUfuuggucaUfcUfggacusgs 1411
 GCAGTCCAGATGACCAAATT 1768 + 1184266 gsgsa c GGC AD-
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 ACATTGCCAAAAGGCTTCTA 1770 + 1184268 csgsa u CGC AD-
 ususgcc(Ahd)AfaAfGfGfcuucua 1059 VPusGfscgaAfgaagccuUfuUfggcaasus 1414
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CTCTTGTGCAGAATGAATTC 1777 + 1184275 uscsa g TCG AD-
ususgug(Chd)AfgAfAfUfgaauc 1066 VPusCfsgagAfauucauuCfuGfcacaasgs 1421
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CTTGTGCAGAATGAATTCTC 1779 + 1184277 gsusa g GTA AD-
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csasгаа(Uhd)GfaAfUfUfucuguaa 1071 VPusAfsгуuAfcgagaauUfcAfuucugscs 1426
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usasauc(Uhd)CfaCfAfUfagcauc 1072 VPusAfsagaUfugcuauUfgAfgauuasas 1427
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uscsuca(Chd)AfuAfGfCfaucuuu 1073 VPusUfsuuaAfgauugcuAfuGfugagasus 1428
AATCTCACATAGCAATCTTT 1785 + 1184283 asasa u AAT AD-
csuscac(Ahd)UfaGfCfAfaucuuua 1074 VPusAfsuuaAfagauugcUfaUfgugagsas 1429
ATCTCACATAGCAATCTTTA 1786 + 1184284 asusa u ATC AD-
uscsaca(Uhd)AfgCfAfAfucuuuaa 1075 VPusGfsauuAfaagauugCfuAfugugasgs 1430
TCTCACATAGCAATCTTTAA 1787 + 1184285 uscsa a TCA AD-
csascau(Ahd)GfcAfAfUfcuuuaau 1076 VPusUfsgauUfaaagauGfcUfaugugsas 1431
CTCACATAGCAATCTTTAAT 1788 + 1184286 csasa g CAG AD-
gsasgcc(Chd)UfaAfUfGfuguaaaa 1077 VPusAfsauuUfuacacauUfaGfggcucsus 1432
AAGAGCCCTAATGTGTAAAA 1789 + 1184287 ususa u TTA AD-
gscscu(Ahd)AfuGfUfGfuuaaauc 1078 VPusUfsuuaUfuuuacacAfuUfagggcsus 1433
GAGCCCTAATGTGTAAAATT 1790 + 1184288 asasa c AAT AD-
cscscua(Ahd)UfgUfGfUfaaaauua 1079 VPusAfsuuaAfuuuuacaCfaUfuagggscs 1434
AGCCCTAATGTGTAAAATTA 1791 + 1184289 asusa u ATT AD-
cscsuua(Uhd)GfuGfUfAfaaaauua 1080 VPusAfsauuAfauuuacAfcAfuuaggsgs 1435
GCCCTAATGTGTAAAATTA 1792 + 1184290 ususa c TTT AD-
cscscu(Ghd)UfgAfUfUfuuaauag 1081 VPusAfsгcuAfuuaaaauCfaCfaugggsgs 1436
TCCCCATGTGATTTTAATAG 1793 + 1184291 csusa a CTT

(634) TABLE-US-00005 TABLE 4 Unmodified Sense and Antisense Strand
Coronavirus dsRNA Sequences Strand of the Viral RNA Start in End in targeted
BetaCoV/Wuhan-Hu- BetaCoV/Wuhan-Hu- Targets Targets Targets by the Duplex Id Sense
Sequence 5' to 3' Antisense Sequence 5' to 3' 1/2019|EP1_ISL_402125
1/2019|EP1_ISL_402125 SARS2? SARS? MERS? Agent AD-1231467
UACUCAUUCGUUUCGGAAGAA UUCUUCCGAAACGAAUGAGUACA 26246 26268 TRUE
TRUE FALSE + (SEQ ID NO: 1794) (SEQ ID NO: 1834) AD-1231468

CUUCUGGUCUACAAACUAA UAGUUUUUAGACCAGAAAG 26459 26481 TRUE
TRUE FALSE + (SEQ ID NO: 1795) (SEQ ID NO: 1835) AD-1231469
UUCUUGCUUUCGUGGUAAUUA UGAAUACCACGAAAGCAAGAAAA 26301 26323 TRUE
TRUE FALSE + (SEQ ID NO: 1796) (SEQ ID NO: 1836) AD-1231470
UUAUCUCACAUAAGUAAUCUA UAGAUAUAUGUGAGAUUAAAG 29659 29681
TRUE TRUE FALSE + (SEQ ID NO: 1797) (SEQ ID NO: 1837) AD-1231471
UAACUUUAAUCUCAUAUAGCA UGCUAUUAUGAGAUUAAAGUUAAC 29654 29676
TRUE TRUE FALSE + (SEQ ID NO: 1798) (SEQ ID NO: 1838) AD-1231472
CCUGAUCUUCUGGUUUAACA UGUUUAACCAGAAGAUCAGGAA 26453 26475 TRUE
TRUE FALSE + (SEQ ID NO: 1799) (SEQ ID NO: 1839) AD-1231473
UGUACAGUAAGUGAUAAACAGA UCUGUUAUCACUACUGUACAAG 27180 27202 TRUE
TRUE FALSE + (SEQ ID NO: 1800) (SEQ ID NO: 1840) AD-1231474
UUCCUGAUCUUCUGGUCUAAA UUUAGACCAGAAGAUCAGGAACU 26451 26473 TRUE
TRUE FALSE + (SEQ ID NO: 143) (SEQ ID NO: 498) AD-1231475
UAUGUACUCAUUCGUUUCGGA UCCGAAACGAAUGAGUACAUAAG 26242 26264 TRUE
TRUE FALSE + (SEQ ID NO: 296) (SEQ ID NO: 651) AD-1231476
ACUGUACAGUCUAAAAUGUCA UGACAUUUUAGACUGUACAGUGG 11832 11854 TRUE
TRUE FALSE + (SEQ ID NO: 7) (SEQ ID NO: 8) AD-1231477
CCCAUGUGAUUUUAAUAGCUA UAGCUAUUAAAAUCACAUGGGGA 29834 29856 TRUE
TRUE FALSE + (SEQ ID NO: 1801) (SEQ ID NO: 1841) AD-1231478
UUCGUGGUAAUUCUACUAGUA UACUAGUAAGAAUACCACGAAAG 26309 26331 TRUE
TRUE FALSE + (SEQ ID NO: 1802) (SEQ ID NO: 1842) AD-1231479
UCUUCUGGUCUAAAUGAACUA UAGUUCAUUUAGACCAGAAGAUC 26458 26480 TRUE
TRUE FALSE + (SEQ ID NO: 1803) (SEQ ID NO: 1843) AD-1231480
GUACUGCUGCAAUAUUGUUA UUAACAAUAUUGCAGCAGUACGC 26365 26387 TRUE
TRUE FALSE + (SEQ ID NO: 310) (SEQ ID NO: 665) AD-1231481
UUUAAUCUCACAUAAGCAAUCA UGAUUGCUAUGUGAGAUUAAAGU 29658 29680
TRUE TRUE FALSE + (SEQ ID NO: 1804) (SEQ ID NO: 1844) AD-1231482
UACAACUCCUCAAGGAACAA UUGUUCCUUGAGGAAGUUGUAGC 28747 28769 TRUE
TRUE FALSE + (SEQ ID NO: 162) (SEQ ID NO: 517) AD-1231483
CUGCUAUUCCUUGUUUUAAUA UAUUAAAACAAGGAAUAGCAGAA 27802 27824 TRUE
TRUE FALSE + (SEQ ID NO: 1805) (SEQ ID NO: 1845) AD-1231484
CUCACAUAAGCAAUCUUUAAUA UAUUAAAGAUUGCUAUGUGAGAU 29664 29686
TRUE TRUE FALSE + (SEQ ID NO: 1806) (SEQ ID NO: 1846) AD-1231485
UCGUGCUACAACUUUCUCAA UUGAGAAAGUUGUAGCACGAUU 28741 28763 TRUE
TRUE FALSE + (SEQ ID NO: 1807) (SEQ ID NO: 1847) AD-1231486
CAGAUGGUACACUUAUGAUUA UAAUCAUAAGUGUACCAUCUGUU 15987 16009 TRUE
TRUE FALSE + (SEQ ID NO: 1808) (SEQ ID NO: 1848) AD-1231487
UAACAAUGUUGCUIIUCAAAA UUUUGAAAAGCAACAUUGUUAGU 14644 14666
TRUE TRUE FALSE + (SEQ ID NO: 1809) (SEQ ID NO: 1849) AD-1231488
UAAUAGUUAUAGCGUACUUA UAAGUACGCUAUUAACUAUUAAC 26277 26299
TRUE TRUE FALSE + (SEQ ID NO: 1810) (SEQ ID NO: 1850) AD-1231489
CAUACAAUGCUAGUUAACAA UUGUUUAACUAGCAUUGUAUGUU 15884 15906
TRUE TRUE FALSE + (SEQ ID NO: 1811) (SEQ ID NO: 1851) AD-1231490
UUUUCAAACUGUCAACCCGA UCGGGUUUGACAGUUUGAAAAGC 14656 14678 TRUE
TRUE FALSE + (SEQ ID NO: 1812) (SEQ ID NO: 1852) AD-1231491
CUUAUGUACUCAUUCGUUUA UGAAACGAAUGAGUACAUAAGUU 26240 26262 TRUE
TRUE FALSE + (SEQ ID NO: 1813) (SEQ ID NO: 1853) AD-1231492
GUUGUGUACACACAUUGGUAA UUACCAAUGUGUGUACACAACAU 13154 13176 TRUE
TRUE FALSE + (SEQ ID NO: 1814) (SEQ ID NO: 1854) AD-1231493

AACUUAUUGUACAUUCGUUA UAACGAAUGAGUACAUAAUGUC 26238 26260 TRUE
TRUE FALSE + (SEQ ID NO: 1815) (SEQ ID NO: 1855) AD-1231494
CUGCUGCAAUAUUGUUAACGA UCGUUAACAAUAUUGCAGCAGUA 26368 26390 TRUE
TRUE FALSE + (SEQ ID NO: 1816) (SEQ ID NO: 1856) AD-1231495
UUCUUCGUAAGAACGGUAAUA UAUUACCGUUCUACGAAGAAGA 628 650 TRUE
TRUE FALSE + (SEQ ID NO: 27) (SEQ ID NO: 382) AD-1231496
CGUGCUACAACUUCUUCAAGA UCUUGAAGAAGUUGUAGCACGAU 28742 28764 TRUE
TRUE FALSE + (SEQ ID NO: 1817) (SEQ ID NO: 1857) AD-1231497
UGAAAUUGUUGACAUUGUGAA UUCACAAUGUCAACAAUUUCAGC 17572 17594 TRUE
TRUE FALSE + (SEQ ID NO: 1818) (SEQ ID NO: 1858) AD-1231498
ACAAUGUUGCUUUUUAAACUA UAGUUUAAAAGCAACAUUGUUA 14646 14668
TRUE TRUE FALSE + (SEQ ID NO: 1819) (SEQ ID NO: 1859) AD-1231499
AAAUUGUUGACACUAUGAGUA UACUCAUAGUGUCAACAAUUUCA 17574 17596 TRUE
TRUE FALSE + (SEQ ID NO: 1820) (SEQ ID NO: 1860) AD-1231500
UCUUCGUAAGAACGGUAAUAA UUAUUACCGUUCUACGAAGAAG 629 651 TRUE
TRUE FALSE + (SEQ ID NO: 28) (SEQ ID NO: 383) AD-1231501
CUGCAAUAUUGUUAACGUGAA UUCACGUUAACAAUAUUGCAGCA 26371 26393 TRUE
TRUE FALSE + (SEQ ID NO: 1821) (SEQ ID NO: 1861) AD-1231502
GCUGAAAUUGUUGAUACUGUA UACAGUAUCAACAAUUUCAGCAG 17570 17592 TRUE
TRUE FALSE + (SEQ ID NO: 1822) (SEQ ID NO: 1862) AD-1231503
CUGCUGAAAUUGUUGACACUA UAGUGUCAACAAUUUCAGCAGGA 17568 17590 TRUE
TRUE FALSE + (SEQ ID NO: 1823) (SEQ ID NO: 1863) AD-1231504
UUGUGAUGAUGAUUAUUUCA UUGAAAUAUCAUCAACAACA 17578 17600 TRUE
TRUE FALSE + (SEQ ID NO: 228) (SEQ ID NO: 583) AD-1231505
UGUUGACACUGUGAAUGCUUA UAAGCAUUCACAGUGUCAACAAU 26268 26290 TRUE
TRUE FALSE + (SEQ ID NO: 1824) (SEQ ID NO: 1864) AD-1231506
CAGGUACGUUAAUAGUUAUA UAUUAACUAUUAACGUACCUGUC 28767 28789 TRUE
TRUE FALSE + (SEQ ID NO: 300) (SEQ ID NO: 655) AD-1231507
ACAUUGCCAAAAGGUUUCUAA UUAGAAACCUUUUGGCAAUGUUG 26370 26392 TRUE
TRUE FALSE + (SEQ ID NO: 1825) (SEQ ID NO: 1865) AD-1231508
GCUGCAAUAUUGUUAACGUGA UCACGUUAACAAUAUUGCAGCAG 29666 29688 TRUE
TRUE FALSE + (SEQ ID NO: 313) (SEQ ID NO: 668) AD-1231509
CACAUAGCAAUCUUAUAUCA UUGAUUAAAGAUUGCUAUGUGAG 14824 14846
TRUE TRUE FALSE + (SEQ ID NO: 1826) (SEQ ID NO: 1866) AD-1231510
AAUGUGUGAUUAUCAGACAACA UGUUGUCUGAUUAUCACACAUGU 26451 26473 TRUE
TRUE FALSE + (SEQ ID NO: 1827) (SEQ ID NO: 1867) AD-1231511
GUUCCUGAUCUUCUGGUCUAA UUAGACCAGAAGAUCAGGAACUC 26450 26472 TRUE
TRUE FALSE + (SEQ ID NO: 142) (SEQ ID NO: 497) AD-1231512
UUGCCAAAAGGCUUCUACGCA UGCGUAGAAGCCUUUUGGCAAUG 28770 28792 TRUE
TRUE FALSE + (SEQ ID NO: 349) (SEQ ID NO: 704) AD-1231513
UUCUGCUAAUCUUGUUGC UAA UUAGCAACAAGAUUAGCAGAAGC 24620 24642 TRUE
TRUE FALSE + (SEQ ID NO: 1828) (SEQ ID NO: 1868) AD-1231514
ACCUAGUAAUAGGUUUCUAA UUAGGAAACCUAUUACUAGGUUC 26582 26604 TRUE
TRUE FALSE + (SEQ ID NO: 1829) (SEQ ID NO: 1869) AD-1231515
CUGC UAAUCUUGCUGCUACUA UAGUAGCAGCAAGAUUAGCAGAA 24622 24644 TRUE
TRUE FALSE + (SEQ ID NO: 121) (SEQ ID NO: 476) AD-1231516
CACUCAUAAAGUCUGUGUUGA UCAACACAGACUUUAUGAGUGUC 15609 15631 TRUE
FALSE FALSE - (SEQ ID NO: 1830) (SEQ ID NO: 1870) AD-1231517
CAUAGGAAUAAAUCUUCUAA UUAGAAGAUUUUAUUCCUAUGGA 20413 20435
TRUE FALSE FALSE - (SEQ ID NO: 281) (SEQ ID NO: 636) AD-1231518

UUCUACCAACACUUAUAAAGAA UUCUUUAAGAGUUUGUGUAGAAU 3745 3767 TRUE
FALSE FALSE – (SEQ ID NO: 210) (SEQ ID NO: 565) AD-1231519
AUCUUGUUUUCUCUAUUCAAA UUUGAAUAGAGAAAACAAGAUGA 3929 3951 TRUE
FALSE FALSE – (SEQ ID NO: 1831) (SEQ ID NO: 1871) AD-1231520
CAGUCAUAAUCUAUAUUA AAAA UUUUAAUAUAGAUAUGACUGUG 10501 10523
TRUE FALSE FALSE – (SEQ ID NO: 1832) (SEQ ID NO: 1872) AD-1231521
UGGUAACACUAAUAAUAAAAA UUUUUAUAUAUAGUGUUACCACA 23712 23734
TRUE FALSE FALSE – (SEQ ID NO: 1833) (SEQ ID NO: 1873)
(635) TABLE-US-00006 TABLE 5 Modified Sense and Antisense Strand dsRNA
Sequences Targeting COVID-19 SEQ ID SEQ ID Duplex Id Sense Sequence 5' to
3' NO: Antisense Sequence 5' to 3' NO: AD-1231467 usascuca(Uhd)uCfGfUfuucggaagsasa
1874 VPusUfscudTc(Cgn)gaaacgAfaUfgaguascsa 1925 AD-1231468
csusuc(Uhd)gGfuCfUfAfaacaacusasa 1875 VPusUfsagdTudTguuuagAfcCfagaagsasu 1926 AD-
1231469 ususcugCfuUfUfCfugg(Uhd)auuscsa 1876 VPusGfsaadTa(Cgn)cacgaaAfgCfaagaasasa
1927 AD-1231470 ususaau(Chd)UfcAfCfAfuagaaucsusa 1877
VPusAfsagdTudAcuauguGfaGfauuaasasg 1928 AD-1231471
usasacu(Uhd)UfaAfUfCfucauauagscsa 1878 VPusGfscudAudAugagauUfaAfaguuasasc 1929 AD-
1231472 cscsuga(Uhd)CfulifCfUfgguuuuaascsa 1879 VPusGfsuudTadAaccagaAfgAfucaggsasa
1930 AD-1231473 usgsua(Chd)aGfuAfAfGfugauaacasgsa 1880
VPusCfsugdTudAucacuuAfcUfguacasasg 1931 AD-1231474
ususccugAfuCfUfUfcugg(Uhd)cuasasa 1881 VPusUfsuadGa(Cgn)cagaagAfuCfaggaascsu 1932
AD-1231475 usasuguaCfuCfAfUfucgu(Uhd)ucgsgsa 1882 VPusCfscgaAfacgaugAfgUfacauasasg
1361 AD-1231476 ascsugua(Chd)aGfUfCfuaaaauguscsa 1883
VPusGfsacaUfuuuagacUfgUfacagusgsg 12 AD-1231477 cscscaugUfgAfUfUfuuaa(Uhd)agcsusa
1884 VPusAfsghcuAfuuaaaauCfaCfaugggsgsa 1436 AD-1231478
ususcgugGfuAfUfUfcuua(Chd)uagsusa 1885 VPusAfsudAgdTaagaauAfcCfacgaasasg 1933 AD-
1231479 uscsuuc(Uhd)GfgUfCfUfaaagaacsusa 1886 VPusAfsugdTcdAuuaagaCfcAfgaagasusc
1934 AD-1231480 gsusacugCfuGfCfAfauau(Uhd)guusasa 1887
VPusUfsaacAfauauugcAfgCfaguacsgsc 1375 AD-1231481 ususuua(Uhd)CfuCfAfCfauagcaauscsa
891 VPusGfsaudTg(Cgn)uagugAfgAfuaaasgsu 1935 AD-1231482
usascaa(Chd)UfuCfCfUfcaaggaacsasa 872 VPusUfsgudTc(Cgn)uugaggAfaGfuuguasgsc 1936 AD-
1231483 csusgcuaUfuCfCfUfuguu(Uhd)uaasusa 1888 VPusAfsuuaAfaacaaggAfaUfagcagsasa
1406 AD-1231484 csuscacaUfaGfCfAfauu(Uhd)uaasusa 1889
VPusAfsuuaAfagauugcUfaUfgugagsasu 1429 AD-1231485 uscsugug(Chd)UfaCfAfAfcuuucucasasa
1890 VPusUfsugdAgdAaaguugUfaGfcacgasusu 1937 AD-1231486
csasgaugGfuAfCfAfcuua(Uhd)gaususa 1891 VPusAfsaucAfuaaguguAfcCfaucugsusu 1320 AD-
1231487 usasacaaUfgUfUfGfcuuu(Uhd)caasasa 1892 VPusUfsuugAfaaagcaaCfaUfuguuasgsu 10
AD-1231488 usasauagUfuAfAfUfagcg(Uhd)acususa 1893 VPusAfsaguAfcgcuauuAfaCfuauuasasc
1367 AD-1231489 csasua(Chd)aAfuGfCfUfaguuaaacsasa 1894
VPusUfsguuUfaacuagcAfuUfguugsusu 1318 AD-1231490 usuuu(Chd)aAfaCfUfGfucaaaccsgsa
1895 VPusCfsgggUfuugacagUfuUfgaaaasgsc 1296 AD-1231491
csusuaug(Uhd)aCfUfCfauucguuuscsa 1896 VPusGfsaaaCfgaugagUfaCfauaagsusu 1359 AD-
1231492 gsusugugUfaCfAfCfacau(Uhd)ggusasa 1897 VPusUfsacdCadAugugugUfaCfacaacsasu
1938 AD-1231493 asascuuaUfgUfAfCfucau(Uhd)cgususa 1898
VPusAfsacgAfaugaguaCfaUfaaguuscsa 1357 AD-1231494 csusgcugCfaAfUfAfuugu(Uhd)aacsgsa
1899 VPusCfsguuAfacaauuUfgCfagcagsusa 1376 AD-1231495
ususcuu(Chd)GfuAfAfGfaacgguaasusa 737 VPusAfsuudAc(Cgn)guucuuAfcGfaagaasgsa 1939
AD-1231496 csgsugc(Uhd)AfcAfAfCfuucuucaasgsa 1900
VPusCfsuudGadAgaaguGfuAfgcacgsasu 1940 AD-1231497
usgsaaa(Uhd)UfgUfUfGfacauugugsasa 1901 VPusUfscadCadAugucaaCfaAfuucacgsc 1941 AD-

1231498 ascsaug(Uhd)uGfCfUfuuuuaacsusa 1902 VPusAfsgudTudAaaaagcAfaCfauugususa
 1942 AD-1231499 asasauugUfuGfAfCfacua(Uhd)gagsusa 1903
 VPusAfsdudCadTagugucAfaCfaauuuscsa 1943 AD-1231500
 uscsuucgUfaAfGfAfacgg(Uhd)aausasa 1904 VPusUfsaudTa(Cgn)cguucuUfaCfgaagasasg 1944
 AD-1231501 csusgcaaUfaUfUfGfuuaa(Chd)gugsasa 1905 VPusUfscacGfuuaacaaUfaUfugcagscsa
 1379 AD-1231502 gscsugaaAfuUfGfUfugaua(Chd)ugsusa 1906
 VPusAfsdudGudAucaacaAfuUfucagcsasg 1945 AD-1231503
 csusgc(Uhd)gAfaAfUfUfuguagacacsusa 1907 VPusAfsgudGu(Cgn)aacaauUfuCfagcagsgsa 1946
 AD-1231504 ususgugaUfgAfUfGfauua(Uhd)uucsasa 1908 VPusUfsgaaAfuaucanCfaUfcacaascsa
 1293 AD-1231505 usgsuugaCfaCfUfGfugaa(Uhd)gcususa 1909
 VPusAfsagdCadTucacagUfgUfcaacasasu 1947 AD-1231506
 csasgguaCfGfUfUfAfauag(Uhd)uaasusa 1910 VPusAfsuuaAfcuaauuaaCfGfUfaccugsusc 1365 AD-
 1231507 ascsauugCfcAfAfAfaggu(Uhd)ucusasa 1911 VPusUfsagdAadAccuuuuGfgCfaaugusug
 1948 AD-1231508 gscsug(Chd)aAfuAfUfUfguuaacgusgsa 1912
 VPusCfsacgUfuaacaauAfuUfgcagcsasg 1378 AD-1231509 csasca(Uhd)aGfcAfAfUfcuuuaacsusa
 1913 VPusUfsgauUfaaagauuGfcUfaugugsasg 1431 AD-1231510
 asasugug(Uhd)gAfUfAfucagacaascsa 1914 VPusGfsuudGu(Cgn)ugauauCfaCfacauusgsu 1949 AD-
 1231511 gsusucc(Uhd)GfaUfCfUfucuggucusasa 852 VPusUfsagdAc(Cgn)agaagaUfcAfggaacsusc
 1950 AD-1231512 ususgccaAfaAfGfGfcuuc(Uhd)acgscsa 1915
 VPusGfscguAfgaagccuUfuUfggcaasusg 1414 AD-1231513 ususcug(Chd)UfaAfUfCfuuguugcusasa
 1916 VPusUfsagdCadAcaagauUfaGfcagaasgsc 1951 AD-1231514
 ascscuagUfaAfUfAfgguu(Uhd)ccusasa 1917 VPusUfsaggAfaaccuauUfaCfuaggususc 1388 AD-
 1231515 csusgcuaAfuCfUfUfgcug(Chd)uacsusa 1918
 VPusAfsgudAg(Cgn)agcaagAfuUfagcagsasa 1952 AD-1231516
 csascucaUfaAfAfGfucug(Uhd)guusgsa 1919 VPusCfsaacAfcagacuuUfaUfgagugsusc 1315 AD-
 1231517 csasuaggAfaUfAfAfauc(Uhd)ucusasa 1920 VPusUfsagaAfgauuuuaUfuCfcuaugsgsa
 1346 AD-1231518 uscsuaca(Chd)aAfAfCfucuuuaagsasa 1921
 VPusUfscuuUfaagaguuUfgUfguagasusa 1275 AD-1231519 asuscuugUfuUfUfCfucua(Uhd)ucasasa
 1922 VPusUfsugdAadTagagaaAfaCfaagaugsgsa 1953 AD-1231520
 csasgucaUfaAfUfCfuaua(Uhd)uaasasa 1923 VPusUfsuudAadTauagauUfaUfgacugsusg 1954 AD-
 1231521 usgsguaaCfaCfUfAfauaa(Uhd)aaasasa 1924 VPusUfsuudTadTuauuagUfgUfuaccascsa
 1955

(636) TABLE-US-00007 TABLE 6 Single Dose In Vitro Screens in Cos-7 Cells % of Message %
 of Message Remaining- Remaining- 10 nM 10 nM Duplex ID Concatenate-02 STDEV
 Concatenate-21 STDEV AD-1184048.1 2.63 0.37 10.90 2.58 AD-1184130.1 4.52 0.51 15.30 3.51
 AD-1184064.1 5.13 2.78 18.41 12.53 AD-1184050.1 5.58 2.20 14.85 7.91 AD-1184202.1 5.58 2.27
 7.20 2.28 AD-1184100.1 6.41 1.86 1.63 1.97 AD-1184098.1 6.46 0.74 1.51 1.09 AD-1184123.1
 6.48 0.59 19.79 1.49 AD-1184223.1 6.63 0.25 8.39 1.65 AD-1184219.1 6.66 0.52 6.38 2.51 AD-
 1184062.1 6.67 4.16 4.12 0.32 AD-1184070.1 6.72 0.66 16.86 4.27 AD-1184061.1 6.78 0.65 10.62
 2.56 AD-1184216.1 6.82 0.44 11.90 0.70 AD-1184255.1 6.88 0.67 2.84 0.96 AD-1184237.1 6.90
 0.16 5.26 0.45 AD-1184218.1 7.05 0.15 10.23 0.16 AD-1184215.1 7.15 0.17 15.26 3.28 AD-
 1184137.1 7.17 1.30 18.05 4.06 AD-1184271.1 7.19 0.52 4.76 2.43 AD-1184166.1 7.57 0.13 N/A
 N/A AD-1184291.1 7.60 0.68 3.12 1.19 AD-1184256.1 7.68 2.71 4.95 0.80 AD-1184260.1 7.69
 1.24 10.95 3.08 AD-1184051.1 7.71 0.32 17.00 2.76 AD-1184063.1 7.71 3.85 10.87 3.90 AD-
 1184213.1 7.71 0.22 14.68 9.77 AD-1184124.1 7.85 1.08 18.09 1.81 AD-1184230.1 7.85 0.71
 11.08 1.15 AD-1184099.1 7.94 0.91 1.24 0.61 AD-1184189.1 7.94 0.98 6.87 1.66 AD-1184152.1
 8.01 0.63 45.16 29.64 AD-1184080.1 8.03 3.13 20.44 9.44 AD-1184261.1 8.04 0.33 10.46 5.96
 AD-1184138.1 8.11 1.81 34.34 17.00 AD-1184221.1 8.28 0.89 14.34 3.37 AD-1184284.1 8.28 0.72
 0.55 0.15 AD-1184144.1 8.31 0.21 19.46 14.43 AD-1184272.1 8.43 0.68 2.04 0.80 AD-1184285.1
 8.55 2.60 N/A N/A AD-1184167.1 8.55 0.22 26.83 12.68 AD-1184110.1 8.57 0.27 32.02 18.77 AD-

1184113.1 8.67 0.36 42.10 37.37 AD-1184141.1 8.67 0.76 32.39 13.05 AD-1184226.1 18.70 0.62
10.59 3.78 AD-1184077.1 8.72 0.18 16.74 1.13 AD-1184175.1 8.79 0.38 17.35 1.43 AD-1184053.1
8.85 1.05 26.88 9.12 AD-1184114.1 8.96 0.95 35.26 15.77 AD-1184091.1 9.06 0.96 35.70 15.75
AD-1184228.1 9.10 1.23 9.24 4.10 AD-1184150.1 9.15 2.15 11.22 2.79 AD-1184222.1 9.27 1.29
11.23 2.32 AD-1184173.1 9.32 3.48 12.47 5.60 AD-1184040.1 9.46 3.27 53.64 29.25 AD-
1184125.1 9.62 2.10 27.24 1.20 AD-1184151.1 9.65 5.07 20.35 6.60 AD-1184214.1 9.66 0.52
13.01 2.02 AD-1184235.1 9.74 2.34 4.91 0.68 AD-1184217.1 9.79 0.95 15.78 4.08 AD-1184055.1
9.83 0.63 38.63 12.62 AD-1184169.1 9.92 2.16 28.77 14.24 AD-1184227.1 10.11 4.84 9.70 1.00
AD-1184195.1 10.11 8.02 45.22 21.80 AD-1183971.1 10.43 1.92 4.64 1.24 AD-1184085.1 10.45
0.59 17.89 2.27 AD-1184212.1 10.59 1.87 14.04 0.85 AD-1184231.1 10.67 4.28 16.42 4.86 AD-
1184148.1 10.87 8.58 18.19 3.78 AD-1183945.1 10.95 0.72 19.06 1.18 AD-1184268.1 11.00 0.54
6.03 2.13 AD-1184054.1 11.12 2.65 34.41 9.52 AD-1184071.1 11.14 2.81 23.38 8.83 AD-
1184232.1 11.16 1.04 9.87 2.12 AD-1184078.1 11.19 2.29 16.35 2.82 AD-1184229.1 11.44 0.22
15.36 1.87 AD-1184018.1 11.92 2.94 11.18 5.66 AD-1184004.1 11.93 0.06 30.41 15.72 AD-
1183979.1 12.07 0.81 20.29 8.12 AD-1184259.1 12.15 3.41 7.92 1.24 AD-1184252.1 12.20 6.36
9.64 1.21 AD-1184224.1 12.21 0.77 11.43 2.61 AD-1184037.1 12.26 2.11 36.01 8.76 AD-
1184112.1 12.57 1.41 53.76 36.30 AD-1184019.1 12.74 5.26 6.57 3.27 AD-1184211.1 12.84 0.95
15.37 1.00 AD-1183946.1 13.10 2.42 16.50 4.85 AD-1184270.1 13.15 0.69 17.48 13.18 AD-
1184234.1 13.16 1.74 12.12 2.73 AD-1184111.1 13.20 0.51 27.70 3.35 AD-1184017.1 13.22 6.55
7.64 1.71 AD-1183944.1 13.24 7.54 22.04 3.53 AD-1184045.1 13.26 2.03 26.78 12.50 AD-
1184016.1 13.28 0.81 18.27 7.54 AD-1184146.1 13.56 5.67 24.87 17.23 AD-1184168.1 13.78
11.08 14.98 1.72 AD-1184136.1 13.78 0.38 18.39 3.38 AD-1184147.1 13.87 8.89 16.79 1.97 AD-
1184020.1 13.90 5.93 4.01 0.50 AD-1183981.1 14.01 5.51 45.10 24.43 AD-1184220.1 14.03 2.59
14.74 1.03 AD-1184086.1 14.29 1.54 20.17 3.50 AD-1184282.1 14.32 4.56 1.75 0.61 AD-
1184106.1 14.34 0.80 2.75 0.47 AD-1184047.1 14.47 0.30 45.66 19.02 AD-1184174.1 14.51 9.37
34.31 12.21 AD-1184008.1 14.69 0.12 41.14 11.84 AD-1184283.1 14.78 4.90 1.91 0.22 AD-
1184052.1 14.90 0.63 58.07 17.97 AD-1184209.1 15.39 0.52 14.16 2.43 AD-1184233.1 15.40 3.88
20.15 2.08 AD-1183962.1 15.63 1.31 54.76 30.80 AD-1184083.1 15.83 4.95 22.37 1.47 AD-
1184286.1 16.01 2.27 2.00 0.03 AD-1184015.1 16.20 11.40 29.61 8.40 AD-1183989.1 16.34 6.20
13.86 3.56 AD-1184057.1 16.41 3.28 49.66 9.68 AD-1184088.1 17.10 6.67 33.62 21.10 AD-
1184060.1 17.11 10.60 15.28 14.57 AD-1184044.1 17.75 1.12 45.51 28.15 AD-1184210.1 18.20
2.59 18.08 2.59 AD-1184172.1 18.41 11.26 30.65 27.58 AD-1183938.1 18.45 3.98 38.39 12.64
AD-1184097.1 19.01 1.36 23.80 10.22 AD-1183982.1 19.21 9.72 47.72 16.24 AD-1184269.1 19.30
8.09 13.74 1.68 AD-1184056.1 19.38 5.22 29.08 13.13 AD-1184038.1 19.44 1.60 11.35 2.52 AD-
1184139.1 19.53 1.18 27.71 12.16 AD-1184243.1 19.77 1.94 19.11 1.57 AD-1184140.1 19.85 5.99
48.11 27.84 AD-1184254.1 20.28 1.40 12.15 1.63 AD-1184039.1 20.38 0.98 12.30 6.12 AD-
1184242.1 20.41 0.99 33.51 12.48 AD-1184102.1 20.41 3.10 4.37 1.78 AD-1184082.1 21.24 0.58
33.45 16.23 AD-1184236.1 21.31 0.68 9.04 1.66 AD-1184006.1 21.33 14.37 17.11 3.28 AD-
1184205.1 21.55 1.62 4.37 1.37 AD-1184042.1 21.83 5.52 33.36 12.05 AD-1184065.1 21.90 2.60
58.16 25.42 AD-1183969.1 21.90 10.09 5.17 2.16 AD-1184225.1 23.07 3.35 17.73 2.00 AD-
1184034.1 23.11 0.49 7.40 3.24 AD-1184075.1 23.96 0.96 12.73 6.25 AD-1184207.1 24.80 2.68
14.52 3.96 AD-1184258.1 25.77 5.86 18.80 1.87 AD-1184103.1 26.50 4.94 3.15 1.78 AD-
1184180.1 27.78 2.01 4.08 2.97 AD-1184238.1 28.23 0.71 20.35 2.33 AD-1183980.1 28.37 20.86
27.84 18.41 AD-1184142.1 28.71 0.80 14.04 2.14 AD-1184043.1 29.12 4.83 26.00 10.19 AD-
1184198.1 29.63 8.04 9.62 1.92 AD-1184132.1 29.71 7.08 3.65 1.37 AD-1183935.1 30.45 1.06
13.92 7.44 AD-1183970.1 30.50 22.12 16.61 16.60 AD-1184109.1 30.85 2.81 3.10 0.65 AD-
1184002.1 31.09 2.55 30.36 6.41 AD-1184129.1 31.47 6.67 33.15 11.16 AD-1183998.1 32.57 2.99
22.22 11.73 AD-1184251.1 33.06 0.66 2.68 1.11 AD-1184120.1 33.85 4.79 9.82 3.79 AD-
1184032.1 33.94 3.79 10.30 1.57 AD-1184186.1 34.00 11.85 9.38 1.87 AD-1184067.1 34.35 11.07
14.02 8.43 AD-1184161.1 34.50 0.31 25.68 15.70 AD-1184084.1 34.60 5.69 43.25 4.15 AD-

1184119.1 34.69 3.51 16.92 11.78 AD-1184026.1 34.98 4.17 20.63 4.42 AD-1184003.1 34.98
24.54 24.07 1.36 AD-1183959.1 35.01 0.67 17.15 10.10 AD-1184108.1 35.13 1.60 14.10 6.45 AD-
1184164.1 35.44 12.85 20.94 6.85 AD-1184277.1 35.50 4.03 6.23 1.22 AD-1184116.1 36.10 7.48
4.91 3.04 AD-1184184.1 36.25 2.52 14.56 7.78 AD-1184249.1 36.26 6.98 2.06 0.48 AD-1183972.1
36.48 24.57 16.50 10.24 AD-1184143.1 37.10 4.12 60.26 47.27 AD-1184279.1 37.93 0.75 3.81
2.02 AD-1184191.2 37.93 0.64 13.99 0.87 AD-1184278.1 38.05 2.23 5.47 2.54 AD-1184160.1
38.14 10.62 48.46 19.14 AD-1184046.1 38.51 1.35 60.87 21.93 AD-1184206.1 38.57 7.93 4.42
0.27 AD-1183984.1 38.80 5.36 9.39 1.60 AD-1184247.1 39.35 2.55 2.45 0.56 AD-1184133.1 39.92
7.07 5.26 1.58 AD-1184154.1 40.25 8.75 15.70 4.99 AD-1184101.1 40.47 5.58 17.96 3.62 AD-
1184192.1 40.75 3.21 47.06 22.51 AD-1184204.1 41.20 5.72 1.46 0.62 AD-1184094.1 41.25 8.86
15.40 3.72 AD-1184281.1 41.82 2.91 5.81 1.47 AD-1184248.1 41.98 1.67 1.41 0.85 AD-1184115.1
42.06 2.95 19.78 11.59 AD-1184250.1 42.26 4.20 2.73 1.36 AD-1184041.1 42.48 3.19 7.06 0.96
AD-1184027.1 42.83 6.09 30.80 14.97 AD-1184122.1 42.88 2.78 21.18 3.28 AD-1183956.1 43.02
4.15 27.77 0.81 AD-1183936.1 43.22 5.77 14.20 7.54 AD-1183983.1 43.73 2.34 23.48 6.77 AD-
1184239.1 43.79 11.20 65.40 24.69 AD-1184117.1 43.91 2.03 33.46 19.25 AD-1184090.1 44.17
10.78 17.42 3.89 AD-1183996.1 44.48 2.38 18.83 6.96 AD-1184159.1 44.54 3.99 71.46 60.74 AD-
1183986.1 45.32 11.06 15.98 1.72 AD-1184171.1 45.52 3.49 15.87 9.86 AD-1184107.1 45.87 2.08
10.22 2.57 AD-1184265.1 46.01 9.43 12.04 2.84 AD-1184275.1 46.21 6.74 16.22 7.54 AD-
1184014.1 46.37 5.74 14.22 1.87 AD-1184263.1 47.01 8.63 9.79 3.27 AD-1184183.1 47.07 6.75
9.20 4.87 AD-1184012.1 47.42 3.84 11.60 0.86 AD-1183999.1 47.62 2.37 33.42 15.40 AD-
1184203.1 47.81 2.60 2.76 0.77 AD-1184118.1 47.81 1.54 14.60 1.57 AD-1183940.1 48.07 4.28
15.84 5.02 AD-1184191.1 48.37 18.93 13.85 2.40 AD-1184127.1 48.59 4.03 9.19 2.45 AD-
1184049.1 48.71 1.62 77.51 44.26 AD-1183993.1 48.74 9.36 19.86 1.34 AD-1184121.1 48.98
21.86 19.80 13.18 AD-1184165.1 49.21 1.14 6.48 2.56 AD-1184093.1 49.84 3.10 15.27 2.93 AD-
1184280.1 49.97 1.27 5.25 0.01 AD-1184073.1 50.08 1.97 37.32 14.73 AD-1184182.1 50.14 0.70
32.68 11.12 AD-1183943.1 50.21 4.56 18.62 2.35 AD-1184276.1 50.40 10.18 7.22 0.40 AD-
1184163.1 50.64 1.71 25.14 15.54 AD-1184179.1 50.74 11.32 9.27 4.91 AD-1184135.1 50.93 8.21
13.80 2.70 AD-1183992.1 51.42 8.33 42.18 23.25 AD-1183967.1 51.71 6.86 57.35 28.94 AD-
1184158.1 52.02 1.89 14.09 2.71 AD-1184157.1 52.16 6.95 58.46 31.63 AD-1184266.1 52.38 6.11
25.86 8.79 AD-1184246.1 52.39 12.83 2.66 0.61 AD-1184190.1 52.42 12.47 35.31 12.80 AD-
1184092.1 53.02 2.85 24.54 13.96 AD-1184264.1 53.03 2.78 6.81 0.99 AD-1184005.1 53.20 11.03
71.42 11.07 AD-1184177.1 53.21 14.56 20.72 15.48 AD-1184104.1 53.27 10.42 26.83 14.02 AD-
1183978.1 53.56 2.47 24.02 16.27 AD-1184194.1 54.03 4.33 249.38 121.89 AD-1184033.1 54.25
14.79 16.32 7.81 AD-1183975.1 54.47 21.12 42.79 19.29 AD-1184290.1 54.65 3.02 5.15 0.39 AD-
1184176.1 54.77 2.23 18.76 13.79 AD-1184030.1 54.81 1.50 69.90 25.45 AD-1184105.1 54.84
8.20 9.56 4.30 AD-1184187.1 55.39 15.91 42.43 31.40 AD-1183990.1 56.00 9.90 9.63 5.29 AD-
1184196.1 56.24 11.11 21.62 1.09 AD-1184188.1 56.26 22.13 19.16 4.83 AD-1184024.1 56.46
11.22 28.76 10.49 AD-1184087.1 56.55 13.61 32.17 11.27 AD-1184193.1 56.61 4.42 85.01 31.27
AD-1183995.1 56.69 5.48 53.57 47.57 AD-1184074.1 56.98 9.01 33.24 13.16 AD-1184267.1 57.34
2.47 14.58 3.09 AD-1183966.1 57.41 3.21 22.22 9.46 AD-1183977.1 57.68 4.84 19.98 15.03 AD-
1184273.1 57.94 2.34 3.97 0.64 AD-1183985.1 58.54 1.43 12.79 1.24 AD-1184011.1 58.67 6.24
31.15 5.26 AD-1184021.1 58.73 12.50 19.65 8.36 AD-1184066.1 58.74 2.53 9.67 6.29 AD-
1184178.1 58.81 21.97 23.61 18.10 AD-1184274.1 59.03 3.54 10.66 9.31 AD-1184208.1 59.10
17.95 21.32 1.63 AD-1184149.1 59.11 24.90 42.83 36.12 AD-1184245.1 59.13 10.08 3.51 0.74
AD-1183937.1 59.99 5.18 29.11 16.30 AD-1183963.1 60.24 8.46 53.73 20.46 AD-1183942.1 60.39
11.40 26.33 8.27 AD-1184069.1 60.49 1.93 5.64 0.53 AD-1184289.1 60.67 1.35 20.43 17.67 AD-
1184023.1 61.62 18.34 57.01 30.89 AD-1184185.1 62.17 11.09 64.99 62.12 AD-1184096.1 62.37
2.21 23.80 9.93 AD-1184197.1 62.64 17.03 57.02 9.62 AD-1184156.1 62.66 5.82 33.70 14.37 AD-
1184013.1 62.74 2.59 27.30 20.05 AD-1184162.1 62.79 17.70 14.73 1.96 AD-1184240.1 63.69
3.43 58.69 22.89 AD-1183997.1 63.76 10.95 42.99 22.78 AD-1183957.1 65.30 19.84 39.22 5.07

AD-1183952.1 65.47 3.48 13.78 2.17 AD-1184076.1 65.58 17.26 28.83 2.54 AD-1184181.1 66.59 12.10 100.82 84.44 AD-1184068.1 67.28 16.70 12.10 4.59 AD-1184089.1 67.93 15.70 25.15 2.99 AD-1183941.1 67.94 1.68 36.29 6.11 AD-1184244.1 68.07 9.26 7.51 3.41 AD-1183965.1 69.26 9.89 25.51 8.59 AD-1184001.1 69.46 10.20 19.18 5.13 AD-1184145.1 72.78 31.76 79.63 52.62 AD-1183958.1 72.98 22.53 24.20 4.22 AD-1184134.1 73.15 13.38 68.22 25.29 AD-1183987.1 73.70 32.04 33.56 10.90 AD-1184153.1 75.12 42.83 132.33 72.85 AD-1183974.1 75.42 24.31 48.58 13.88 AD-1184262.1 77.77 12.69 10.06 1.53 AD-1184000.1 78.82 13.60 61.86 32.12 AD-1184241.1 78.94 8.87 46.74 4.99 AD-1184288.1 81.27 21.61 13.77 4.01 AD-1184095.1 82.94 23.57 22.66 5.47 AD-1183939.1 85.76 3.23 40.92 18.61 AD-1184022.2 86.03 4.66 48.90 2.36 AD-1184287.1 86.16 15.61 4.36 2.08 AD-1183994.1 87.40 24.94 91.29 76.63 AD-1184007.1 88.09 33.36 31.42 3.87 AD-1184155.1 88.35 42.97 20.59 2.82 AD-1184028.1 88.83 19.76 87.84 3.75 AD-1184025.1 90.53 20.18 54.27 23.14 AD-1183991.1 90.88 42.68 30.34 4.53 AD-1184025.2 92.98 12.32 50.82 18.55 AD-1184022.1 93.46 48.39 52.21 8.69 AD-1183960.1 97.09 73.05 27.29 2.02 AD-1184031.1 108.42 12.44 87.48 8.47 AD-1183988.1 110.39 51.89 98.51 74.09 AD-1183976.1 137.05 47.58 10.82 0.28

(637) TABLE-US-00008 TABLE 7 Single Dose In Vitro Screens in Cos-7 Cells 10 nM 10 nM 1 nM 1 nM 0.1 nM 0.1 nM Duplex ID Avg STDEV Avg STDEV Avg STDEV AD-1231493.1 25.3 6.2 47.6 10.4 103.4 12.8 AD-1231505.1 21.6 11.7 18.4 6.3 72.0 26.6 AD-1231488.1 13.3 1.3 48.3 2.9 116.3 9.3 AD-1231486.1 11.5 4.3 25.9 3.9 98.8 33.2 AD-1231502.1 7.9 2.9 37.5 8.2 100.4 5.6 AD-1231478.1 30.9 18.8 53.0 9.9 75.0 6.4 AD-1231499.1 46.4 26.3 51.4 24.0 77.9 12.6 AD-1231470.1 8.0 6.3 15.7 7.4 78.4 15.8 AD-1231477.1 13.7 7.4 36.0 9.5 104.2 12.0 AD-1231515.1 51.9 6.6 79.3 15.4 113.3 24.4 AD-1231503.1 58.9 14.7 91.2 11.9 139.0 7.0 AD-1231479.1 15.4 3.7 50.2 13.1 123.5 24.1 AD-1231498.1 15.5 9.0 17.0 2.8 78.8 22.3 AD-1231483.1 16.7 3.0 31.0 11.8 78.9 2.7 AD-1231484.1 14.7 7.3 28.2 4.7 80.1 19.1 AD-1231506.1 45.8 13.2 52.3 5.1 112.1 14.4 AD-1231495.1 20.4 8.1 51.1 6.8 98.9 25.0 AD-1231516.1 109.2 32.1 129.8 28.9 102.8 22.3 AD-1231508.1 30.6 5.6 65.3 8.9 121.5 8.5 AD-1231475.1 8.7 4.3 14.8 4.0 70.9 12.4 AD-1231490.1 23.2 19.6 32.0 5.9 86.5 11.7 AD-1231494.1 42.3 9.1 89.6 9.6 128.1 16.4 AD-1231473.1 12.1 5.5 35.2 4.6 108.2 18.5 AD-1231496.1 8.5 3.6 51.5 10.9 112.9 11.9 AD-1231491.1 33.4 26.2 38.9 17.4 88.1 4.4 AD-1231469.1 46.2 8.2 37.9 5.2 82.2 14.7 AD-1231476.1 18.3 17.0 34.7 7.4 89.7 21.5 AD-1231481.1 10.6 1.0 36.2 6.6 107.7 6.3 AD-1231512.1 61.1 37.0 63.7 9.3 94.9 11.2 AD-1231471.1 12.1 5.3 26.7 7.9 99.6 24.4 AD-1231510.1 12.5 5.9 49.9 1.8 106.0 19.4 AD-1231472.1 11.3 6.2 29.0 7.1 80.3 19.1 AD-1231480.1 49.7 25.7 73.5 12.6 107.0 19.1 AD-1231492.1 36.8 24.7 73.8 15.3 105.4 40.6 AD-1231507.1 61.0 22.1 73.5 1.5 100.8 10.4 AD-1231517.1 87.4 18.0 99.2 25.5 109.4 25.0 AD-1231511.1 13.7 4.8 39.3 10.6 108.1 7.9 AD-1231513.1 60.8 12.9 70.9 11.0 98.8 13.1 AD-1231514.1 57.8 18.2 86.1 15.6 91.7 11.5 AD-1231468.1 8.9 0.1 46.8 18.4 93.5 19.1 AD-1231500.1 43.4 16.2 50.8 7.8 85.0 16.7 AD-1231497.1 15.1 13.6 24.9 2.8 80.1 18.9 AD-1231501.1 20.7 7.8 65.5 10.7 122.5 5.7 AD-1231467.1 35.9 8.6 62.0 15.1 102.7 10.7 AD-1231518.1 126.4 19.5 131.1 15.7 141.9 22.7 AD-1231504.1 9.6 6.0 29.1 12.9 89.7 16.4 AD-1231509.1 28.9 25.3 44.1 13.6 87.6 13.3 AD-1231482.1 28.0 10.4 54.0 3.5 136.1 31.3 AD-1231489.1 12.9 3.0 21.4 2.0 90.5 9.6 AD-1231474.1 59.0 26.0 66.1 17.5 86.9 19.8 AD-1231519.1 85.2 19.5 107.1 12.6 116.2 24.4 AD-1231485.1 22.8 17.8 42.3 3.9 80.5 21.0 AD-1231520.1 111.6 15.3 103.8 24.6 99.7 15.5 AD-1231487.1 22.5 9.9 51.5 14.2 96.5 17.8 AD-1231521.1 107.1 20.2 100.7 30.3 115.6 24.2

Example 3. In Vivo Screening of dsRNA Duplexes in Mice

(638) siRNA molecules targeting the coronavirus genome, identified from the above in vitro studies, are evaluated in vivo.

(639) Mice previously infected with a coronavirus, e.g., severe acute respiratory syndrome-2 (SARS-2)-CoV-2, are administered, via pulmonary system or subcutaneous delivery, a dsRNA molecule at a dose of 0.1 mg/kg, 1 mg/kg or 10 mg/kg. Uptake of dsRNA in bronchioles and alveoli and expression level of target gene in whole lung of treated mice are measured. Expression

level of coronavirus target genes are further evaluated by in situ hybridization in mice bronchus and bronchiole.

Example 4. In Vitro SARS-CoV-2 Replication Screening of siRNA Duplexes

(640) In Vitro screening of siRNAs in SARS-CoV-2 Infection Assay

(641) Vero E6 cells cultured in DMEM supplemented with 10% FBS (VWR) and 1× Penicillin/Streptomycin (Thermo Fisher Scientific) were reverse transfected with siRNAs in white 96-well plates using RNAiMax transfection reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. Twenty-four hours later, cells were infected with SARS-CoV-2 (isolate USA-WA1/2020, passage 2, MOI 0.001) in a BSL-3 facility. The viral inoculum was removed after 4 hours, cells were washed with phosphate-buffered saline (PBS, pH 7.4) and media was replaced. At 48 hours post-infection, the cell supernatant was collected for RNA extraction and RT-qPCR analysis. The cells were fixed for in-cell ELISA analysis (below).

(642) Quantification of Extracellular SARS-CoV-2 Genomes by RT-qPCR

(643) Viral RNA was extracted from the cell culture supernatant using the NucleoSpin 96 Virus kit (Macherey-Nagel). Quantification of viral genomes was performed using the Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs) with a primer/probeset binding in the M region (forward: CACTATTCTGACCAGACCGCTTCT (SEQ ID NO: 1956), reverse: CCTGCTACACGCTGCGAAG (SEQ ID NO: 1957), probe: TCACGAACGCTTTCTT (SEQ ID NO: 1958))). A standard curve of defined dilutions of a synthetic SARS-CoV-2 RNA (Twist Bioscience) was used for normalization.

(644) Quantification of Intracellular Viral Nucleocapsid Protein by In-Cell ELISA

(645) Cells were fixed with 4% paraformaldehyde for 30 minutes, followed by two PBS (pH 7.4) washes and permeabilization with 0.125% Triton X-100 in PBS for 30 min. After blocking in 2% milk powder/PBS for 30 minutes, cells were incubated with a primary antibody targeting SARS-CoV-2 nucleocapsid protein (Sino Biological, cat. 40143-R001) at a 1:3000 dilution for 1 hour. After washing and incubation with a secondary HRP-labeled antibody for 1 hour, SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Fisher Scientific) was added to the cells and luminescence signal was read on an Ensign plate reader (Perkin-Elmer). After subtraction of background (uninfected) controls, all signals were normalized to the mock transfected controls.

Results

(646) The RT-qPCR assay quantifying viral genome copy number based on the M (membrane) ORF, identified 47 siRNAs capable of reducing viral RNA ≥ 2 -log.sub.10s at 10 nM, with 10 siRNAs reducing viral RNA ≥ 2 -log.sub.10s at 0.1 nM. Reduction in viral RNA correlated with reduction in intracellular nucleocapsid protein. A total of 11 siRNAs had >80% reduction of viral nucleocapsid at a 0.1 nM dose. As expected, the control siRNA (targeting firefly luciferase transcripts) had no significant effect on SARS-CoV2 viral replication. The results of these assays are provided in Table 8 and FIG. 3.

(647) TABLE-US-00009 TABLE 8 Single Dose In Vitro SARS-COV-2 Replication Screens RT-qPCR in-cell ELISA (% mock transfected) (% mock transfected) Duplex ID 10 nM 1 nM 0.1 nM 10 nM 1 nM 0.1 nM Target AD-1184106.1 0.12 0.21 3.46 8.5 8.6 24.0 AD-1184102.1 0.13 0.17 2.65 8.5 8.4 25.0 AD-1184136.1 0.17 41.04 25.26 9.6 77.7 54.9 AD-1184151.1 0.05 0.10 16.96 10.5 10.0 33.8 AD-1184130.1 0.14 0.05 36.95 11.0 9.5 63.4 AD-1184137.1 0.06 0.04 0.03 10.2 10.6 10.2 ORF1ab AD-1184144.1 0.30 0.26 66.10 9.9 9.4 118.1 AD-1184189.1 0.05 0.06 33.02 7.7 7.6 84.5 AD-1184123.1 0.13 0.80 81.37 6.3 13.2 95.6 AD-1184168.1 0.08 0.08 33.20 8.4 7.5 70.2 AD-1184124.1 0.06 0.07 174.39 9.0 9.2 110.3 AD-1184131.1 18.05 45.93 48.79 47.1 78.8 81.0 (-)strand AD-1184147.1 5.74 0.03 0.04 26.7 10.2 10.2 ORF1ab AD-1184170.1 26.55 38.87 150.04 74.2 66.1 123.1 (-)strand AD-1184199.1 12.44 38.26 185.16 47.2 91.5 119.0 (-)strand AD-1184126.1 8.75 103.16 32.55 42.9 120.5 80.3 (-)strand AD-1184148.1 0.08 0.08 27.76 8.8 9.4 74.0 AD-1184200.1 15.87 75.29 120.16 51.7 101.5 105.0 (-)strand AD-1184128.1 26.30 93.02 225.36 71.9 94.9 112.0 (-)strand AD-1184150.1 0.05 0.07 0.20 10.3 10.2 10.0 ORF1ab AD-1184173.1 0.95

3.84 166.25 8.9 9.4 119.1 AD-1184202.1 0.74 30.76 51.06 10.7 61.0 87.7 AD-1184210.1 0.09 0.10
2.41 9.7 8.1 25.5 ORF3AE AD-1184218.1 0.07 0.71 79.43 11.9 15.5 98.8 AD-1184232.1 0.29 0.10
22.09 9.1 10.5 46.1 siLuc 24.50 79.00 351.04 73.3 98.0 131.0 mock tr 116.89 35.49 79.73 102.3
72.8 116.8 mock tr 118.33 83.41 166.15 118.5 81.5 108.1 AD-1184255.1 5.15 33.33 50.18 47.0
87.9 88.6 AD-1184270.1 0.23 2.32 0.03 9.1 22.6 88.9 N AD-1184211.1 0.09 0.39 28.21 10.1 13.9
89.8 AD-1184219.1 12.94 1.54 75.97 82.3 19.9 96.7 AD-1184226.1 0.06 0.60 22.51 9.3 14.4 81.1
AD-1184233.1 1.04 5.11 0.72 37.5 27.9 10.6 E AD-1184256.1 89.78 45.86 31.97 148.0 76.4 83.1
AD-1184271.1 0.03 0.02 1.26 7.5 8.0 22.5 N AD-1184286.1 0.26 2.20 38.59 9.3 19.5 86.2 AD-
1184212.1 0.07 0.57 3.56 8.5 14.9 22.1 ORF3AE AD-1184234.1 0.18 1.01 27.60 12.9 19.2 72.6
AD-1184213.1 0.04 0.04 10.47 7.4 8.8 55.0 AD-1184220.1 0.06 1.47 35.19 8.7 17.9 70.4 AD-
1184227.1 0.10 1.02 29.57 7.8 13.4 80.7 AD-1184235.1 0.16 0.09 110.96 9.3 8.6 108.8 AD-
1184272.1 0.04 0.03 4.82 7.7 7.6 50.5 AD-1184221.1 0.06 0.09 23.30 12.3 9.2 68.5 AD-1184228.1
0.10 0.56 1.75 7.3 15.6 15.4 E AD-1184259.1 11.19 11.00 43.81 50.6 45.4 92.3 AD-1184215.1 0.09
0.81 15.71 9.8 24.5 59.9 AD-1184229.1 0.07 0.20 8.25 9.2 9.6 65.3 AD-1184237.1 0.04 0.07 37.98
8.5 8.8 88.0 AD-1184252.1 1.63 9.54 45.25 38.0 82.0 111.9 AD-1184260.1 53.62 25.57 6.91 117.5
65.9 119.8 AD-1184282.1 0.10 0.04 48.94 8.9 6.9 99.7 siLuc 6.59 8.32 72.62 45.3 32.0 104.8 mock
tr 1.98 50.59 65.01 12.4 65.0 99.3 mock tr 132.78 85.04 166.58 114.7 103.5 117.6 AD-1184216.1
20.99 0.27 9.84 52.2 7.4 48.0 AD-1184223.1 0.38 0.02 16.14 8.4 10.1 32.1 E AD-1184261.1
323.03 96.87 107.35 178.0 98.1 92.5 AD-1184268.1 88.37 no result 95.18 112.9 31.9 83.7 AD-
1184283.1 0.09 3.19 99.85 8.3 17.7 93.3 AD-1184209.1 15.92 60.57 138.32 78.1 92.9 92.0 AD-
1184217.1 0.18 1.82 38.03 12.6 14.0 79.4 AD-1184224.1 0.52 2.86 257.68 7.6 20.8 95.8 AD-
1184254.1 156.78 31.46 109.25 121.2 45.3 83.7 AD-1184269.1 1.67 3.94 31.99 16.6 26.5 71.1 AD-
1184284.1 0.36 0.07 25.81 7.1 7.1 42.5 AD-1231467.1 0.19 0.12 21.93 12.7 7.7 52.6 AD-
1231475.1 0.06 no result 37.33 8.4 13.3 73.6 AD-1231482.1 0.11 no result 44.60 10.3 9.8 78.7 AD-
1231490.1 0.14 0.07 0.06 7.6 7.5 6.3 AD-1231512.1 0.23 4.74 241.32 8.5 25.2 90.0 AD-1231520.1
113.26 146.18 54.93 107.1 90.5 58.9 AD-1231468.1 1.29 18.06 161.56 23.3 56.2 84.8 AD-
1231476.1 0.22 0.23 75.68 8.8 7.6 57.7 AD-1231491.1 0.39 0.92 16.39 8.9 10.2 40.8 AD-
1231506.1 0.45 1.96 194.85 13.0 16.6 104.6 AD-1231513.1 0.10 1.12 1.76 8.5 8.7 9.4 AD-
1231521.1 130.20 106.72 399.69 114.6 85.9 101.9 AD-1231469.1 12.77 21.10 66.09 50.1 44.0
63.3 AD-1231484.1 0.08 0.11 72.20 7.2 9.1 107.4 siLuc 55.81 27.22 143.92 117.5 52.2 95.6 mock
tr 102.03 78.63 199.25 88.9 95.0 126.0 mock tr 66.37 55.42 98.31 80.1 89.1 120.9 AD-1231492.1
0.28 0.78 70.62 9.4 11.0 86.6 AD-1231514.1 0.01 132.16 79.46 39.0 116.7 84.5 AD-1231485.1
0.16 no result 0.50 11.3 11.5 9.4 AD-1231500.1 56.30 85.87 10.97 108.2 108.1 42.2 AD-1231507.1
0.62 0.23 2.89 8.8 9.5 20.2 AD-1231471.1 0.13 0.22 0.16 8.1 8.0 8.3 AD-1231494.1 0.14 0.17 1.98
10.1 11.0 100.5 AD-1231501.1 0.11 no result 53.02 11.7 26.2 72.7 AD-1231508.1 0.25 2.32 no
result 9.5 21.7 58.9 AD-1231516.1 32.57 50.07 119.43 50.4 77.9 86.8 AD-1231472.1 0.23 1.00
53.86 10.2 17.6 81.8 AD-1231480.1 0.18 12.95 52.14 12.2 60.2 90.8 AD-1231487.1 0.13 0.14 8.03
8.4 8.1 24.8 AD-1231495.1 10.79 no result 109.05 44.8 8.1 94.1 AD-1231509.1 0.58 2.36 46.14 9.7
16.1 61.0 AD-1231517.1 50.31 51.27 42.30 105.6 82.7 69.1 AD-1231473.1 0.46 20.26 55.45 19.6
48.4 73.3 AD-1231496.1 0.23 0.19 0.85 8.4 9.2 10.8 AD-1231510.1 0.15 12.10 57.03 10.3 41.1
70.3 AD-1231518.1 49.92 92.40 57.36 122.1 103.1 69.1 AD-1231474.1 no result 59.84 42.40 105.4
70.6 88.3 AD-1231497.1 0.19 0.24 0.16 5.4 7.7 8.5 AD-1231504.1 1.71 0.15 41.15 8.3 6.4 60.3
AD-1231511.1 no result 0.48 12.27 8.6 9.0 27.4 AD-1231519.1 23.98 24.46 113.17 76.6 78.7 124.1
siLuc 35.83 46.93 121.44 89.0 74.5 115.7 mock tr 112.40 0.16 60.57 84.8 4.8 79.7 mock tr 116.06
99.56 111.85 107.6 105.2 117.5

Example 5. Evaluation of siRNA Duplex Against Resistance Mutants

(648) Because of their high mutation rates, viruses, such as SARS-CoV-2, have the potential to elude host defense systems as well as antiviral drugs and vaccines.

(649) Accordingly, in order to identify duplexes, or combinations or duplexes, that are able to evade or delay the emergence of antiviral resistance, a resistance selection analysis was performed

and, subsequently, agents targeting the coronavirus genome were assessed for their ability to inhibit expression and inhibit infection of host cells and infectious virus particles in a focus-forming assay using these resistant cells.

(650) The materials and methods for this example are described below.

(651) Resistance Selection Using Fixed Concentrations

(652) The selection of variants in the presence of fixed concentrations of AD-1184150 or a combination of AD-1184150 with AD-1184137 was conducted in VeroE6 cells cultured in DMEM supplemented with 10% FBS (VWR) and 1× Penicillin/Streptomycin (Thermo Fisher Scientific). 1.2×10^5 VeroE6 cells per well in a 24-well plate were reverse transfected with 5×, 10× or 20× EC₅₀ of AD-1184150 (330 pM, 660 pM or 1320 pM, respectively) or of an equimolar mixture of AD-1184150 and AD-1184137 at the same combined total concentrations. As non-targeting control, VeroE6 cells were reverse transfected with the highest concentration (1320 pM) of an siRNA targeting the luciferase gene. All transfections used RNAiMax transfection reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. Six hours post reverse transfection, cells were incubated with SARS-CoV-2 (isolate USA-WA1/2020, passage 2) at an MOI (multiplicity of infection) of 0.01 in infection medium (DMEM, 10% FBS) for 1 hour at 37° C. After viral adsorption, cells were washed with DMEM and overlaid with infection media. For each condition, three independent wells were transfected and infected, and the supernatants were pooled together at the time of harvest. Infected cells were monitored visually for cytopathic effect (CPE) daily. When the cells in the luciferase-targeting control wells exhibited >50% CPE, the culture supernatants were harvested, diluted 1:10 and added to fresh VeroE6 cells in 24-well plates that had been reverse-transfected with the equivalent amounts of siRNA(s) as used for the initial passage. Selection continued for a total of 5 passages. At each passage, supernatant was aliquoted and frozen at -80° C. for further analyses.

(653) Determination of Viral Titer by Focus-Forming Assay

(654) Viral titers were determined using a focus-forming assay (FFA) on VeroE6 cells. One day prior to infection, 1.5×10^4 VeroE6 cells were plated in black-walled, clear bottomed 96-well plates. The next day, undiluted or 4-point 10-fold serially diluted virus samples using 10% FBS-containing media were adsorbed onto VeroE6 cells for one hour at 37° C. The cells were washed once and overlaid with 1% methylcellulose (Sigma-Aldrich) in serum-containing medium. At 24 hours post-infection, the methylcellulose overlay was removed and cells were washed with PBS. Cells were fixed with 4% PFA, incubated for 30 minutes at room temperature, then washed with PBS to remove residual PFA. The cells were permeabilized with 100 µL of 0.25% Triton X-100 (Sigma-Aldrich) in PBS for 30 minutes at room temperature. The Triton X-100 was removed, cells were washed twice with PBS, and incubated with 50 µL of SARS-CoV-2 nucleocapsid (N) antibody (Sino Biological) at 1:2,000 in blocking buffer (2% milk powder/PBS) for 1 hour at room temperature. Plates were washed three times with PBS and then incubated for 1 hour at room temperature with 50 µL/well of goat anti-rabbit-Alexa647 (Thermo Fisher Scientific) secondary antibody at 1:1,000 in blocking buffer along with 1 µg/ml Hoechst33342 (Thermo Fisher Scientific). After washing three times with PBS, plates were imaged on a Cytation5 (BioTek) plate reader (12 images at 4× magnification) with fluorescence detected in DAPI (377,447 nm) and Cy5 (628,685 nm) channels. Nucleocapsid-positive foci were counted from images and used to determine focus-forming units/mL supernatant (FFU/mL).

(655) Evaluation of Antiviral Activity Against Selected Virus

(656) Cell supernatants from viral passages containing detectable virus as determined by FFA were evaluated for a shift in EC₅₀ values in an siRNA antiviral activity assay. 7-point 5-fold serial dilutions of AD-1184150 and AD-1184137 were prepared in PBS and VeroE6 cells were reverse transfected in 96-well plates with each dilution in duplicates (range: 41250 to 3 pM final concentration). Twenty-four hours later, cells were infected with 20 FFUs of the virus-containing cell supernatants generated during passaging. The viral inoculum was removed after 1 hour and

media was replaced. At 24 hours post-infection, the cells were fixed with 4% PFA and viral nucleocapsid protein was immunostained as described above. For automated image acquisition and quantification, cells were imaged on a Cytation 5 Cell Imaging reader with 12 images per well to cover the complete well. Nuclei and AlexaFluor647-positive cells were counted using the manufacturer's provided software. After subtraction of background (uninfected) controls, all signals were normalized to the mock transfected controls.

(657) Sequencing of siRNA Binding Sites within the SARS-CoV-2 Genome

(658) To isolate nucleic acid from the supernatant of viral passages, 300 μ L of cell supernatant was added to 900 μ L of Trizol and stored at -80° C. for further analysis. Trizol collected samples were subjected to RNA isolation using PureLink RNA Mini Kit with the incorporation of on-column PureLink DNase Treatment, following manufacturer's instructions. Reverse transcription reactions were performed with 6 μ L of purified RNA and random hexamer primers using the NEB ProtoScript II First Strand cDNA Synthesis kit, according to manufacturer's instructions. The resulting cDNA was used as a template for PCR amplification of the siRNA binding sites, two primer pairs per site, using KapaBiosystems polymerase (KAPA HiFi HotStart ReadyMix) with primers, 5'-acgctgcttctggtatc-3' (SEQ ID NO: 1959) plus 5'-agaaacccttagacacagc-3' (SEQ ID NO: 1960), and 5'-ctagataaacgcactacgtg-3' (SEQ ID NO: 1961) and 5'-cctgagcaaagaagaagtg-3' (SEQ ID NO: 1962) for the AD-1184150 binding site, and 5'-agatgccttcaaactcaac-3' (SEQ ID NO: 1963) plus 5'-ttctactctgagttgttg-3' (SEQ ID NO: 1964), and 5'-ttggtggcaaacctgtatc-3' (SEQ ID NO: 1965) plus 5'-tgtgtaactggacacattg-3' (SEQ ID NO: 1966) for the AD-1184137 binding site. Each PCR with a primer pair was carried out independently, then PCR products from each treatment were pooled for purification and subsequent library preparation. Amplification conditions included an initial 3 minutes at 95° C., followed by 24 cycles with 20 seconds at 98° C., 15 seconds at 60° C. and 72° C. for 15 seconds, with a final 1 minute at 72° C. Pooled PCR products were purified using AMPure XP beads following manufacturer's instructions. The size of the amplicon was confirmed by analyzing 2 μ L of PCR products using the Agilent D1000 ScreenTape System. Products were quantified by analyzing 1 μ L with the Quant-iT dsDNA High-Sensitivity Assay Kit. Fifty ng of purified PCR product was used as input for library construction using the NEBNext Ultra II DNA Library Prep kit following manufacturer's instructions. NEBNext Multiplex Oligos for Illumina Dual Index Primer Set 1 was used for library construction, with a total of 4 PCR cycles. Libraries size was determined using the Agilent D1000 ScreenTape System and quantified with the Quant-iT dsDNA High-Sensitivity Assay Kit. Equal amounts of each library were pooled together for multiplexing and 'Protocol A: Standard Normalization Method' of the Illumina library preparation guide was used to prepare 8 pM final multiplexed library with 1% PhiX spike-in for sequencing. The MiSeq Reagent Kit v3 (600-cycle) was used for sequencing the libraries on the Illumina MiSeq platform, with 150 cycles for Read 1, 150 cycles for Read 2, 8 cycles for Index 1, and 8 cycles for Index 2.

(659) Sequence Analysis of siRNA Binding Sites

(660) Paired-end reads were trimmed to 2×150 upon inspection with FastQC to retain segments of high quality ($>Q30$). Illumina adapters were clipped using Trimmomatic. Read alignment to SARS-CoV-2 Wuhan-Hu-1 reference (NCBI: NC_045512.2) was performed with Burrows-Wheeler Aligner (BWA). Variants were called with LoFreq* upon indel realignment and base quality recalibration, using a frequency threshold of 1%. Primers were excluded from variant calling. A consensus sequence was generated, mapped back to reference sequence coordinates and used to align reads a second time. The incorporation of variants from the first mapping iteration into a consensus sequence facilitates read alignment in case of mismatches with the reference. Variants were called again against the reference sequence from reads mapped to the consensus sequence. A third iteration of consensus generation read alignment and variant calling was performed. The variant call set from this last iteration was retained as final. Nucleotide variants were annotated with SnpEff to generate amino acid variants. Extensive QCs were performed at read, alignment and

variant level using FastQC, samtools, picard, mosdepth, bcftools and MultiQC. An end-to-end workflow was automated using NextFlow. All programs are available through the Bioconda Initiative (bioconda.github.io).

(661) Quantification of Extracellular SARS-CoV-2 Genomes by RT-qPCR

(662) Viral RNA was extracted from the cell culture supernatant using the NucleoSpin 96 Virus kit (Macherey-Nagel). Quantification of viral genomes was performed using the Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs) with a primer/probeset binding in the orflab region (forward: CCCTGTGGGTTTTACACTTAA (SEQ ID NO: 1967), reverse:

ACGATTGTGCATCAGCTGA (SEQ ID NO: 1968), probe:

CCGTCTGCGGTATGTGGAAAGGTTATGG (SEQ ID NO: 1969)). A standard curve of defined dilutions of a synthetic SARS-CoV-2 RNA (Twist Bioscience) was used for normalization.

Results

(663) Duplexes AD-1184137; AD-1184150; AD-1184151; AD-1184284; AD-1184212; AD-1231490; and the combinations of duplexes AD-1184137 and AD-1184150; AD-1184137 and AD-1184151; and AD-1184137, AD-1184150, AD-1184284, and AD-1184212 were assessed for their ability to inhibit coronavirus genome expression and focus formation in resistance selected cells.

(664) As depicted in FIG. 4, all of the duplexes and duplex combinations inhibited expression of the SARS-CoV2 genome in the resistance selected cells and, as depicted in FIG. 5, all of the duplexes and duplex combinations inhibited SARS-CoV2 cell infection of the resistance selected cells. FIG. 6 is an exemplary immunofluorescence assay confirming the results of the q-PCR and focus-forming assays that the duplexes inhibit formation of viral particles in resistance selected cells. The duplexes that were most effective were AD-1184137 and AD-1184150, as was the combination of duplexes AD-1184137 and AD-1184150 (see Table 9 below and FIGS. 4 and 5).

(665) TABLE-US-00010

TABLE 9	EC.sub.50	EC.sub.50	Genome	Genome	EC.sub.95	EC.sub.95
Reactivity*	Reactivity*	siRNA (pM;	PCR) (pM;	IFA) (0 mm)	(1 mm)	AD-1184150
42	66					
99.91%	100.00%	1183	763	AD-1184137	86	118
99.89%	99.98%	702	608	*N = 4386 genomes analyzed		

Example 6. Intranasal Delivery of siRNA Duplexes Prevents Coronavirus Infection

(666) Experimental Design

(667) To determine the efficacy of dsRNA agents administered intranasally, fifty-four (54) Male Syrian Golden hamsters, approximately 6-8 weeks of age were divided among seven groups, according to Table 10, below, in groups of 6 animals. Group 1 was a control group administered PBS via intranasal (IN) dosing on day -7 pre-challenge. Group 2 was a control group administered a dsRNA agent targeting luciferase via intranasal (IN) dosing on day -7 pre-challenge. Groups 3-6 were administered either a combination of AD-1184150 and AD-1184137, both targeting COVID-19, or an iRNA agent targeting ACE2, (see Table 10) via intranasal (IN) dosing on day -7 pre-challenge. Group 7 was administered a combination of AD-1184150 and AD-1184137, via subcutaneous (SQ) dosing on day -7 pre-challenge.

(668) Animals were challenged on study day 0 with SARS-CoV-2 via the intranasal route. Animals were monitored to Day 7 post-challenge. Oral swabs were collected in the post-challenge period, days 1, 3, and 5. Terminal oral swabs, blood, and tissue collection occurred on day 7 post-challenge.

(669) TABLE-US-00011

TABLE 10	Experimental Design	Dose at each Treatment Group	N
Treatment	Route	Treatment Days	1
6	PBS	IN	0 mg/kg SD -7
2	6	iRNA agent targeting	IN 30 mg/kg SD -7
3	6	AD-1184150 + AD-1184137	IN 30 mg/kg SD -7
4	6	iRNA agent targeting	ACE2 IN 30 mg/kg SD -7
5	6	AD-1184150 + AD-1184137	IN 10 mg/kg SD -7
6	6	AD-1184150 + AD-1184137	IN 1 mg/kg SD -7
7	5	AD-1184150 + AD-1184137	SQ 30 mg/kg SD -7

(670) For animals receiving a combination of AD-1184150 and AD-1184137, the two duplexes were mixed together and the weight administered to each animal, as indicated in Table 10, is the total weight of the mixture of the two duplexes.

(671) Each animal received a dose volume for IN dosing of 100 µl per animal (50 µl per nostril) or

200 µl per animal for SQ dosing.

(672) Virus Challenge with SARS-CoV-2

(673) The intranasal inoculation (IN) was performed on Ketamine/Xylazine anesthetized hamsters. Administration of virus was conducted as follows: using a calibrated P200 pipettor, 50 µL of the viral inoculum was administered dropwise into each nostril, for a total of 100 µL per animal. Anesthetized animals were held upright such that the nostrils of the hamster were pointing towards the ceiling. The tip of the syringe was placed into the first nostril and virus inoculum was slowly injecting into the nasal passage, and then removed. This was repeated for the second nostril. The animal's head was tilted back for about 20 seconds and then returned to its housing unit and monitored until fully recovered.

(674) Body weights were determined each day post-challenge through Day 7 post-challenge to assess the effectiveness of the duplexes as assessed by the weight of the animals.

(675) The results are provided in FIGS. 7 and 8 and demonstrate that intranasal administration of a single 10 mg/kg dose or 30 mg/kg dose of the combination of AD-1184137 and AD-1184150 prevents SARS-CoV-2 infection as demonstrated by the maintenance of the weights of the hamsters (FIG. 7) and that intranasal administration of a single 30 mg/kg dose of the combination of AD-1184137 and AD-1184150 is superior to subcutaneous administration of a single 30 mg/kg dose of the combination of AD-1184137 and AD-1184150 in preventing SARS-CoV-2 infection (FIG. 8).

EQUIVALENTS

(676) Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

Claims

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of a coronavirus genome in a cell, or a salt thereof, wherein the dsRNA agent, or a salt thereof, comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a nucleotide sequence differing by no more than three bases from any one of the antisense strand nucleotide sequences selected from the group consisting of (a) 5'-CCGGGUUUGACAGUUUGAAAAGC-3' of SEQ ID NO: 586; (b) 5'-GAUUAAGAUAUGCUAUGUGAGAU-3' of SEQ ID NO: 719; and (c) 5'-UCGGGUUUGACAGUUUGAAAAGC-3' of SEQ ID NO: 1852.
2. An isolated cell containing the dsRNA agent, or a salt thereof, of claim 1.
3. A pharmaceutical composition for inhibiting expression of a coronavirus genome, comprising the dsRNA agent, or a salt thereof, of claim 1.
4. The dsRNA agent, or a salt thereof, of claim 1, wherein the sense strand is conjugated to one or more lipophilic moieties.
5. The dsRNA agent, or a salt thereof, of claim 4, wherein the one or more lipophilic moieties is selected from the group consisting of lipid, cholesterol, retinoic acid, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-bis-O (hexadecyl) glycerol, geranyloxyhexanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, 03-(oleoyl) lithocholic acid, 03-(oleoyl) cholenic acid, dimethoxytrityl, or phenoxazine.
6. The dsRNA agent, or a salt thereof, of claim 5, wherein the one or more lipophilic moieties is an aliphatic, alicyclic, or polyalicyclic compound.
7. The dsRNA agent, or a salt thereof, of claim 6, wherein the one or more lipophilic moieties contains a saturated or unsaturated C4-C30 hydrocarbon chain.
8. The dsRNA agent, or a salt thereof, of claim 7, wherein the one or more lipophilic moieties contains a saturated or unsaturated C16 hydrocarbon chain.

9. The dsRNA agent, or a salt thereof, of claim 8, wherein the saturated or unsaturated C16 hydrocarbon chain is conjugated to position 6, counting from the 5'-end of the strand.
10. The dsRNA agent, or a salt thereof, of claim 9, wherein the saturated or unsaturated C16 hydrocarbon chain is conjugated to a nucleobase, a sugar moiety, or an internucleoside linkage.
11. The dsRNA agent, or a salt thereof, of claim 10, wherein the saturated or unsaturated C16 hydrocarbon chain comprises a 2'-O-hexadecyl-adenosine-3'-phosphate nucleotide.
12. The dsRNA agent, or a salt thereof, of claim 1, wherein all of the nucleotides of sense strand and all of the nucleotides of the antisense strand comprise a nucleotide modification.
13. The dsRNA agent, or a salt thereof, of claim 1, wherein the dsRNA agent, or a salt thereof, further comprises at least one phosphorothioate internucleotide linkage.
14. The dsRNA agent, or a salt thereof, of claim 1, wherein the dsRNA agent, or a salt thereof, further comprises a phosphate or phosphate mimic at the 5'-end of the antisense strand.
15. The dsRNA agent, or a salt thereof, of claim 14, wherein the phosphate mimic is a 5'-vinyl phosphonate (VP).
16. The dsRNA agent, or a salt thereof, of claim 1, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.
17. The dsRNA agent, or a salt thereof, of claim 1, wherein the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-UUUUCAAACUGUCAACCCGG-3' of SEQ ID NO: 231 and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-CCGGGUUGACAGUUUGAAAAGC-3' of SEQ ID NO: 586.
18. The dsRNA agent, or a salt thereof, of claim 1, wherein the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-CUCACAUAGCAAUCUUUAAUC-3' of SEQ ID NO: 364 and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-GAUUAAGAUAUGCUAUGUGAGAU-3' of SEQ ID NO: 719.
19. The dsRNA agent, or a salt thereof, of claim 1, wherein the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-UUUUCAAACUGUCAACCCGA-3' of SEQ ID NO: 1812 and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-UCGGGUUGACAGUUUGAAAAGC-3' SEQ ID NO: 1852.
20. The dsRNA agent, or a salt thereof, of claim 1, wherein the sense strand comprises the nucleotide sequence 5'-ususuc(Ahd)AfaCfUfGfucaaaccsgsa-3' of SEQ ID NO:941 and the antisense strand comprises the nucleotide sequence 5'-VPusCfsgggUfuugacagUfuUfgaaaasgsc-3' of SEQ ID NO:1296, wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U; s is a phosphorothioate linkage; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; and VP is Vinyl-phosphonate.
21. The dsRNA agent, or a salt thereof, of claim 1, wherein the sense strand comprises the nucleotide sequence 5'-csuscac(Ahd)UfaGfCfAfaucuuuaasusa-3' of SEQ ID NO: 1074 and the antisense strand comprises the nucleotide sequence 5'-VPusAfsuuaAfagauugcUfaUfgugagsasu-3' of SEQ ID NO:1429, wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U; s is a phosphorothioate linkage; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; and VP is Vinyl-phosphonate.
22. The dsRNA agent, or a salt thereof, of claim 1, wherein the sense strand comprises the nucleotide sequence 5'-ususuu(Chd)aAfaCfUfGfucaaaccsgsa-3' of SEQ ID NO: 1895 and the antisense strand comprises the nucleotide sequence 5'-VPusCfsgggUfuugacagUfuUfgaaaasgsc-3' of SEQ ID NO:1296 wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U; s is a phosphorothioate linkage; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; and VP is Vinyl-phosphonate.
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