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(54) Title: COMPOSITIONS AND METHODS FOR SILENCING MYOC EXPRESSION

(57) Abstract: The disclosure relates to double-stranded ribonucleic acid (dsRNA) compositions targeting MYOC, and methods of using such dsRNA compositions to alter (e.g., inhibit) expression of MYOC.

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COMPOSITIONS AND METHODS FOR SILENCING MYOC EXPRESSION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 63/215,804, filed on June 28, 2021, claims the benefit of priority to U.S. Provisional Application No. 63/287,404, filed on December 8, 2021, and claims the benefit of priority to U.S. Provisional Application No. 63/351,033, filed on June 10, 2022. The entire contents of the foregoing applications are hereby incorporated herein by reference.

SEQUENCE LISTING

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 June 22, 2022 is named A108868_1490WO_SL.txt and is 596,467 bytes in size.

FIELD OF THE DISCLOSURE

The disclosure relates to the specific inhibition of the expression of the MYOC.

BACKGROUND

Glaucoma (*e.g.*, primary open angle glaucoma (POAG)) is a major cause of irreversible vision loss in today's aging population. MYOC protein misfolding occludes its secretion from trabecular meshwork cells, leading to elevated eye pressure that in turn compresses and damages the optic nerve reducing its ability to transmit visual information to the brain, which results in vision loss. New treatments for glaucoma are needed.

SUMMARY

The present disclosure describes methods and iRNA compositions for modulating the expression of MYOC. In certain embodiments, expression of MYOC is reduced or inhibited using a MYOC-specific iRNA. Such inhibition can be useful in treating disorders related to MYOC expression, such as ocular disorders (*e.g.*, glaucoma, *e.g.*, primary open angle glaucoma (POAG)).

Accordingly, described herein are compositions and methods that effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of MYOC, such as in a cell or in a subject (*e.g.*, in a mammal, such as a human subject). Also described are compositions and methods for treating a disorder related to expression of MYOC, such as glaucoma (*e.g.*, primary open angle glaucoma (POAG))

The iRNAs (*e.g.*, dsRNAs) included in the compositions featured herein include an RNA strand (the antisense strand) having a region, *e.g.*, a region that is 30 nucleotides or less, generally 19-24 nucleotides in length, that is substantially complementary to at least part of an mRNA transcript of MYOC (*e.g.*, a human MYOC) (also referred to herein as a "MYOC-specific iRNA"). In some embodiments, the MYOC mRNA transcript is a human MYOC mRNA transcript, *e.g.*, SEQ ID NO: 1 herein.

In some embodiments, the iRNA (*e.g.*, dsRNA) described herein comprises an antisense strand having a region that is substantially complementary to a region of a human MYOC mRNA. In some embodiments, the human MYOC mRNA has the sequence NM_000261.2 (SEQ ID NO: 1). The sequence of NM_000261.2 is also herein incorporated by reference in its entirety. The reverse complement of SEQ ID NO: 1 is provided as SEQ ID NO: 2 herein.

In some aspects, the present disclosure provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of myocilin (MYOC), 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 a coding strand of human MYOC 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 a non-coding strand of human MYOC such that the sense strand is complementary to the at least 15 contiguous nucleotides in the antisense strand.

In some aspects, the present disclosure provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of MYOC, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of nucleotide sequence of SEQ ID NO: 2 such that the sense strand is complementary to the at least 15 contiguous nucleotides in the antisense strand.

In some aspects, the present disclosure provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of MYOC, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1565804 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1565804.

In some aspects, the present disclosure provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of MYOC, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1565837 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1565837.

In some aspects, the present disclosure provides a human cell or tissue comprising a reduced level of MYOC mRNA or a level of MYOC protein as compared to an otherwise similar untreated cell or tissue, wherein optionally the cell or tissue is not genetically engineered (*e.g.*, wherein the cell or tissue comprises one or more naturally arising mutations, *e.g.*, MYOC mutations), wherein optionally the level is reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%. In some embodiments, the human cell or tissue is a trabecular meshwork tissue, a ciliary body, a retinal pigment epithelium (RPE), a retinal tissue, an astrocyte, a pericyte, a Müller cell, a ganglion cell, an endothelial cell, a photoreceptor cell, a retinal blood vessel (e.g., including endothelial cells and vascular smooth muscle cells), or choroid tissue, e.g., a choroid vessel.

The present disclosure also provides, in some aspects, a cell containing the dsRNA agent described herein.

In another aspect, provided herein is a human ocular cell, *e.g.*, (a cell of the trabecular meshwork, a cell of the ciliary body, an RPE cell, a retinal cell, an astrocyte, a pericyte, a Müller cell, a ganglion cell, an endothelial cell, or a photoreceptor cell) comprising a reduced level of MYOC mRNA or a level of MYOC protein as compared to an otherwise similar untreated cell.

In some embodiments, the level is reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%.

In some aspects, the present disclosure also provides a pharmaceutical composition for inhibiting expression of a gene encoding MYOC, comprising a dsRNA agent described herein.

The present disclosure also provides, in some aspects, a method of inhibiting expression of MYOC in a cell, the method comprising:

(a) contacting the cell with the dsRNA agent described herein, or a pharmaceutical composition described herein; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of MYOC, thereby inhibiting expression of the MYOC in the cell.

The present disclosure also provides, in some aspects, a method of inhibiting expression of MYOC in a cell, the method comprising:

(a) contacting the cell with the dsRNA agent described herein, or a pharmaceutical composition described herein; and

(b) maintaining the cell produced in step (a) for a time sufficient to reduce levels of MYOC mRNA, MYOC protein, or both of MYOC mRNA and protein, thereby inhibiting expression of the MYOC in the cell.

The present disclosure also provides, in some aspects, a method of inhibiting expression of MYOC in an ocular cell or tissue, the method comprising:

(a) contacting the cell or tissue with a dsRNA agent that binds MYOC; and

(b) maintaining the cell or tissue produced in step (a) for a time sufficient to reduce levels of MYOC mRNA, MYOC protein, or both of MYOC mRNA and protein, thereby inhibiting expression of MYOC in the cell or tissue.

The present disclosure also provides, in some aspects, a method of treating a subject diagnosed with MYOC-associated disorder comprising administering to the subject a therapeutically effective amount of the dsRNA agent described herein or a pharmaceutical composition described herein, thereby treating the disorder.

In any of the aspects herein, *e.g.*, the compositions and methods above, any of the embodiments herein (*e.g.*, below) may apply.

In some embodiments, the coding strand of human MYOC has the sequence of SEQ ID NO: 1. In some embodiments, the non-coding strand of human MYOC has the sequence of SEQ ID NO: 2.

In some embodiments, the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, or 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO: 1.

In some embodiments, the dsRNA agent comprises a sense strand and an antisense strand, wherein the antisense strand comprises a nucleotide sequence comprising at least 17 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of nucleotide sequence of SEQ ID NO: 2 such that the sense strand is complementary to the at least 17 contiguous nucleotides in the antisense strand. In some embodiments, the sense strand comprises a nucleotide sequence comprising at least 17 contiguous nucleotides, with 0, or 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO: 1.

In some embodiments, the dsRNA agent comprises a sense strand and an antisense strand, wherein the antisense strand comprises a nucleotide sequence comprising at least 19 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of nucleotide sequence of SEQ ID NO: 2 such that the sense strand is complementary to the at least 19 contiguous nucleotides in the antisense strand. In some embodiments, the sense strand comprises a nucleotide sequence comprising at least 19 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO: 1.

In some embodiments, the dsRNA agent comprises a sense strand and an antisense strand, wherein the antisense strand comprises a nucleotide sequence comprising at least 21 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of nucleotide sequence of SEQ ID NO: 2 such that the sense strand is complementary to the at least 21 contiguous nucleotides in the antisense strand. In some embodiments, the sense strand comprises a nucleotide sequence comprising at least 21 contiguous nucleotides, with 0, or 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO: 1.

In some embodiments, the portion of the sense strand is a portion within a sense strand in any one of Tables 2A and 2B.

In some embodiments, the portion of the antisense strand is a portion within an antisense strand in any one of Tables 2Aand 2B.

In some embodiments, the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from one of the antisense sequences listed in any one of Tables 2A and 2B. In some embodiments, the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from a sense sequence listed in any one of Tables 2A and 2B that corresponds to the antisense sequence.

In some embodiments, the antisense strand comprises a nucleotide sequence comprising at least 17 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from one of the antisense sequences listed in any one of Tables 2A and 2B. In some embodiments, the sense strand comprises a nucleotide sequence comprising at least 17 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from a sense sequence listed in any one of Tables 2A and 2B that corresponds to the antisense sequence.

In some embodiments, the antisense strand comprises a nucleotide sequence comprising at least 19 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from one of the antisense sequences listed in any one of Tables 2A and 2B. In some embodiments, the sense strand comprises a nucleotide sequence comprising at least 19 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from a sense sequence listed in any one of Tables 2A and 2B that corresponds to the antisense sequence.

In some embodiments, the antisense strand comprises a nucleotide sequence comprising at least 21 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from one of the antisense sequences listed in any one of Tables 2A and 2B. In some embodiments, the sense strand comprises a nucleotide sequence comprising at least 21 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from a sense sequence listed in any one of Tables 2A and 2B that corresponds to the antisense sequence.

In some embodiments, the sense strand of the dsRNA agent is at least 23 nucleotides in length, *e.g.*, 23-30 nucleotides in length.

In some embodiments, at least one of the sense strand and the antisense strand is conjugated to one or more lipophilic moieties. In some embodiments, the lipophilic moiety is conjugated to one or more positions in the double stranded region of the dsRNA agent. In some embodiments, the lipophilic moiety is conjugated via a linker or carrier. In some embodiments, lipophilicity of the lipophilic moiety, measured by $\log K_{ow}$, exceeds 0.

In some embodiments, 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. In some embodiments, the plasma protein binding assay is an electrophoretic mobility shift assay using human serum albumin protein.

In some embodiments, the dsRNA agent comprises at least one modified nucleotide. In some embodiments, no more than five of the sense strand nucleotides and not more than five of the nucleotides of the antisense strand are unmodified nucleotides. In some embodiments, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In some embodiments, 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 phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a glycol modified nucleotide, and a 2'-O-(N-methylacetamide) modified nucleotide; and combinations thereof. In some embodiments, no more than five of the sense strand nucleotides and not more than five of the nucleotides of the antisense strand include modifications other than 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, unlocked nucleic acids (UNA) or glycerol nucleic acid (GNA).

In some embodiments, the dsRNA comprises a non-nucleotide spacer (wherein optionally the non-nucleotide spacer comprises a C3-C6 alkyl) between two of the contiguous nucleotides of the sense strand or between two of the contiguous nucleotides of the antisense strand.

In some embodiments, each strand is no more than 30 nucleotides in length. In some embodiments, at least one strand comprises a 3' overhang of at least 1 nucleotide. In some

embodiments, at least one strand comprises a 3' overhang of at least 2 nucleotides. In some embodiments, at least one strand comprises a 3' overhang of 2 nucleotides.

In some embodiments, the double stranded region is 15-30 nucleotide pairs in length. In some embodiments, the double stranded region is 17-23 nucleotide pairs in length. In some embodiments, the double stranded region is 17-25 nucleotide pairs in length. In some embodiments, the double stranded region is 23-27 nucleotide pairs in length. In some embodiments, the double stranded region is 19-21 nucleotide pairs in length. In some embodiments, the double stranded region is 21-23 nucleotide pairs in length. In some embodiments, each strand has 19-30 nucleotides. In some embodiments, each strand has 19-23 nucleotides. In some embodiments, each strand has 21-23 nucleotides.

In some embodiments, the agent comprises at least one phosphorothioate or methylphosphonate internucleotide linkage. In some embodiments, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. In some embodiments, the strand is the antisense strand. In some embodiments, the strand is the sense strand.

In some embodiments, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. In some embodiments, the strand is the antisense strand. In some embodiments, the strand is the sense strand.

In some embodiments, each of the 5'- and 3'-terminus of one strand comprises a phosphorothioate or methylphosphonate internucleotide linkage. In some embodiments, the strand is the antisense strand.

In some embodiments, the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

In some embodiments, the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

In some embodiments, one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand. In some embodiments, the one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand via a linker or carrier.

In some embodiments, the internal positions include all positions except the terminal two positions from each end of the at least one strand. In some embodiments, the internal positions

include all positions except the terminal three positions from each end of the at least one strand. In some embodiments, the internal positions exclude a cleavage site region of the sense strand. In some embodiments, the internal positions include all positions except positions 9-12, counting from the 5'-end of the sense strand. In some embodiments, the internal positions include all positions except positions 11-13, counting from the 3'-end of the sense strand. In some embodiments, the internal positions exclude a cleavage site region of the antisense strand. In some embodiments, the internal positions include all positions except positions 12-14, counting from the 5'-end of the antisense strand. In some embodiments, 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.

In some embodiments, 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. In some embodiments, 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.

In some embodiments, the positions in the double stranded region exclude a cleavage site region of the sense strand.

In some embodiments, 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. In some embodiments, the lipophilic moiety is conjugated to position 21, position 20, position 15, position 1, or position 7 of the sense strand. In some embodiments, the lipophilic moiety is conjugated to position 21, position 20, or position 15 of the sense strand. In some embodiments, the lipophilic moiety is conjugated to position 20 or position 15 of the sense strand. In some embodiments, the lipophilic moiety is conjugated to position 16 of the antisense strand. In some embodiments, the lipophilic moiety is conjugated to position 6, counting from the 5'-end of the sense strand.

In some embodiments, the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound. In some embodiments, 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, geranyloxyhexyanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine. In some embodiments, 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 alkyne. In some embodiments, the lipophilic moiety contains a saturated or unsaturated C6-C18 hydrocarbon chain. In some embodiments, the lipophilic moiety contains a saturated or unsaturated C16 hydrocarbon chain.

In some embodiments, 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. In some embodiments, 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.

In some embodiments, 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.

In some embodiments, the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

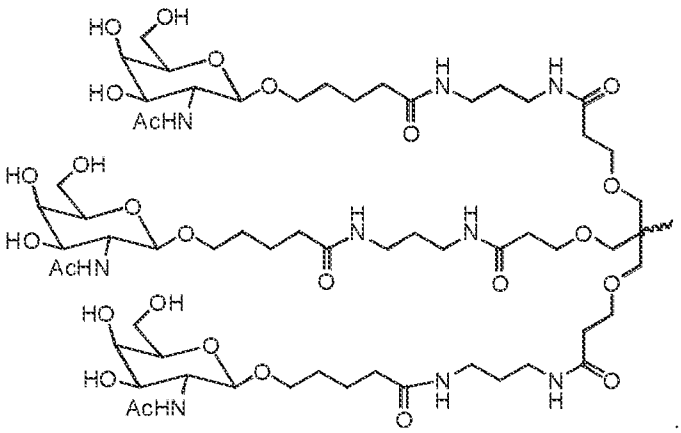
In some embodiments, the lipophilic moiety or targeting ligand is conjugated via a bio-cleavable linker selected from the group consisting of DNA, RNA, disulfide, amide, functionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, mannose, and combinations thereof.

In some embodiments, 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.

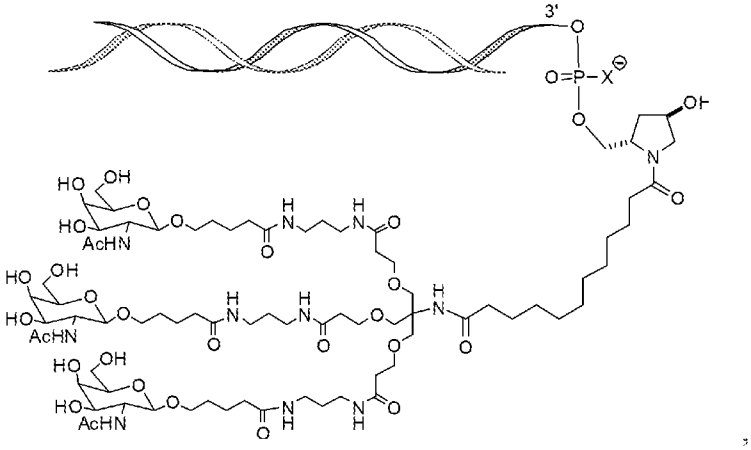
In some embodiments, the dsRNA agent further comprises a targeting ligand, *e.g.*, a ligand that targets an ocular tissue or a liver tissue. In some embodiments, the ocular tissue is a trabecular meshwork tissue, a ciliary body, a retinal tissue, a retinal pigment epithelium (RPE) or choroid tissue, e.g., a choroid vessel.

In some embodiments, the ligand is conjugated to the sense strand. In some embodiments, the ligand is conjugated to the 3' end or the 5' end of the sense strand. In some embodiments, the ligand is conjugated to the 3' end of the sense strand.

In some embodiments, the ligand comprises N-acetylgalactosamine (GalNAc). In some embodiments, the targeting ligand comprises one or more GalNAc conjugates or one or more GalNAc derivatives. In some embodiments, the ligand is one or more GalNAc conjugates or one or more GalNAc derivatives are attached through a monovalent linker, or a bivalent, trivalent, or tetravalent branched linker. In some embodiments, the ligand is



In some embodiments, the dsRNA agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S. In some embodiments, the X is O.

In some embodiments, 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.

In some embodiments, 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.

In some embodiments, 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.

In some embodiments, 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.

In some embodiments, 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.

In some embodiments, the dsRNA agent further comprises a phosphate or phosphate mimic at the 5'-end of the antisense strand. In some embodiments, the phosphate mimic is a 5'-vinyl phosphonate (VP).

In various embodiments of the aforementioned dsRNA agents, the dsRNA agent targets a hotspot region of an mRNA encoding MYOC.

In another aspect, the present invention provides a dsRNA agent that targets a hotspot region of a myocilin (MYOC) mRNA.

In some embodiments, a cell described herein, *e.g.*, a human cell, was produced by a process comprising contacting a human cell with the dsRNA agent described herein.

In some embodiments, a pharmaceutical composition described herein comprises the dsRNA agent and a lipid formulation.

In some embodiments (*e.g.*, embodiments of the methods described herein), the cell is within a subject. In some embodiments, the subject is a human. In some embodiments, the level of MYOC mRNA is inhibited by at least 50%. In some embodiments, the level of MYOC protein is inhibited by at least 50%. In some embodiments, the expression of MYOC is inhibited

by at least 50%. In some embodiments, inhibiting expression of MYOC decreases the MYOC protein level in a biological sample (*e.g.*, an aqueous ocular fluid sample) from the subject by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%. In some embodiments, inhibiting expression of MYOC gene decreases the MYOC mRNA level in a biological sample (*e.g.*, an aqueous ocular fluid sample) from the subject by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%.

In some embodiments, the subject has been diagnosed with a MYOC-associated disorder. In some embodiments, the subject meets at least one diagnostic criterion for a MYOC-associated disorder. In some embodiments, the MYOC associated disorder is glaucoma. In some embodiments, the MYOC associated disorder is primary open angle glaucoma (POAG).

In some embodiments, the ocular cell or tissue is a trabecular meshwork tissue, a ciliary body, RPE, a retinal cell, an astrocyte, a pericyte, a Müller cell, a ganglion cell, an endothelial cell, a photoreceptor cell, a retinal blood vessel (e.g., including endothelial cells and vascular smooth muscle cells), or choroid tissue, e.g., a choroid vessel.

In some embodiments, the MYOC-associated disorder is a glaucoma. In some embodiments, the glaucoma is caused by or associated with an elevated eye pressure. In some embodiments, the glaucoma primary open angle glaucoma (POAG)).

In some embodiments, treating comprises amelioration of at least one sign or symptom of the disorder. In some embodiments, the at least one sign or symptom includes a measure of one or more of optic nerve damage, vision loss, tunnel vision, blurred vision, eye pain or presence, level, or activity of MYOC (*e.g.*, MYOC gene, MYOC mRNA, or MYOC protein).

In some embodiments, a level of the MYOC that is higher than a reference level is indicative that the subject has glaucoma. In some embodiments, treating comprises prevention of progression of the disorder. In some embodiments, the treating comprises one or more of (a) inhibiting or reducing the expression or activity of MYOC; (b) reducing the level of misfolded MYOC protein; (c) reducing trabecular meshwork cell death; (d) decreasing intraocular pressure; or (e) increasing visual acuity.

In some embodiments, the treating results in at least a 30% mean reduction from baseline of MYOC mRNA in the trabecular meshwork tissue, ciliary body, retina, RPE, a retinal blood vessel (e.g., including endothelial cells and vascular smooth muscle cells), or choroid tissue, e.g., a choroid vessel. In some embodiments, the treating results in at least a 60% mean reduction

from baseline of MYOC mRNA in the trabecular meshwork tissue, ciliary body, retina, RPE, a retinal blood vessel (e.g., including endothelial cells and vascular smooth muscle cells), or choroid tissue, e.g., a choroid vessel. In some embodiments, the treating results in at least a 90% mean reduction from baseline of MYOC mRNA in the trabecular meshwork tissue, ciliary body, retina, RPE, a retinal blood vessel (e.g., including endothelial cells and vascular smooth muscle cells), or choroid tissue, e.g., a choroid vessel.

In some embodiments, after treatment the subject experiences at least an 8-week duration of knockdown following a single dose of dsRNA as assessed by MYOC protein in the retina. In some embodiments, treating results in at least a 12-week duration of knockdown following a single dose of dsRNA as assessed by MYOC protein in the retina. In some embodiments, treating results in at least a 16-week duration of knockdown following a single dose of dsRNA as assessed by MYOC protein in the retina.

In some embodiments, the subject is human.

In some embodiments, the dsRNA agent is administered at a dose of about 0.01 mg/kg to about 50 mg/kg.

In some embodiments, the dsRNA agent is administered to the subject intraocularly. In some embodiments, the intraocular administration comprises intravitreal administration, e.g., intravitreal injection; transscleral administration, e.g., transscleral injection; subconjunctival administration, e.g., subconjunctival injection; retrobulbar administration, e.g., retrobulbar injection; intracameral administration, e.g., intracameral injection, or subretinal administration, e.g., subretinal injection.

In some embodiments, the dsRNA agent is administered to the subject intravenously. In some embodiments, the dsRNA agent is administered to the subject topically.

In some embodiments, a method described herein further comprises measuring a level of MYOC (*e.g.*, MYOC gene, MYOC mRNA, or MYOC protein) in the subject. In some embodiments, measuring the level of MYOC in the subject comprises measuring the level of MYOC protein in a biological sample from the subject (*e.g.*, an aqueous ocular fluid sample). In some embodiments, a method described herein further comprises performing a blood test, an imaging test, or an aqueous ocular fluid biopsy (e.g., an aqueous humor tap).

In some embodiments, a method described herein further measuring a level of MYOC (*e.g.*, MYOC gene, MYOC mRNA, or MYOC protein) in the subject is performed prior to

treatment with the dsRNA agent or the pharmaceutical composition. In some embodiments, upon determination that a subject has a level of MYOC that is greater than a reference level, the dsRNA agent or the pharmaceutical composition is administered to the subject. In some embodiments, measuring level of MYOC in the subject is performed after treatment with the dsRNA agent or the pharmaceutical composition.

In some embodiments, a method described herein further comprises treating the subject with a therapy suitable for treatment or prevention of a MYOC-associated disorder, *e.g.*, wherein the therapy comprises laser trabeculoplasty surgery, trabeculectomy surgery, a minimally invasive glaucoma surgery, or placement of a drainage tube in the eye. In some embodiments, a method described herein further comprises administering to the subject an additional agent suitable for treatment or prevention of a MYOC-associated disorder. In some embodiments, the additional agent comprises a carbonic anhydrase inhibitor, a prostaglandin, a beta blocker, an alpha-adrenergic agonist, a carbonic anhydrase inhibitor, a Rho kinase inhibitor, or a cholinergic agent, or any combination thereof. In some embodiments, the additional agent comprises an oral medication or an eye drop.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several features of the present disclosure.

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1A shows the experimental setup for testing the effect of human *MYOC* siRNA AD-822899 on intraocular pressure (IOP) in SAM mice comprising a humanized *MYOC* locus comprising a Y437H mutation (SAM-MYOC mice, homozygous for each allele) treated with AAV2.Y3F-SAM-g4.

Figure 1B shows intraocular pressure (IOP) in SAM mice comprising a humanized *MYOC* locus comprising a Y437H mutation (SAM-MYOC mice, homozygous for each allele) treated with AAV2.Y3F-SAM-g4 or nothing (naïve), and in which the AAV2.Y3F-SAM-g4-

treated mice were subsequently treated with either human *MYOC* siRNA or a control luciferase siRNA.

Figure 2A shows the experimental setup for testing the effect of human *MYOC* siRNAs AD-822899, AD-1565804, AD-1565837, AD-1193175, and AD-1565503 on intraocular pressure (IOP) in SAM mice comprising a humanized *MYOC* locus comprising a Y437H mutation (SAM-MYOC mice, homozygous for each allele) treated with LV-SAM-g4.

Figure 2B shows intraocular pressure (IOP) in SAM mice comprising a humanized *MYOC* locus comprising a Y437H mutation (SAM-MYOC mice, homozygous for each allele) treated with LV-SAM-g4 or PBS, and in which the LV-SAM-g4-treated mice were subsequently treated with human *MYOC* siRNA AD-822899, AD-1565804, AD-1565837, AD-1193175, or AD-1565503.

Figure 2C shows qPCR results showing the percentage of human *MYOC* mRNA expression relative to *Gapdh* in SAM mice comprising a humanized *MYOC* locus comprising a Y437H mutation (SAM-MYOC mice, homozygous for each allele) treated with LV-SAM-g4 or PBS, and in which the LV-SAM-g4-treated mice were subsequently treated with human *MYOC* siRNA AD-822899, AD-1565804, AD-1565837, AD-1193175, or AD-1565503.

Figure 2D shows RNASCOPE® analysis of human *MYOC* mRNA expression in eyes from SAM mice comprising a humanized *MYOC* locus comprising a Y437H mutation (SAM-MYOC mice, homozygous for each allele) treated with LV-SAM-g4 or PBS, and in which the LV-SAM-g4-treated mice were subsequently treated with human *MYOC* siRNA AD-1565804 or AD-1565837.

Figure 3A shows the experimental setup for testing the effect of human *MYOC* siRNAs AD-1565804 or AD-1565837 on intraocular pressure (IOP) in SAM mice comprising a humanized *MYOC* locus comprising a Y437H mutation (SAM-MYOC mice, homozygous for each allele) treated with LV-SAM-g4.

Figure 3B shows intraocular pressure (IOP) in SAM mice comprising a humanized *MYOC* locus comprising a Y437H mutation (SAM-MYOC mice, homozygous for each allele) treated with LV-SAM-g4 or PBS, and in which the LV-SAM-g4-treated mice were subsequently treated with human *MYOC* siRNA AD-1565804 or AD-1565837.

Figure 3C shows qPCR results showing the percentage of human *MYOC* mRNA expression relative to *Gapdh* in SAM mice comprising a humanized *MYOC* locus comprising a

Y437H mutation (SAM-MYOC mice, homozygous for each allele) treated with LV-SAM-g4 or PBS, and in which the LV-SAM-g4-treated mice were subsequently treated with human *MYOC* siRNA AD-1565804 or AD-1565837.

Figure 4 shows the percent MYOC protein remaining relative to PBS in the TM at day 85 after dosing with either AD-1565837 duplex or AD-1565804 duplex in the non-human primate (NHP) model. Results are shown for two different MYOC antibodies, R&D and Abnova.

Figure 5 depicts the percent MYOC protein remaining in the aqueous humor relative to pre-dose at days -35, 22, 50, and 85 after dosing with either AD-1565837 duplex or AD-1565804 duplex in the non-human primate (NHP) model.

Figure 6A shows the percent MYOC protein remaining relative to PBS in the vitreous humor and ciliary body at day 85 after dosing with either AD-1565837 duplex or AD-1565804 duplex in the non-human primate (NHP) model.

Figure 6B shows the percent MYOC protein remaining relative to PBS in the iris and sclera at day 85 after dosing with either AD-1565837 duplex or AD-1565804 duplex in the non-human primate (NHP) model.

Figure 7 depicts the percent MYOC mRNA remaining in the aqueous humor relative to PBS at day 85 after dosing with either AD-1565837 duplex or AD-1565804 duplex in the non-human primate (NHP) model.

The details of various embodiments of the disclosure are set forth in the description below. Other features, objects, and advantages of the disclosure will be apparent from the description and the drawings, and from the claims.

DETAILED DESCRIPTION

iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). Described herein are iRNAs and methods of using them for modulating (*e.g.*, inhibiting) the expression of MYOC. Also provided are compositions and methods for treatment of disorders related to MYOC expression, such as glaucoma (*e.g.*, primary open angle glaucoma (POAG)).

Human MYOC is a secreted glycoprotein of approximately 57 kDa that regulates the activation of several signaling pathways in adjacent cells to control different processes including

cell adhesion, cell-matrix adhesion, cytoskeleton organization, and cell migration. MYOC is typically expressed and secreted by a variety of tissues including the retina and the structures involved in aqueous humor regulation such as the trabecular meshwork tissue and the ciliary body. Aberrant MYOC is associated with glaucoma, for instance primary open angle glaucoma (POAG). Without wishing to be bound by theory, aberrant MYOC may exacerbate the pathogenesis of glaucoma, *e.g.*, by impeding the drainage of aqueous humor consequently leading to an increased intraocular pressure.

The following description discloses how to make and use compositions containing iRNAs to modulate (*e.g.*, inhibit) the expression of MYOC, as well as compositions and methods for treating disorders related to expression of MYOC.

In some aspects, pharmaceutical compositions containing MYOC iRNA and a pharmaceutically acceptable carrier, methods of using the compositions to inhibit expression of MYOC, and methods of using the pharmaceutical compositions to treat disorders related to expression of MYOC (*e.g.*, glaucoma, *e.g.*, primary open angle glaucoma (POAG)) are featured herein.

I. Definitions

For convenience, the meaning of certain terms and phrases used in the specification, examples, and appended claims, are provided below. If there is an apparent discrepancy between the usage of a term in other parts of this specification and its definition provided in this section, the definition in this section shall prevail.

The term "about" when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary from, for example, between 1% and 15% of the stated number or numerical range.

The terms "or more' and "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 17 nucleotides of a 20 nucleotide nucleic acid molecule" means that 17, 18, 19, or 20 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.

As used herein, "or less" and "no more than" are understood as including the value adjacent to the phrase and logical lower values or integers, as logical from context, to zero. For example, a duplex with mismatches to a target site of "no more than 2 nucleotides" has a 2, 1, or 0 mismatches. 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, "less than" is understood as not including the value adjacent to the phrase and including logical lower values or integers, as logical from context, to zero. For example, a duplex with mismatches to a target site of "less than 3 nucleotides" has 2, 1, or 0 mismatches. When "less than" is present before a series of numbers or a range, it is understood that "less than" can modify each of the numbers in the series or range.

As used herein, "more than" is understood as not including the value adjacent to the phrase and including logical higher values or integers, as logical from context, to infinity. For example, a duplex with mismatches to a target site of "more than 3 nucleotides" has 4, 5, 6, or more mismatches. When "more than" is present before a series of numbers or a range, it is understood that "more than" can modify each of the numbers in the series or range.

As used herein, "up to" as in "up to 10" is understood as up to and including 10, *i.e.*, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

Ranges provided herein are understood to include all individual integer values and all subranges within the ranges.

The terms "activate," "enhance," "up-regulate the expression of," "increase the expression of," and the like, in so far as they refer to a MYOC gene, herein refer to the at least partial activation of the expression of a MYOC gene, as manifested by an increase in the amount of MYOC mRNA, which may be isolated from or detected in a first cell or group of cells in which a MYOC gene is transcribed and which has or have been treated such that the expression of a MYOC gene is increased, 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).

In some embodiments, expression of a MYOC gene is activated by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by administration of an iRNA as described herein. In some embodiments, a MYOC gene is activated by at least about 60%, 70%, or 80% by administration of an iRNA featured in the disclosure. In some embodiments, expression of a

MYOC gene is activated by at least about 85%, 90%, or 95% or more by administration of an iRNA as described herein. In some embodiments, the MYOC gene expression is increased by at least 1-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1000-fold or more in cells treated with an iRNA as described herein compared to the expression in an untreated cell. Activation of expression by small dsRNAs is described, for example, in Li *et al.*, 2006 *Proc. Natl. Acad. Sci. U.S.A.* 103:17337-42, and in US2007/0111963 and US2005/226848, each of which is incorporated herein by reference.

The terms "silence," "inhibit expression of," "down-regulate expression of," "suppress expression of," and the like, in so far as they refer to MYOC, herein refer to the at least partial suppression of the expression of MYOC, as assessed, *e.g.*, based on MYOC mRNA expression, MYOC protein expression, or another parameter functionally linked to MYOC expression. For example, inhibition of MYOC expression may be manifested by a reduction of the amount of MYOC mRNA which may be isolated from or detected in a first cell or group of cells in which MYOC is transcribed and which has or have been treated such that the expression of MYOC is inhibited, as compared to a control. The control may be a second cell or group of cells substantially identical to the first cell or group of cells, except that the second cell or group of cells have not been so treated (control cells). The degree of inhibition is usually expressed as a percentage of a control level, *e.g.*,

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} * 100\%$$

Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to MYOC expression, *e.g.*, the amount of protein encoded by a MYOC gene. The reduction of a parameter functionally linked to MYOC expression may similarly be expressed as a percentage of a control level. In principle, MYOC silencing may be determined in any cell expressing MYOC, either constitutively or by genomic engineering, and by any appropriate assay.

For example, in certain instances, expression of MYOC is suppressed by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by administration of an iRNA disclosed herein. In some embodiments, MYOC is suppressed by at least about 60%, 65%, 70%, 75%, or 80% by administration of an iRNA disclosed herein. In some embodiments, MYOC is

suppressed by at least about 85%, 90%, 95%, 98%, 99%, or more by administration of an iRNA as described herein.

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.

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, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches may be in the internal or terminal regions of the molecule. In some embodiments, the region of complementarity comprises 0, 1, or 2 mismatches.

The term "sense strand" or "passenger strand" as used herein, refers to the strand of an iRNA that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

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.

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. Such conditions can, for example, be stringent conditions, where stringent conditions may include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing. Other conditions, such as physiologically relevant conditions as may 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.

Complementary sequences within an iRNA, *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 may 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, may yet be referred to as "fully complementary" for the purposes described herein.

Complementary sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs includes, but are not limited to, G:U Wobble or Hoogstein base pairing.

The terms "complementary," "fully complementary" and "substantially complementary" herein may 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 an iRNA agent and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding a MYOC protein). For example, a polynucleotide is complementary to at least a part of a MYOC mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding MYOC. The term "complementarity" refers to the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

As used herein, the term "region of complementarity" refers to the region of one nucleotide sequence agent that is substantially complementary to another sequence, *e.g.*, the region of a sense sequence and corresponding antisense sequence of a dsRNA, or the antisense strand of an iRNA and a target sequence, *e.g.*, a MYOC 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 antisense strand of the iRNA. 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 iRNA agent.

"Contacting," as used herein, includes directly contacting a cell, as well as indirectly contacting a cell. For example, a cell within a subject may be contacted when a composition comprising an iRNA is administered (*e.g.*, intraocularly, topically, or intravenously) to the subject.

"Introducing into a cell," when referring to an iRNA, means facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an iRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells *in vitro*; an iRNA may also be "introduced into a cell," wherein the cell is part of a living organism. In such an instance, introduction into the cell will include the delivery to the organism. For example, for *in vivo* delivery, iRNA can be injected into a tissue site or administered systemically. *In vivo* delivery can also be by a β-glucan delivery system, such as those described in U.S. Patent Nos. 5,032,401 and 5,607,677, and U.S. Publication No. 2005/0281781, which are hereby incorporated by reference in their entirety. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or known in the art. As used herein, a "disorder related to MYOC expression," a "disease related to MYOC expression," a "pathological process related to MYOC expression," "a MYOC-associated disorder," "a MYOC-associated disease," or the like includes any condition, disorder, or disease in which MYOC expression is altered (*e.g.*, decreased or increased relative to a reference level, e.g., a level characteristic of a non-diseased subject). In some embodiments, MYOC expression is decreased. In some embodiments, MYOC expression is increased. In some embodiments, the decrease or increase in MYOC expression is detectable in a tissue sample from the subject (*e.g.*, in an aqueous ocular fluid sample). The decrease or increase may be assessed relative the level observed in the same individual prior to

the development of the disorder or relative to other individual(s) who do not have the disorder. The decrease or increase may be limited to a particular organ, tissue, or region of the body (*e.g.*, the eye). MYOC-associated disorders include, but are not limited to, glaucoma (*e.g.*, primary open angle glaucoma (POAG)).

The term "glaucoma", as used herein, means any disease of the eye that is caused by or associated with damage to the optic nerve. In some embodiments, the glaucoma is associated with elevated intraocular pressure. In some embodiments, the glaucoma is asymptomatic. In other embodiments, the glaucoma has one or more symptoms, e.g., loss of peripheral vision, tunnel vision, or blind spots. A non-limiting example of glaucoma that is treatable using methods provided herein is primary open angle glaucoma (POAG).

The term "double-stranded RNA," "dsRNA," or "siRNA" as used herein, refers to an iRNA that includes an RNA molecule or complex of molecules having a hybridized duplex region that comprises two anti-parallel and substantially complementary nucleic acid strands, which will be referred to as having "sense" and "antisense" orientations with respect to a target RNA. The duplex region can be of any length that permits specific degradation of a desired target RNA, *e.g.*, through a RISC pathway, but will typically range from 9 to 36 base pairs in length, *e.g.*, 15-30 base pairs in length. Considering a duplex between 9 and 36 base pairs, the duplex can be any length in this range, for example, 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 and any sub-range therein between, including, but not limited to 15-30 base pairs, 15-26 base pairs, 15-23 base pairs, 15-22 base pairs, 15-21 base pairs, 15-20 base pairs, 15-19 base pairs, 15-18 base pairs, 15-17 base pairs, 18-30 base pairs, 18-26 base pairs, 18-23 base pairs, 18-22 base pairs, 18-21 base pairs, 18-20 base pairs, 19-30 base pairs, 19-26 base pairs, 19-23 base pairs, 19-22 base pairs, 19-21 base pairs, 19-20 base pairs, 20-30 base pairs, 20-26 base pairs, 20-25 base pairs, 20-24 base pairs, 20-23 base pairs, 20-22 base pairs, 20-21 base pairs, 21-30 base pairs, 21-26 base pairs, 21-25 base pairs, 21-24 base pairs, 21-23 base pairs, or 21-22 base pairs. dsRNAs generated in the cell by processing with Dicer and similar enzymes are generally in the range of 19-22 base pairs in length. One strand of the duplex region of a dsDNA comprises a sequence that is substantially complementary to a region of a target RNA. The two strands forming the duplex structure can be from a single RNA molecule having at least one self-complementary region, or can be formed from two or more separate RNA molecules. Where the duplex region is formed

from two strands of a single molecule, the molecule can have a duplex region separated by a single stranded chain of nucleotides (herein referred to as a "hairpin loop") between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure. The hairpin loop can comprise at least one unpaired nucleotide; in some embodiments the hairpin loop can comprise at least 3, 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. Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. In some embodiments, the two strands are connected covalently by means other than a hairpin loop, and the connecting structure is a linker.

In some embodiments, the iRNA agent may be a "single-stranded siRNA" that is introduced into a cell or organism to inhibit a target mRNA. In some embodiments, single-stranded RNAi agents can bind to the RISC endonuclease Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are generally 15-30 nucleotides and are optionally chemically modified. The design and testing of single-stranded siRNAs are described in U.S. Patent 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 (*e.g.*, sequences provided in Tables 2A or 2B) may be used as a single-stranded siRNA as described herein and optionally as chemically modified, *e.g.*, as described herein, *e.g.*, by the methods described in Lima *et al.*, (2012) *Cell* 150:883-894.

In some embodiments, an RNA interference agent includes a single stranded RNA that interacts with a 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.*, *Genes Dev.* 2001, 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 cleaves the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). Thus, in some embodiments, the disclosure relates

to a single stranded RNA that promotes the formation of a RISC complex to effect silencing of the target gene.

"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 terms "deoxyribonucleotide," "ribonucleotide," or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil may 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 may base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine may be replaced in the nucleotide sequences of dsRNA featured in the disclosure 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 disclosure.

As used herein, the term "iRNA," "RNAi", "iRNA agent," or "RNAi agent" or "RNAi molecule" refers to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript, *e.g.*, via an RNA-induced silencing complex (RISC) pathway. In some embodiments, an iRNA as described herein effects inhibition of MYOC expression, *e.g.*, in a cell or mammal. Inhibition of MYOC expression may be assessed based on a reduction in the level of MYOC mRNA or a reduction in the level of the MYOC protein.

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.

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

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.*, $\log K_{ow}$) value of the lipophilic moiety.

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.

In some embodiments, 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/US2019/031170. 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.

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.

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. Patent 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.

As used herein, the term "modulate the expression of," refers to an at least partial "inhibition" or partial "activation" of a gene (*e.g.*, MYOC gene) expression in a cell treated with an iRNA composition as described herein compared to the expression of the corresponding gene in a control cell. A control cell includes an untreated cell, or a cell treated with a non-targeting control iRNA.

The skilled artisan will recognize that the term "RNA molecule" or "ribonucleic acid molecule" encompasses not only RNA molecules as expressed or found in nature, but also analogs and derivatives of RNA comprising one or more ribonucleotide/ribonucleoside analogs or derivatives as described herein or as known in the art. Strictly speaking, a "ribonucleoside" includes a nucleoside base and a ribose sugar, and a "ribonucleotide" is a ribonucleoside with one, two or three phosphate moieties or analogs thereof (*e.g.*, phosphorothioate). However, the terms "ribonucleoside" and "ribonucleotide" can be considered to be equivalent as used herein. The RNA can be modified in the nucleobase structure, in the ribose structure, or in the ribose-phosphate backbone structure, *e.g.*, as described herein below. However, the molecules comprising ribonucleoside analogs or derivatives must retain the ability to form a duplex. As non-limiting examples, an RNA molecule can also include at least one modified ribonucleoside including but not limited to a 2'-O-methyl modified nucleoside, a nucleoside comprising a 5' phosphorothioate group, a terminal nucleoside linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a locked nucleoside, an abasic nucleoside, an acyclic nucleoside, a glycol nucleotide, a 2'-deoxy-2'-fluoro modified nucleoside, a 2'-amino-modified nucleoside, 2'-alkyl-modified nucleoside, morpholino nucleoside, a phosphoramidate or a non-natural base comprising nucleoside, or any combination thereof. Alternatively, or in combination, an RNA molecule can comprise at least two modified ribonucleosides, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20 or more, up to the entire length of the dsRNA molecule. The modifications need not be the same for each of such a plurality of modified ribonucleosides in an RNA molecule. In some embodiments, modified RNAs contemplated for use in methods and compositions described herein are peptide nucleic acids (PNAs) that have the ability to form the required duplex structure and that permit or mediate the specific degradation of a target RNA, *e.g.*, via a RISC pathway. For clarity, it is

understood that the term "iRNA" does not encompass a naturally occurring double stranded DNA molecule or a 100% deoxynucleoside-containing DNA molecule.

In some aspects, a modified ribonucleoside includes a deoxyribonucleoside. In such an instance, an iRNA agent can comprise one or more deoxynucleosides, including, for example, a deoxynucleoside overhang(s), or one or more deoxynucleosides within the double stranded portion of a dsRNA. In certain embodiments, the RNA molecule comprises a percentage of deoxyribonucleosides of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95% or higher (but not 100%) deoxyribonucleosides, *e.g.*, in one or both strands.

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of an iRNA, *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, or 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) may 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 some embodiments, the antisense strand of a dsRNA has a 1-10 nucleotide overhang at the 3' end and/or the 5' end. In some embodiments, the sense strand of a dsRNA has a 1-10 nucleotide overhang at the 3' end and/or the 5' end. In some embodiments, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of a therapeutic agent (*e.g.*, an iRNA) and a pharmaceutically acceptable carrier. As used herein, "pharmacologically effective amount," "therapeutically effective amount" or simply "effective amount" refers to that amount of an agent (*e.g.*, iRNA) effective to produce the intended pharmacological, therapeutic or preventive result. For example, in a method of treating a disorder related to MYOC expression (*e.g.*, glaucoma, *e.g.*, primary open angle glaucoma (POAG)), an effective amount includes an amount effective to reduce one or more symptoms associated with the disorder (*e.g.*, an amount effective to; (a) inhibit or reduce the expression or activity of MYOC; (b) reduce the level of misfolded MYOC protein; (c) reduce trabecular

meshwork cell death; (d) decrease intraocular pressure; or (e) increase visual acuity. For example, if a given clinical treatment is considered effective when there is at least a 10% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to obtain at least a 10% reduction in that parameter. For example, a therapeutically effective amount of an iRNA targeting MYOC can reduce a level of MYOC mRNA or a level of MYOC protein by any measurable amount, *e.g.*, by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%.

The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The term specifically excludes cell culture medium. For drugs administered orally, pharmaceutically acceptable carriers include, but are not limited to pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract. Agents included in drug formulations are described further herein below.

As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle. A SNALP represents a vesicle of lipids coating a reduced aqueous interior comprising a nucleic acid such as an iRNA or a plasmid from which an iRNA is transcribed. SNALPs are described, *e.g.*, in U.S. Patent Application Publication Nos. 2006/0240093, 2007/0135372, and in International Application No. WO 2009/082817. These applications are incorporated herein by reference in their entirety. In some embodiments, the SNALP is a SPLP. As used herein, the term "SPLP" refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle.

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.

As used herein, a "subject" to be treated according to the methods described herein, includes a human or non-human animal, *e.g.*, a mammal. The mammal may be, for example, a rodent (*e.g.*, a rat or mouse) or a primate (*e.g.*, a monkey). In some embodiments, the subject is a human.

A "subject in need thereof" includes a subject having, suspected of having, or at risk of developing a disorder related to MYOC expression, e.g., overexpression (*e.g.*, glaucoma). In some embodiments, the subject has, or is suspected of having, a disorder related to MYOC expression or overexpression. In some embodiments, the subject is at risk of developing a disorder related to MYOC expression or overexpression.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a gene, *e.g.*, MYOC, 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. For example, the target sequence will generally be from 9-36 nucleotides in length, *e.g.*, 15-30 nucleotides in length, including all sub-ranges therebetween. As non-limiting examples, the target sequence can be from 15-30 nucleotides, 15-26 nucleotides, 15-23 nucleotides, 15-22 nucleotides, 15-21 nucleotides, 15-20 nucleotides, 15-19 nucleotides, 15-18 nucleotides, 15-17 nucleotides, 18-30 nucleotides, 18-26 nucleotides, 18-23 nucleotides, 18-22 nucleotides, 18-21 nucleotides, 18-20 nucleotides, 19-30 nucleotides, 19-26 nucleotides, 19-23 nucleotides, 19-22 nucleotides, 19-21 nucleotides, 19-20 nucleotides, 20-30 nucleotides, 20-26 nucleotides, 20-25 nucleotides, 20-24 nucleotides, 20-23 nucleotides, 20-22 nucleotides, 20-21 nucleotides, 21-30 nucleotides, 21-26 nucleotides, 21-25 nucleotides, 21-24 nucleotides, 21-23 nucleotides, or 21-22 nucleotides.

As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" and the like refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of any disorder or pathological process related to MYOC expression (*e.g.*, glaucoma, *e.g.*, primary open angle glaucoma (POAG)). The specific amount that is therapeutically effective may vary depending on factors known in the art, such as, for example, the type of disorder or pathological process, the patient's history and age, the stage of the disorder or pathological process, and the administration of other therapies.

In the context of the present disclosure, the terms "treat," "treatment," and the like mean to prevent, delay, relieve or alleviate at least one symptom associated with a disorder related to MYOC expression, or to slow or reverse the progression or anticipated progression of such a disorder. For example, the methods featured herein, when employed to treat a glaucoma, may serve to reduce or prevent one or more symptoms of the glaucoma, as described herein, or to reduce the risk or severity of associated conditions. Thus, unless the context clearly indicates otherwise, the terms "treat," "treatment," and the like are intended to encompass prophylaxis, *e.g.*, prevention of disorders and/or symptoms of disorders related to MYOC expression. Treatment can also mean prolonging survival as compared to expected survival in the absence of treatment.

By "lower" in the context of a disease marker or symptom is meant any decrease, *e.g.*, a statistically or clinically significant decrease in such level. The decrease can be, for example, at least 10%, at least 20%, at least 30%, at least 40%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%. The decrease can be down to a level accepted as within the range of normal for an individual without such disorder.

As used herein, "MYOC" refers to "myocilin" the corresponding mRNA ("MYOC mRNA"), or the corresponding protein ("MYOC protein"). The sequence of a human MYOC mRNA transcript can be found at SEQ ID NO: 1.

II. iRNA Agents

Described herein are iRNA agents that modulate (*e.g.*, inhibit) the expression of MYOC.

In some embodiments, the iRNA agent activates the expression of MYOC in a cell or mammal.

In some embodiments, the iRNA agent includes double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of MYOC in a cell or in a subject (*e.g.*, in a mammal, *e.g.*, in a human), where the dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of MYOC, and where the region of complementarity is 30 nucleotides or less in length, generally 19-24 nucleotides in length, and where the dsRNA, upon contact with a cell expressing MYOC, inhibits the expression of MYOC, *e.g.*, by at least 10%, 20%, 30%, 40%, or 50%.

The modulation (*e.g.*, inhibition) of expression of MYOC can be assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by Western blot. Expression of MYOC in cell culture, such as in COS cells, ARPE-19 cells, hTERT RPE-1 cells, HeLa cells, primary hepatocytes, HepG2 cells, primary cultured cells or in a biological sample from a subject can be assayed by measuring MYOC mRNA levels, such as by bDNA or TaqMan assay, or by measuring protein levels, such as by immunofluorescence analysis, using, for example, Western Blotting or flow cytometric techniques.

A dsRNA typically includes two RNA strands that are sufficiently complementary to hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) typically includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence, derived from the sequence of an mRNA formed during the expression of MYOC. The other strand (the sense strand) typically 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. Generally, the duplex structure is between 15 and 30 inclusive, more generally between 18 and 25 inclusive, yet more generally between 19 and 24 inclusive, and most generally between 19 and 21 base pairs in length, inclusive. Similarly, the region of complementarity to the target sequence is between 15 and 30 inclusive, more generally between 18 and 25 inclusive, yet more generally between 19 and 24 inclusive, and most generally between 19 and 21 nucleotides in length, inclusive.

In some embodiments, the dsRNA is between 15 and 20 nucleotides in length, inclusive, and in other embodiments, the dsRNA is between 25 and 30 nucleotides in length, inclusive. As the ordinarily skilled person will recognize, the targeted 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 be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway). dsRNAs having duplexes as short as 9 base pairs can, under some circumstances, mediate RNAi-directed RNA cleavage. Most often a target will be at least 15 nucleotides in length, *e.g.*, 15-30 nucleotides in length.

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 9 to 36, *e.g.*, 15-30 base pairs. Thus, in some

embodiments, 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 some embodiments, then, an miRNA is a dsRNA. In some embodiments, a dsRNA is not a naturally occurring miRNA. In some embodiments, an iRNA agent useful to target MYOC expression is not generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein may further include one or more single-stranded nucleotide overhangs. The 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.

In some embodiments, MYOC is a human MYOC.

In specific embodiments, the dsRNA comprises a sense strand that comprises or consists of a sense sequence selected from the sense sequences provided in Tables 2A or 2B and an antisense strand that comprises or consists of an antisense sequence selected from the antisense sequences provided in Tables 2A or 2B.

In some aspects, a dsRNA will include at least sense and antisense nucleotide sequences, whereby the sense strand is selected from the sequences provided in Tables 2A or 2B and the corresponding antisense strand is selected from the sequences provided in Tables 2A or 2B.

In these aspects, 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 an mRNA generated by the expression of MYOC. As such, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand, and the second oligonucleotide is described as the corresponding antisense strand. 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.

The skilled person is well aware that dsRNAs having a duplex structure of between 20 and 23, but specifically 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, *EMBO* 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can be effective as well.

In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Tables 2A and 2B, dsRNAs described herein can include at least one strand of a length of minimally 19 nucleotides. It can be reasonably expected that shorter duplexes having one of the sequences of Tables 2A and 2B minus only a few nucleotides on one or both ends will be similarly effective as compared to the dsRNAs described above.

In some embodiments, the dsRNA has a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Tables 2A or 2B.

In some embodiments, the dsRNA has an antisense sequence that comprises at least 15, 16, 17, 18, or 19 contiguous nucleotides of an antisense sequence provided in Tables 2A or 2B and a sense sequence that comprises at least 15, 16, 17, 18, or 19 contiguous nucleotides of a corresponding sense sequence provided in Tables 2A or 2B.

In some embodiments, the dsRNA comprises an antisense sequence that comprises at least 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleotides of an antisense sequence provided in Tables 2A or 2B and a sense sequence that comprises at least 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides of a corresponding sense sequence provided in Tables 2A or 2B.

In some such embodiments, the dsRNA, although it comprises only a portion of the sequences provided in Tables 2A or 2B is equally effective in inhibiting a level of MYOC expression as is a dsRNA that comprises the full-length sequences provided in Tables 2A or 2B. In some embodiments, the dsRNA differs in its inhibition of a level of expression of MYOC by not more than 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 % inhibition compared with a dsRNA comprising the full sequence disclosed herein.

In some embodiments, an iRNA described herein comprises an antisense strand comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of nucleotide sequence of SEQ ID NO: 2. In some embodiments, an iRNA described herein comprises a sense strand comprising at least 15 contiguous nucleotides, with 0, or 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO: 1.

A human MYOC mRNA may have the sequence of SEQ ID NO: 1 provided herein. Homo sapiens myocilin (MYOC), mRNA

GAGCCAGCAAGGCCACCCATCCAGGCACCTCTCAGCACAGCAGAGCTTTCCAGAGGAAGCCTCA
CCAAGCCTCTGCAATGAGGTTCTTCTGTGCACGTTGCTGCAGCTTTGGGCCTGAGATGCCAGCT
GTCCAGCTGCTGCTTCTGGCCTGCCTGGTGTGGGATGTGGGGGCCAGGACAGCTCAGCTCAGGA
AGGCCAATGACCAGAGTGGCCGATGCCAGTATACCTTCAGTGTGGCCAGTCCCAATGAATCCAG

CTGCCCAGAGCAGAGCCAGGCCATGTCAGTCATCCATAACTTACAGAGAGACAGCAGCACCCAA
CGCTTAGACCTGGAGGCCACCAAAGCTCGACTCAGCTCCCTGGAGAGCCTCCTCCACCAATTGA
CCTTGGACCAGGCTGCCAGGCCCCAGGAGACCCAGGAGGGGCTGCAGAGGGAGCTGGGCACCCT
GAGGCGGGAGCGGGACCAGCTGGAAACCCAAACCAGAGAGTTGGAGACTGCCTACAGCAACCTC
CTCCGAGACAAGTCAGTTCTGGAGGAAGAGAAGAAGCGACTAAGGCAAGAAAATGAGAATCTGG
CCAGGAGGTTGGAAAGCAGCAGCCAGGAGGTAGCAAGGCTGAGAAGGGGCCAGTGTCCCCAGAC
CCGAGACACTGCTCGGGCTGTGCCACCAGGCTCCAGAGAAGTTTCTACGTGGAATTTGGACACT
TTGGCCTTCCAGGAACTGAAGTCCGAGCTAACTGAAGTTCCTGCTTCCCGAATTTTGAAGGAGA
GCCCATCTGGCTATCTCAGGAGTGGAGAGGGAGACACCGGATGTGGAGAACTAGTTTGGGTAGG
AGAGCCTCTCACGCTGAGAACAGCAGAAACAATTACTGGCAAGTATGGTGTGTGGATGCGAGAC
CCCAAGCCCACCTACCCCTACACCCAGGAGACCACGTGGAGAATCGACACAGTTGGCACGGATG
TCCGCCAGGTTTTTGAGTATGACCTCATCAGCCAGTTTATGCAGGGCTACCCTTCTAAGGTTCA
CATACTGCCTAGGCCACTGGAAAGCACGGGTGCTGTGGTGTACTCGGGGAGCCTCTATTTCCAG
GGCGCTGAGTCCAGAACTGTCATAAGATATGAGCTGAATACCGAGACAGTGAAGGCTGAGAAGG
AAATCCCTGGAGCTGGCTACCACGGACAGTTCCCGTATTCTTGGGGTGGCTACACGGACATTGA
CTTGGCTGTGGATGAAGCAGGCCTCTGGGTCATTTACAGCACCGATGAGGCCAAAGGTGCCATT
GTCCTCTCCAAACTGAACCCAGAGAATCTGGAACTCGAACAAACCTGGGAGACAAACATCCGTA
AGCAGTCAGTCGCCAATGCCTTCATCATCTGTGGCACCTTGTACACCGTCAGCAGCTACACCTC
AGCAGATGCTACCGTCAACTTTGCTTATGACACAGGCACAGGTATCAGCAAGACCCTGACCATC
CCATTCAAGAACCGCTATAAGTACAGCAGCATGATTGACTACAACCCCCTGGAGAAGAAGCTCT
TTGCCTGGGACAACTTGAACATGGTCACTTATGACATCAAGCTCTCCAAGATGTGAAAAGCCTC
CAAGCTGTACAGGCAATGGCAGAAGGAGATGCTCAGGGCTCCTGGGGGGAGCAGGCTGAAGGGA
GAGCCAGCCAGCCAGGGCCCAGGCAGCTTTGACTGCTTTCCAAGTTTTCATTAATCCAGAAGGA
TGAACATGGTCACCATCTAACTATTCAGGAATTGTAGTCTGAGGGCGTAGACAATTTCATATAA
TAAATATCCTTTATCTTCTGTCAGCATTTATGGGATGTTTAATGACATAGTTCAAGTTTTCTTG
TGATTTGGGGCAAAAGCTGTAAGGCATAATAGTTTCTTCCTGAAAACCATTGCTCTTGCATGTT
ACATGGTTACCACAAGCCACAATAAAAAGCATAACTTCTAAAGGAAGCAGAATAGCTCCTCTGG
CCAGCATCGAATATAAGTAAGATGCATTTACTACAGTTGGCTTCTAATGCTTCAGATAGAATAC
AGTTGGGTCTCACATAACCCTTTACATTGTGAAATAAAATTTTCTTACCCAA (SEQ ID NO: 1)

The reverse complement of SEQ ID NO: 1 is provided as SEQ ID NO: 2 herein:

TTGGGTAAGAAAATTTTATTTCACAATGTAAAGGGTTATGTGAGACCCAACTGTATTCTATCTG
AAGCATTAGAAGCCAACTGTAGTAAATGCATCTTACTTATATTCGATGCTGGCCAGAGGAGCTA
TTCTGCTTCCTTTAGAAGTTATGCTTTTTATTGTGGCTTGTGGTAACCATGTAACATGCAAGAG
CAATGGTTTTCAGGAAGAAACTATTATGCCTTACAGCTTTTGCCCCAAATCACAAGAAAACTTG
AACTATGTCATTAAACATCCCATAAATGCTGACAGAAGATAAAGGATATTTATTATATGAAATT
GTCTACGCCCTCAGACTACAATTCCTGAATAGTTAGATGGTGACCATGTTCATCCTTCTGGATT
AATGAAAACTTGGAAAGCAGTCAAAGCTGCCTGGGCCCTGGCTGGCTGGCTCTCCCTTCAGCCT
GCTCCCCCCCAGGAGCCCTGAGCATCTCCTTCTGCCATTGCCTGTACAGCTTGGAGGCTTTTCAC
ATCTTGGAGAGCTTGATGTCATAAGTGACCATGTTCAAGTTGTCCCAGGCAAAGAGCTTCTTCT
CCAGGGGGTTGTAGTCAATCATGCTGCTGTACTTATAGCGGTTCTTGAATGGGATGGTCAGGGT
CTTGCTGATACCTGTGCCTGTGTCATAAGCAAAGTTGACGGTAGCATCTGCTGAGGTGTAGCTG
CTGACGGTGTACAAGGTGCCACAGATGATGAAGGCATTGGCGACTGACTGCTTACGGATGTTTG
TCTCCCAGGTTTGTTCGAGTTCCAGATTCTCTGGGTTCAGTTTGGAGAGGACAATGGCACCTTT
GGCCTCATCGGTGCTGTAAATGACCCAGAGGCCTGCTTCATCCACAGCCAAGTCAATGTCCGTG
TAGCCACCCCAAGAATACGGGAACTGTCCGTGGTAGCCAGCTCCAGGGATTTCCTTCTCAGCCT

TCACTGTCTCGGTATTCAGCTCATATCTTATGACAGTTCTGGACTCAGCGCCCTGGAAATAGAG
GCTCCCCGAGTACACCACAGCACCCGTGCTTTCCAGTGGCCTAGGCAGTATGTGAACCTTAGAA
GGGTAGCCCTGCATAAACTGGCTGATGAGGTCATACTCAAAAACCTGGCGGACATCCGTGCCAA
CTGTGTCGATTCTCCACGTGGTCTCCTGGGTGTAGGGGTAGGTGGGCTTGGGGTCTCGCATCCA
CACACCATACTTGCCAGTAATTGTTTCTGCTGTTCTCAGCGTGAGAGGCTCTCCTACCCAAACT
AGTTCTCCACATCCGGTGTCTCCCTCTCCACTCCTGAGATAGCCAGATGGGCTCTCCTTCAAAA
TTCGGGAAGCAGGAACTTCAGTTAGCTCGGACTTCAGTTCCTGGAAGGCCAAAGTGTCCAAATT
CCACGTAGAAACTTCTCTGGAGCCTGGTGGCACAGCCCGAGCAGTGTCTCGGGTCTGGGGACAC
TGGCCCCTTCTCAGCCTTGCTACCTCCTGGCTGCTGCTTTCCAACCTCCTGGCCAGATTCTCAT
TTTCTTGCCTTAGTCGCTTCTTCTCTTCCTCCAGAACTGACTTGTCTCGGAGGAGGTTGCTGTA
GGCAGTCTCCAACTCTCTGGTTTGGGTTTCCAGCTGGTCCCGCTCCCGCCTCAGGGTGCCCAGC
TCCCTCTGCAGCCCCTCCTGGGTCTCCTGGGGCCTGGCAGCCTGGTCCAAGGTCAATTGGTGGA
GGAGGCTCTCCAGGGAGCTGAGTCGAGCTTTGGTGGCCTCCAGGTCTAAGCGTTGGGTGCTGCT
GTCTCTCTGTAAGTTATGGATGACTGACATGGCCTGGCTCTGCTCTGGGCAGCTGGATTCATTG
GGACTGGCCACACTGAAGGTATACTGGCATCGGCCACTCTGGTCATTGGCCTTCCTGAGCTGAG
CTGTCCTGGCCCCCACATCCCACACCAGGCAGGCCAGAAGCAGCAGCTGGACAGCTGGCATCTC
AGGCCCAAAGCTGCAGCAACGTGCACAGAAGAACCTCATTGCAGAGGCTTGGTGAGGCTTCCTC
TGGAAAGCTCTGCTGTGCTGAGAGGTGCCTGGATGGGTGGCCTTGCTGGCTC (SEQ ID NO: 2)

In some embodiments, an iRNA described herein includes at least 15 contiguous nucleotides from one of the sequences provided in Tables 2A and 2B, and may optionally be coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in MYOC.

While a target sequence is generally 15-30 nucleotides in length, there is wide variation in the suitability of particular sequences in this range for directing cleavage of any given target RNA. Various software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given gene target, but an empirical approach can also be taken in which a "window" or "mask" of a given size (as a non-limiting example, 21 nucleotides) is literally or figuratively (including, *e.g.*, in silico) placed on the target RNA sequence to identify sequences in the size range that may serve as target sequences. By moving the sequence "window" progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified sequences (using assays described herein or known in the art) to identify those sequences that perform optimally can identify those RNA sequences that, when targeted with an iRNA agent, mediate the best inhibition of target gene expression. Thus, it is contemplated that further optimization of inhibition efficiency can be

achieved by progressively "walking the window" one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

Further, it is contemplated that for any sequence identified, *e.g.*, in Tables 2A and 2B, further optimization can be achieved by systematically either adding or removing nucleotides to generate longer or shorter sequences and testing those and sequences generated by walking a window of the longer or shorter size up or down the target RNA from that point. Again, coupling this approach to generating new candidate targets with testing for effectiveness of iRNAs based on those target sequences in an inhibition assay as known in the art or as described herein can lead to further improvements in the efficiency of inhibition. Further still, such optimized sequences can be adjusted by, *e.g.*, the introduction of modified nucleotides as described herein or as known in the art, addition or changes in overhang, or other modifications as known in the art and/or discussed herein to further optimize the molecule (*e.g.*, increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with silencing pathway enzymes, increasing release from endosomes, *etc.*) as an expression inhibitor.

In some embodiments, the disclosure provides an iRNA of Table 2B that is un-modified or un-conjugated. In some embodiments, an RNAi agent of the disclosure has a nucleotide sequence as provided in Table 2A, but lacks one or more ligand or moiety shown in the table. A ligand or moiety (*e.g.*, a lipophilic ligand or moiety) can be included in any of the positions provided in the instant application.

An iRNA as described herein can contain one or more mismatches to the target sequence. In some embodiments, an iRNA as described herein contains no more than 3 mismatches. In some embodiments, when the antisense strand of the iRNA contains mismatches to a target sequence, the area of mismatch is not located in the center of the region of complementarity. In some embodiments, when the antisense strand of the iRNA contains mismatches to the target sequence, the mismatch is restricted to be within the last 5 nucleotides from either the 5' or 3' end of the region of complementarity. For example, for a 23 nucleotide iRNA agent RNA strand which is complementary to a region of MYOC, the RNA strand 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 iRNA containing a mismatch to a target sequence is effective in inhibiting the expression of MYOC. Consideration of the efficacy of iRNAs with

mismatches in inhibiting expression of MYOC is important, especially if the particular region of complementarity in a MYOC gene is known to have polymorphic sequence variation within the population.

In some embodiments, at least one end of a dsRNA has a single-stranded nucleotide overhang of 1 to 4, generally 1 or 2 nucleotides. In some embodiments, dsRNAs having at least one nucleotide overhang have superior inhibitory properties relative to their blunt-ended counterparts. In some embodiments, the RNA of an iRNA (*e.g.*, a dsRNA) is chemically modified to enhance stability or other beneficial characteristics. The nucleic acids featured in the disclosure may be synthesized and/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, (a) end modifications, *e.g.*, 5' end modifications (phosphorylation, conjugation, inverted linkages, *etc.*) 3' end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*), (b) 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, (c) sugar modifications (*e.g.*, at the 2' position or 4' position, or having an acyclic sugar) or replacement of the sugar, as well as (d) backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of RNA compounds useful in this disclosure 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 particular embodiments, the modified RNA will have a phosphorus atom in its internucleoside backbone.

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, mixed salts and free acid forms are also included.

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 US Pat RE39464, each of which is herein incorporated by reference.

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 CH_2 component parts.

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, each of which is herein incorporated by reference.

In other RNA mimetics suitable or contemplated for use in iRNAs, 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, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found, for example, in Nielsen *et al.*, Science, 1991, 254, 1497-1500.

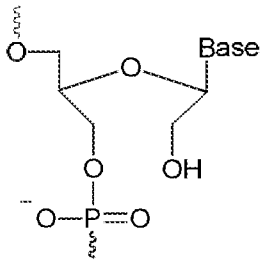
Some embodiments featured in the disclosure include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2--NH--CH_2--, --CH_2--N(CH_3)--O--CH_2--[known as a methylene (methylimino) or MMI backbone], --CH_2--O--N(CH_3)--CH_2--, --CH_2--N(CH_3)--N(CH_3)--CH_2-- and --N(CH_3)--CH_2--CH_2--[wherein the native phosphodiester backbone is represented as --O--P--O--CH_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.

Modified RNAs may also contain one or more substituted sugar moieties. The iRNAs, *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 may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an iRNA, or a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, 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'-dimethylaminooxyethoxy, *i.e.*, a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH_2--O--CH_2--$N(CH_2)_2$.

In other embodiments, an iRNA agent comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides (or nucleosides). In certain embodiments, the sense strand or the antisense strand, or both sense strand and antisense strand, include less than five acyclic nucleotides per strand (*e.g.*, four, three, two or one acyclic nucleotides per strand). The one or more acyclic nucleotides can be found, for example, in the double-stranded region, of the sense or antisense strand, or both strands; at the 5'-end, the 3'-end, both of the 5' and 3'-ends of the sense or antisense strand, or both strands, of the iRNA agent. In some embodiments, one or more acyclic nucleotides are present at positions 1 to 8 of the sense or antisense strand, or both. In some embodiments, one or more acyclic nucleotides are found in the antisense strand at positions 4 to 10 (*e.g.*, positions 6-8) from the 5'-end of the antisense strand. In some embodiments, the one or more acyclic nucleotides are found at one or both 3'-terminal overhangs of the iRNA agent.

The term "acyclic nucleotide" or "acyclic nucleoside" as used herein refers to any nucleotide or nucleoside having an acyclic sugar, *e.g.*, an acyclic ribose. An exemplary acyclic nucleotide or nucleoside can include a nucleobase, *e.g.*, a naturally occurring or a modified nucleobase (*e.g.*, a nucleobase as described herein). In certain embodiments, a bond between any of the ribose carbons (C1, C2, C3, C4, or C5), is independently or in combination absent from the nucleotide. In some embodiments, the bond between C2-C3 carbons of the ribose ring is absent, *e.g.*, an acyclic 2'-3'-seco-nucleotide monomer. In other embodiments, the bond between C1-C2, C3-C4, or C4-C5 is absent (*e.g.*, a 1'-2', 3'-4' or 4'-5'-seco nucleotide monomer). Exemplary acyclic nucleotides are disclosed in US 8,314,227, incorporated herein by reference in its entirely. For example, an acyclic nucleotide can include any of monomers D-J in Figures 1-2 of US 8,314,227. In some embodiments, the acyclic nucleotide includes the following monomer:



wherein Base is a nucleobase, *e.g.*, a naturally occurring or a modified nucleobase (*e.g.*, a nucleobase as described herein).

In certain embodiments, the acyclic nucleotide can be modified or derivatized, *e.g.*, by coupling the acyclic nucleotide to another moiety, *e.g.*, a ligand (*e.g.*, a GalNAc, a cholesterol ligand), an alkyl, a polyamine, a sugar, a polypeptide, among others.

In other embodiments, the iRNA agent includes one or more acyclic nucleotides and one or more LNAs (*e.g.*, an LNA as described herein). For example, one or more acyclic nucleotides and/or one or more LNAs can be present in the sense strand, the antisense strand, or both. The number of acyclic nucleotides in one strand can be the same or different from the number of LNAs in the opposing strand. In certain embodiments, the sense strand and/or the antisense strand comprises less than five LNAs (*e.g.*, four, three, two or one LNAs) located in the double stranded region or a 3'-overhang. In other embodiments, one or two LNAs are located in the double stranded region or the 3'-overhang of the sense strand. Alternatively, or in combination, the sense strand and/or antisense strand comprises less than five acyclic nucleotides (*e.g.*, four, three, two or one acyclic nucleotides) in the double-stranded region or a 3'-overhang. In some embodiments, the sense strand of the iRNA agent comprises one or two LNAs in the 3'-overhang of the sense strand, and one or two acyclic nucleotides in the double-stranded region of the antisense strand (*e.g.*, at positions 4 to 10 (*e.g.*, positions 6-8) from the 5'-end of the antisense strand) of the iRNA agent.

In other embodiments, inclusion of one or more acyclic nucleotides (alone or in addition to one or more LNAs) in the iRNA agent results in one or more (or all) of: (i) a reduction in an off-target effect; (ii) a reduction in passenger strand participation in RNAi; (iii) an increase in specificity of the guide strand for its target mRNA; (iv) a reduction in a microRNA off-target effect; (v) an increase in stability; or (vi) an increase in resistance to degradation, of the iRNA molecule.

Other modifications include 2'-methoxy (2'-OCH_3), 2'-5 aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other

positions on the RNA of an iRNA, 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. iRNAs may 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, and each of which is herein incorporated by reference.

An iRNA may 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 anal 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, these disclosed by Englisch *et al., Angewandte Chemie*, International Edition, 1991, 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 0-6 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.

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. No. 3,687,808, as well as U.S. Pat. Nos. 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; 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, each of which is herein incorporated by reference, and U.S. Pat. No. 5,750,692, also herein incorporated by reference.

The RNA of an iRNA can also be modified to include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 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 (LNAs) (also referred to herein as "locked nucleotides"). In some embodiments, a locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting, *e.g.*, 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, increase thermal stability, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *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.*, US Patent No. 8,278,283); 4'-CH2—N(OCH3)-2' (and analogs thereof; see *e.g.*, US Patent 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.*, US Patent No. 8,278,426). The contents of each of the foregoing are incorporated herein by reference for the methods provided therein. Representative U.S. Patents that teach the preparation of locked nucleic acids include, but are not limited to, the following: U.S. Pat. Nos. 6,268,490; 6,670,461; 6,794,499; 6,998,484; 7,053,207; 7,084,125; 7,399,845, and 8,314,227, each of which is herein incorporated by reference in its entirety. Exemplary LNAs include but are not limited to, a 2', 4'-C methylene bicyclo nucleotide (see for example Wengel *et al.*, International PCT 5 Publication No. WO 00/66604 and WO 99/14226).

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).

A 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)-0-2' bridge. In some embodiments, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

A 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.

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 contents of each of which are hereby incorporated herein by reference for the methods provided therein.

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).

Representative U.S. publications that teach the preparation of UNA include, but are not limited to, US8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the contents of each of which are hereby incorporated herein by reference for the methods provided therein.

In other embodiments, the iRNA agents include one or more (*e.g.,* about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.,* 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in the iRNA molecules can result in enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands.

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 PCT Publication No. WO 2011/005861.

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 contents of which are incorporated herein by reference for the methods provided therein. In one

embodiment, the double stranded RNAi agent of the invention further comprises a 5'-phosphate or a 5'-phosphate mimic at the 5' nucleotide of the antisense strand. In another embodiment, the double stranded RNAi agent further comprises a 5'-phosphate mimic at the 5' nucleotide of the antisense strand. In a specific embodiment, the 5'-phosphate mimic is a 5'-vinyl phosphonate (5'-VP). In one embodiment, the phosphate mimic is a 5'-cyclopropyl phosphonate (VP). In some embodiments, the 5'-end of the antisense strand of the double-stranded iRNA agent does not contain a 5'-vinyl phosphonate (VP).

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), e.g., Ggn, Cgn, Tgn, or Agn, a nucleotide with a 2' phosphate, e.g., G2p, C2p, A2p or U2p, and, a vinyl-phosphonate nucleotide; and combinations thereof. In other embodiments, each of the duplexes of Tables 5 and 6 may be particularly modified to provide another double-stranded iRNA agent of the present disclosure. In one example, the 3'-terminus of each sense duplex may be modified by removing the 3'-terminal L96 ligand and exchanging the two phosphodiester internucleotide linkages between the three 3'-terminal nucleotides with phosphorothioate internucleotide linkages. That is, the three 3'-terminal nucleotides (N) of a sense sequence of the formula:

5'- N1-... -Nn-2Nn-1NnL96 3'

may be replaced with

5'- N1-... -Nn-2sNn-1sNn 3'.

An RNA target may have regions, or spans of the target RNA's nucleotide sequence, which are relatively more susceptible or amenable than other regions of the RNA target to mediating cleavage of the RNA target via RNA interference induced by the binding of an RNAi agent to that region. The increased susceptibility to RNA interference within such "hotspot regions" (or simply "hotspots") means that iRNA agents targeting the region will likely have higher efficacy in inducing iRNA interference than iRNA agents which target other regions of the target RNA. For example, without being bound by theory, the accessibility of a target region of a target RNA may influence the efficacy of iRNA agents which target that region, with some hotspot regions having increased accessibility. Secondary structures, for instance, that form in the RNA target (e.g., within or proximate to hotspot regions) may affect the ability of the iRNA agent to bind the target region and induce RNA interference.

According to certain aspects of the invention, an iRNA agent may be designed to target a hotspot region of any of the target RNAs described herein, including any identified portions of a target RNA (e.g., a particular exon). As used herein, a hotspot region may refer to an approximately 19-200, 19-150, 19-100, 19-75, 19-50, 21-200, 21-150, 21-100, 21-75, 21-50, 50-200, 50-150, 50-100, 50-75, 75-200, 75-150, 75-100, 100-200, or 100-150 nucleotide region of a target RNA sequence for which targeting using RNAi agents provides an observably higher probability of efficacious silencing relative to targeting other regions of the same target RNA. According to certain aspects of the invention, a hotspot region may comprise a limited region of the target RNA, and in some cases, a substantially limited region of the target, including for example, less than half of the length of the target RNA, such as about 5%, 10%, 15%, 20%, 25%, or 30% of the lenth of the target RNA. Conversely, the other regions against which a hotspot is compared may cumulatively comprise at least a majority of the length of the target RNA. For example, the other regions may cumulatively comprise at least about 60%, or at least about 70%, or at least about 80%, or at least about 90%, or at least about 95% of the length of the target RNA.

Compared regions of the target RNA may be empirically evaluated for identification of hotspots using efficacy data obtained from in vitro or in vivo screening assays. For example, RNAi agents targeting various regions that span a target RNA may be compared for frequency of efficacious iRNA agents (e.g., the amount by which target gene expression is inhibited, such as measured by mRNA expression or protein expression) that bind each region. In general, a hotspot can be recognized by observing clustering of multiple efficacious RNAi agents that bind to a limited region of the RNA target. A hotspot may be sufficiently characterized as such by observing efficacy of iRNA agents which cumulatively span at least about 60% of the target region identified as a hotspot, such as about 70%, about 80%, about 90%, or about 95% or more of the length of the region, including both ends of the region (i.e. at least about 60%, 70%, 80%, 90%, or 95% or more of the nucleotides within the region, including the nucleotides at each end of the region, were targeted by an iRNA agent). According to some aspects of the invention, an iRNA agent which demonstrates at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% inhibition over the region (e.g., no more than about 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% mRNA remaining) may be identified as efficacious.

Amenibility to targeting of RNA regions may also be assessed using quantitative comparison of inhibition measurements across different regions of a defined size (e.g, 25, 30, 40, 50, 60, 70, 80, 90, or 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 nts). For example, an average level of inhibition may be determined for each region and the averages of each region may be compared. The average level of inhibition within a hotspot region may be substantially higher than the average of averages for all evaluated regions. According to some aspects, the average level of inhibition in a hotspot region may be at least about 10%, 20%, 30%, 40%, or 50% higher than the average of averages. According to some aspects, the average level of inhibition in a hotspot region may be at least about 1.0, 1.1, 1.2, 1.3, 1.4, 1.5 1.6, 1.7, 1.8. 1.9, or 2.0 standard deviations above the average of averages. The average level of inhibition may be higher by a statistically significant (e.g., $p < 0.05$) amount. According to some aspects, each inhibition measurement within a hotspot region may be above a threshold amount (e.g., at or below a threshold amount of mRNA remaining). According to some aspects, each inhibition measurement within the region may be substantially higher than an average of all inhibition measurements across all the measured regions. For example, each inhibition measurement in a hotspot region may be at least about 10%, 20%, 30%, 40%, or 50% higher than the average of all inhibition measurements. According to some aspects, each inhibition measurement may be at least about 1.0, 1.1, 1.2, 1.3, 1.4, 1.5 1.6, 1.7, 1.8. 1.9, or 2.0 standard deviations above the average of all inhibition measurements. Each inhibition measurement may be higher by a statistically significant (e.g., $p < 0.05$) amount than the average of all inhibition measurements. A standard for evaluating a hotspot may comprise various combinations of the above standards where compatible (e.g., an average level of inhibition of at least about a first amount and having no inhibition measurements below a threshold level of a second amount, lesser than the first amount).

It is therefore expressly contemplated that any iRNA agent, including the specific exemplary iRNA agents described herein, which targets a hotspot region of a target RNA, may be preferably selected for inducing RNA interference of the target mRNA as targeting such a hotspot region is likely to exhibit a robust inhibitory response relative to targeting a region which is not a hotspot region. RNAi agents targeting target sequences that substantially overlap (e.g., by at least about 70%, 75%, 80%, 85%, 90%, 95% of the target sequence length) or, preferably, that reside fully within the hotspot region may be considered to target the hotspot region.

Hotspot regions of the RNA target(s) of the instant invention may include any region for which the data disclosed herein demonstrates higher frequency of targeting by efficacious RNAi agents, including by any of the standards described elsewhere herein, whether or not the range(s) of such hotspot region(s) are explicitly specified.

In various embodiments, a dsRNA agent of the present invention targets a hotspot region of an mRNA encoding MYOC.

III. iRNA Motifs

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 contents of which are incorporated herein by reference for the methods provided therein. 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 moiety or ligand, *e.g.*, a C16 moiety or 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.

In some embodiments, the sense strand sequence may be represented by formula (I):

$5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\ \text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$ (I)

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

each N_a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p and n_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. In some embodiments, YYY is all 2'-F modified nucleotides.

In some embodiments, the N_a and/or N_b comprise modifications of alternating pattern.

In some embodiments, 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 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'-end.

In some embodiments, 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_p-N_a-YYY-N_b-ZZZ-N_a-n_q 3' (Ib);

5' n_p-N_a-XXX-N_b-YYY-N_a-n_q 3' (Ic); or

5' n_p-N_a-XXX-N_b-YYY-N_b-ZZZ-N_a-n_q 3' (Id).

When the sense strand is represented by formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

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

When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. In some embodiments, N_b is 0, 1, 2, 3, 4, 5 or 6. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

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

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

5' n_p-N_a-YYY- N_a-n_q 3' (Ia).

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

In some embodiments, the antisense strand sequence of the RNAi may be represented by formula (Ie):

5' n_q'-N_a'-(Z'Z'Z')$_k$-N_b'-Y'Y'Y'-N_b'-(X'X'X')$_l$-N'$_a$-n_p' 3' (Ie)

wherein:

k and l are each independently 0 or 1;

p' and q' are each independently 0-6;

each N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p' and n_q' independently represent an overhang nucleotide;

wherein N_b' and Y' do not have the same modification;

and

X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one of three identical modification on three consecutive nucleotides.

In some embodiments, the N_a' and/or N_b' comprise modification of alternating pattern.

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 nucleotides 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 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end. In some embodiments, the Y'Y'Y' motif occurs at positions 11, 12, 13.

In some embodiments, Y'Y'Y' motif is all 2'-Ome modified nucleotides.

In on embodiment, k is 1 and l is 0, or k is 0 and l is 1, or both 5 k and l are 1.

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

5' n_q'-N_a'-Z'Z'Z'-N_b'-Y'Y'Y'-N_a'n_p' 3' (Ig);

5' n_q'-N_a'-Y'Y'Y'-N_b'-X'X'X'-n_p' 3' (Ih); or

5' n_q'-N_a'- Z'Z'Z'-N_b'-Y'Y'Y'-N_b'- X'X'X'-N_a'-n_p' 3' (Ii).

When the antisense strand is represented by formula (Ig), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (Ii), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. In some embodiments, N_b is 0, 1, 2, 3, 4, 5 or 6.

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

5' n_p'-N_a'-Y'Y'Y'- N_a'-n_q' 3' (If).

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

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

Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, HNA, CeNA, GNA, 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.

In some embodiments, 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 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st 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.

In some embodiments the antisense strand may Y'Y'Y' motif occurring at positions 11, 12, 13 of the strand, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st 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.

The sense strand represented by any one of the above formulas (Ia), (Ib), (Ic), and (Id) forms a duplex with an antisense strand being represented by any one of formulas (If), (Ig), (Ih), and (Ii), respectively.

Accordingly, certain 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 (Ij):

sense: 5' n_p -N_a-(XXX)i -N_b- YYY -N_b -(ZZZ)j-N_a-n_q 3'

antisense: 3' n_p'-Na'-(X'X'X')k-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')l-N_a'-n_q' 5'

(Ij)

wherein,

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

wherein

each n_p', n_p, n_q', and n_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 modification on three consecutive nucleotides.

In some embodiments, 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 some embodiments, 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.

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

5' n_p -N_a-Y Y Y-N_a-n_q 3'

3' n_p' -N_a'- Y'Y'Y'-N_a'n_q' 5'

(Ik)

5' n_p -N_a -Y Y Y -N_b -Z Z Z -N_a-n_q 3'

3' n_p -N_a'- Y'Y'Y'-N_b'- Z'Z'Z'- Na'-nq' 5'

(Il)

5' n_p -N_a - X X X -N_b- Y Y Y -N_a-n_q 3'

3' n_p -N_a'- X'X'X' -N_b'- Y'Y'Y'- Na'-n_q' 5'

(Im)

5' n_p -N_a - X X X -N_b -Y Y Y - N_b- Z Z Z-N_a-n_q 3'

3' n_p -N_a'- X'X'X'-N_b'- Y'Y'Y'-N_b'- Z'Z'Z'-Na'-n_q' 5'

(In)

When the RNAi agent is represented by formula (Ik), each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented by formula (Il), each N_b independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5 or 1-4 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented as formula (Im), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

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

Each of X, Y and Z in formulas (Ij), (Ik), (Il), (Im), and (In) may be the same or different from each other.

When the RNAi agent is represented by formula (Ij), (Ik), (Il), (Im), and (In), at least one of the Y nucleotides may form a base pair with one of the Y' nucleotides. Alternatively, at least two of the Y nucleotides form base pairs with the corresponding Y' nucleotides; or all three of the Y nucleotides all form base pairs with the corresponding Y' nucleotides.

When the RNAi agent is represented by formula (II) or (In), at least one of the Z nucleotides may form a base pair with one of the Z' nucleotides. Alternatively, at least two of the Z nucleotides form base pairs with the corresponding Z' nucleotides; or all three of the Z nucleotides all form base pairs with the corresponding Z' nucleotides.

When the RNAi agent is represented as formula (Im) or (In), at least one of the X nucleotides may form a base pair with one of the X' nucleotides. Alternatively, at least two of the X nucleotides form base pairs with the corresponding X' nucleotides; or all three of the X nucleotides all form base pairs with the corresponding X' nucleotides.

In some embodiments, the modification on the Y nucleotide is different than the modification on the Y' nucleotide, the modification on the Z nucleotide is different than the modification on the Z' nucleotide, and/or the modification on the X nucleotide is different than the modification on the X' nucleotide.

In some embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications. In some embodiments, when the RNAi agent is represented by formula (In), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications and $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide a via phosphorothioate linkage. In some embodiments, when the RNAi agent is represented by formula (In), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications, $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, and the sense strand is conjugated to one or more moieties or ligands (*e.g.*, one or more lipophilic moieties, optionally one or more C16 moieties, or one or more GalNAc moieties) attached through a bivalent or trivalent branched linker. In some embodiments, when the RNAi agent is represented by formula (In), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications, $n_p' > 0$ and at least one n_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 moieties or ligands (*e.g.*, one or more lipophilic moieties, optionally one or more C16 moieties, or one or more GalNAc moieties) attached through a bivalent or trivalent branched linker.

In some embodiments, when the RNAi agent is represented by formula (IIIa), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications, $n_p' > 0$ and at least one n_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 moieties or ligands

(*e.g.*, one or more lipophilic moieties, optionally one or more C16 moieties, or one or more GalNAc moieties) attached through a bivalent or trivalent branched linker.

In some embodiments, the RNAi agent is a multimer containing at least two duplexes represented by formula (Ij), (Ik), (Il), (Im), and (In), 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.

In some embodiments, the RNAi agent is a multimer containing three, four, five, six or more duplexes represented by formula (Ij), (Ik), (Il), (Im), and (In), 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.

In some embodiments, two RNAi agents represented by formula (Ij), (Ik), (Il), (Im), and (In) 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.

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 US 7858769, the contents of each of which are hereby incorporated herein by reference for the methods provided therein. In certain embodiments, the RNAi agents of the disclosure may include GalNAc ligands.

As described in more detail below, the RNAi agent that contains conjugations of one or more carbohydrate moieties to a 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.

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.

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 and decalin; preferably, the acyclic group is selected from serinol backbone or diethanolamine backbone.

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 2A and 2B. These agents may further comprise a ligand. The ligand can be attached to the sense strand, antisense strand or both strands, at the 3'-end, 5'-end, or both ends. For instance, the ligand may be conjugated to the sense strand, in particular, the 3'-end of the sense strand.

IV. iRNA Conjugates

The iRNA agents disclosed herein can be in the form of conjugates. The conjugate may be attached at any suitable location in the iRNA molecule, *e.g.*, at the 3' end or the 5' end of the sense or the antisense strand. The conjugates are optionally attached via a linker.

In some embodiments, an iRNA agent described herein is chemically linked to one or more ligands, moieties or conjugates, which may confer functionality, *e.g.*, by affecting (*e.g.*, enhancing) the activity, cellular distribution or cellular uptake of the iRNA. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, *Proc. Natl. Acid. Sci. USA*, 1989, 86: 6553-6556), cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 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. Let.*, 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 triethyl-ammonium 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).

In some 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.

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-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Examples 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 α helical peptide.

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-gulucosamine 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.

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, $[MPEG]_2$, 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.

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 an ocular 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-κB.

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, and/or intermediate filaments. The drug can be, for example, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

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 disclosure 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.

Ligand-conjugated oligonucleotides of the disclosure 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.

The oligonucleotides used in the conjugates of the present disclosure 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.

In the ligand-conjugated oligonucleotides and ligand-molecule bearing sequence-specific linked nucleosides of the present disclosure, 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.

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 disclosure 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.

A. *Lipophilic Moieties*

In certain embodiments, the lipophilic moiety is an aliphatic, cyclic such as alicyclic, or polycyclic such as polyalicyclic compound, such as a steroid (*e.g.*, sterol) or a linear or branched aliphatic hydrocarbon. The lipophilic moiety may generally comprise a hydrocarbon chain, which may be cyclic or acyclic. The hydrocarbon chain may comprise various substituents or one or more heteroatoms, such as an oxygen or nitrogen atom. Such lipophilic aliphatic moieties include, without limitation, saturated or unsaturated C_4-C_{30} hydrocarbon (*e.g.*, C_6-C_{18} hydrocarbon), saturated or unsaturated fatty acids, waxes (*e.g.*, monohydric alcohol esters of fatty acids and fatty diamides), terpenes (*e.g.*, C_{10} terpenes, C_{15} sesquiterpenes, C_{20} diterpenes, C_{30} triterpenes, and C_{40} tetraterpenes), and other polyalicyclic hydrocarbons. For instance, the lipophilic moiety may contain a C_4-C_{30} hydrocarbon chain (*e.g.*, C_4-C_{30} alkyl or alkenyl). In some embodiments the lipophilic moiety contains a saturated or unsaturated C_6-C_{18} hydrocarbon

chain (*e.g.*, a linear C_6-C_{18} alkyl or alkenyl). In some embodiments, the lipophilic moiety contains a saturated or unsaturated C16 hydrocarbon chain (*e.g.*, a linear C16 alkyl or alkenyl).

The lipophilic moiety may be attached to the RNAi agent by any method known in the art, including via a functional grouping already present in the lipophilic moiety or introduced into the RNAi agent, such as a hydroxy group (*e.g.*, —CO—CH_2—OH). The functional groups already present in the lipophilic moiety or introduced into the RNAi agent include, but are not limited to, hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

Conjugation of the RNAi agent and the lipophilic moiety may occur, for example, through formation of an ether or a carboxylic or carbamoyl ester linkage between the hydroxy and an alkyl group R—, an alkanoyl group RCO— or a substituted carbamoyl group RNHCO—. The alkyl group R may be cyclic (*e.g.*, cyclohexyl) or acyclic (*e.g.*, straight-chained or branched; and saturated or unsaturated). Alkyl group R may be a butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl or octadecyl group, or the like.

In some embodiments, the lipophilic moiety is conjugated to the double-stranded RNAi agent via a linker a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction (*e.g.*, a triazole from the azide-alkyne cycloaddition), or carbamate.

In another embodiment, the lipophilic moiety is a steroid, such as sterol. Steroids are polycyclic compounds containing a perhydro-1,2-cyclopentanophenanthrene ring system. Steroids include, without limitation, bile acids (*e.g.*, cholic acid, deoxycholic acid and dehydrocholic acid), cortisone, digoxigenin, testosterone, cholesterol, and cationic steroids, such as cortisone. A "cholesterol derivative" refers to a compound derived from cholesterol, for example by substitution, addition or removal of substituents.

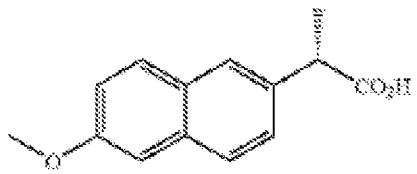
In another embodiment, the lipophilic moiety is an aromatic moiety. In this context, the term "aromatic" refers broadly to mono- and polyaromatic hydrocarbons. Aromatic groups include, without limitation, C_6-C_{14} aryl moieties comprising one to three aromatic rings, which may be optionally substituted; "aralkyl" or "arylalkyl" groups comprising an aryl group covalently linked to an alkyl group, either of which may independently be optionally substituted or unsubstituted; and "heteroaryl" groups. As used herein, the term "heteroaryl" refers to groups having 5 to 14 ring atoms, preferably 5, 6, 9, or 10 ring atoms; having 6, 10, or 14π electrons

shared in a cyclic array, and having, in addition to carbon atoms, one to about three heteroatoms selected from the group consisting of nitrogen (N), oxygen (O), and sulfur (S).

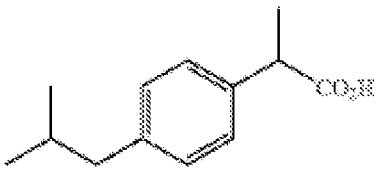
As employed herein, a "substituted" alkyl, cycloalkyl, aryl, heteroaryl, or heterocyclic group is one having one to about four, preferably one to about three, more preferably one or two, non-hydrogen substituents. Suitable substituents include, without limitation, halo, hydroxy, nitro, haloalkyl, alkyl, alkaryl, aryl, aralkyl, alkoxy, aryloxy, amino, acylamino, alkylcarbamoyl, arylcarbamoyl, aminoalkyl, alkoxycarbonyl, carboxy, hydroxyalkyl, alkanesulfonyl, arenesulfonyl, alkanesulfonamido, arenesulfonamido, aralkylsulfonamido, alkylcarbonyl, acyloxy, cyano, and ureido groups.

In some embodiments, the lipophilic moiety is an aralkyl group, *e.g.*, a 2-arylpropanoyl moiety. The structural features of the aralkyl group are selected so that the lipophilic moiety will bind to at least one protein in vivo. In certain embodiments, the structural features of the aralkyl group are selected so that the lipophilic moiety binds to serum, vascular, or cellular proteins. In certain embodiments, the structural features of the aralkyl group promote binding to albumin, an immunoglobulin, a lipoprotein, α-2-macroglubulin, or α-1-glycoprotein.

In certain embodiments, the ligand is naproxen or a structural derivative of naproxen. Procedures for the synthesis of naproxen can be found in U.S. Pat. No. 3,904,682 and U.S. Pat. No. 4,009,197, which are hereby incorporated by reference in their entirety. Naproxen has the chemical name (S)-6-Methoxy-α-methyl-2-naphthaleneacetic acid and the structure is



In certain embodiments, the ligand is ibuprofen or a structural derivative of ibuprofen. Procedures for the synthesis of ibuprofen can be found in US3,228,831, which is incorporated herein by reference for the methods provided therein. The structure of ibuprofen is



Additional exemplary aralkyl groups are illustrated in US 7,626,014, which is incorporated herein by reference for the methods provided therein.

In another embodiment, suitable lipophilic moieties include lipid, cholesterol, retinoic acid, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-bis-O(hexadecyl)glycerol, geranyloxyhexyanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, ibuprofen, naproxen, dimethoxytrityl, or phenoxazine.

In certain embodiments, more than one lipophilic moiety can be incorporated into the double-strand RNAi agent, particularly when the lipophilic moiety has a low lipophilicity or hydrophobicity. In some embodiments, two or more lipophilic moieties are incorporated into the same strand of the double-strand RNAi agent. In some embodiments, each strand of the double-strand RNAi agent has one or more lipophilic moieties incorporated. In some embodiments, two or more lipophilic moieties are incorporated into the same position (i.e., the same nucleobase, same sugar moiety, or same internucleosidic linkage) of the double-strand RNAi agent. This can be achieved by, *e.g.,* conjugating the two or more lipophilic moieties via a carrier, or conjugating the two or more lipophilic moieties via a branched linker, or conjugating the two or more lipophilic moieties via one or more linkers, with one or more linkers linking the lipophilic moieties consecutively.

The lipophilic moiety may be conjugated to the RNAi agent via a direct attachment to the ribosugar of the RNAi agent. Alternatively, the lipophilic moiety may be conjugated to the double-strand RNAi agent via a linker or a carrier.

In certain embodiments, the lipophilic moiety may be conjugated to the RNAi agent via one or more linkers (tethers).

In some embodiments, the lipophilic moiety is conjugated to the double-stranded RNAi agent via a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction (*e.g.,* a triazole from the azide-alkyne cycloaddition), or carbamate.

B. Lipid Conjugates

In some embodiments, the ligand 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 vascular distribution of the conjugate to a target tissue. For

example, the target tissue can be the eye. Other molecules that can bind HSA can also be used as ligands. For example, neproxin 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, and/or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

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.

In some 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.

In some 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.

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).

C. Cell Permeation Agents

In another aspect, the ligand is a cell-permeation agent, such as a helical cell-permeation agent. In some embodiments, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. 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.

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.

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: 3). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 4)) 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: 5)) and the *Drosophila Antennapedia* protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 6)) 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.

An RGD peptide for use in the compositions and methods of the disclosure 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. In some embodiments, conjugates of this ligand target PECAM-1 or VEGF.

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 a iRNA agent to a tumor cell expressing $\alpha_V\beta_3$ (Haubner *et al.*, *Jour. Nucl. Med.*, 42:326-336, 2001).

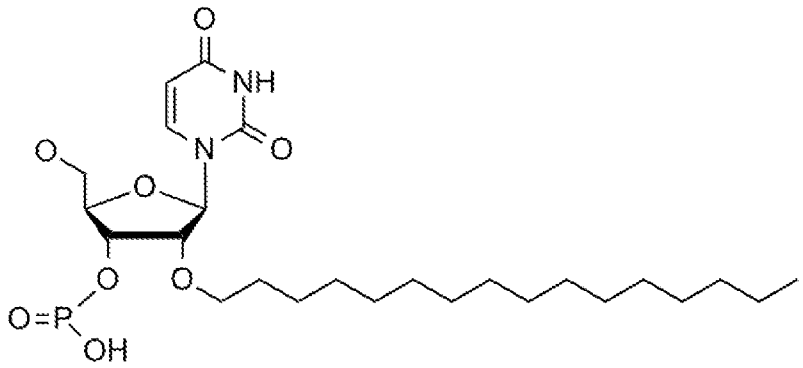
A "cell permeation peptide" is capable of permeating a cell, *e.g.*, a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α-helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing 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).

D. Carbohydrate Conjugates and Ligands

In some embodiments of the compositions and methods of the disclosure, an iRNA oligonucleotide 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 trisaccharides include sugars having two or three monosaccharide units (*e.g.*, C5, C6, C7, or C8).

In certain embodiments, the compositions and methods of the disclosure include a C16 ligand. In exemplary embodiments, the C16 ligand of the disclosure has the following structure (exemplified here below for a uracil base, yet attachment of the C16 ligand is contemplated for a nucleotide presenting any base (C, G, A, etc.) or possessing any other modification as presented herein, provided that 2' ribo attachment is preserved) and is attached at the 2' position of the ribo within a residue that is so modified:



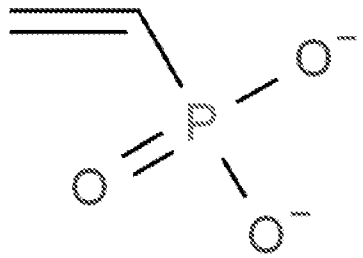
Chemical Formula: $C_{25}H_{43}N_2O_8P$
Exact Mass: 530.2757
Molecular Weight: 530.5913

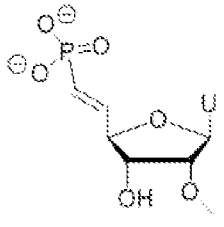
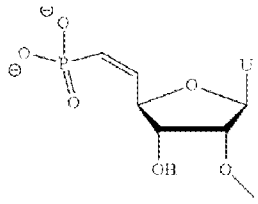
As shown above, a C16 ligand-modified residue presents a straight chain alkyl at the 2'-ribo position of an exemplary residue (here, a Uracil) that is so modified.

In some embodiments, a carbohydrate conjugate of a RNAi agent of the instant disclosure further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator or a cell permeation peptide.

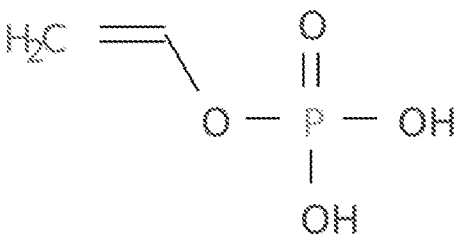
Additional carbohydrate conjugates (and linkers) suitable for use in the present disclosure include those described in WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.

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:



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. The dsRNA agent can comprise a phosphorus-containing group at the 5'-end of the sense strand or antisense strand. The 5'-end phosphorus-containing group can be 5'-end phosphate (5'-P), 5'-end phosphorothioate (5'-PS), 5'-end phosphorodithioate (5'-PS2), 5'-end vinylphosphonate (5'-VP), 5'-end methylphosphonate (MePhos), or 5'-deoxy-5'-C-malonyl. When the 5'-end phosphorus-containing group is 5'-end vinylphosphonate (5'-VP), the 5'-VP can be either 5'-E-VP isomer (i.e., trans-vinylphosphonate, ), 5'-Z-VP isomer (i.e., cis-vinylphosphonate, ), or mixtures thereof.

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

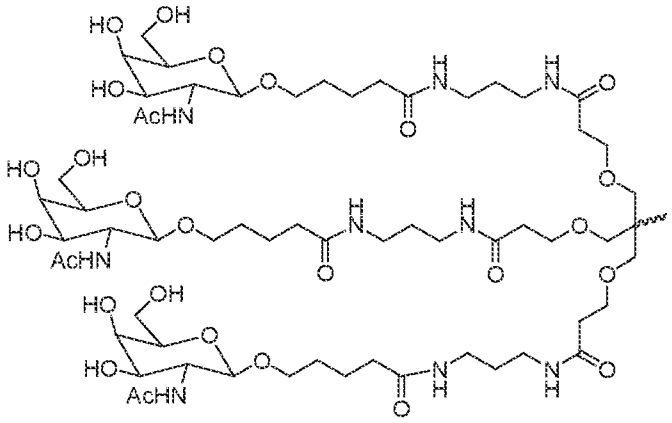


In some embodiments, a carbohydrate conjugate comprises a monosaccharide. In some embodiments, the monosaccharide is an N-acetylgalactosamine (GalNAc). GalNAc conjugates, which comprise one or more N-acetylgalactosamine (GalNAc) derivatives, are described, for

example, in U.S. Patent 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).

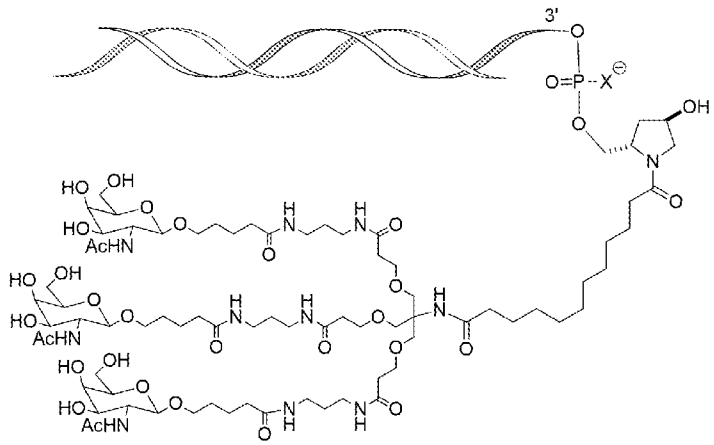
In some embodiments, the carbohydrate conjugate comprises one or more GalNAc derivatives. The GalNAc derivatives may be attached via a linker, *e.g.*, a bivalent or trivalent branched linker. In some embodiments the GalNAc conjugate is conjugated to the 3' end of the sense strand. In some embodiments, 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

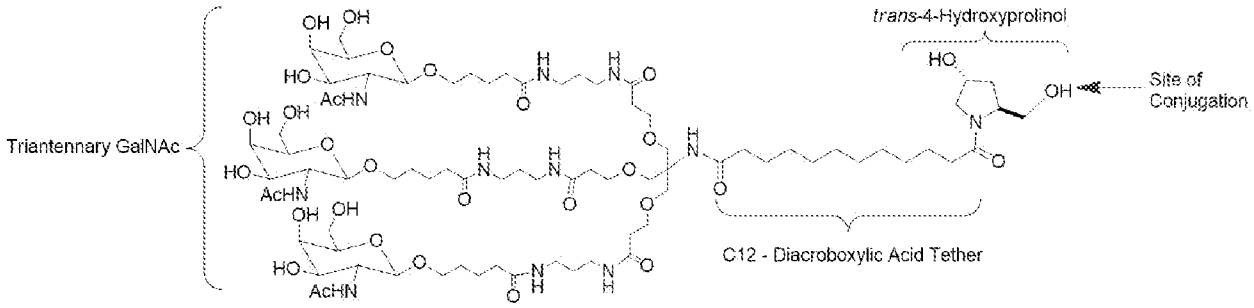


Formula II.

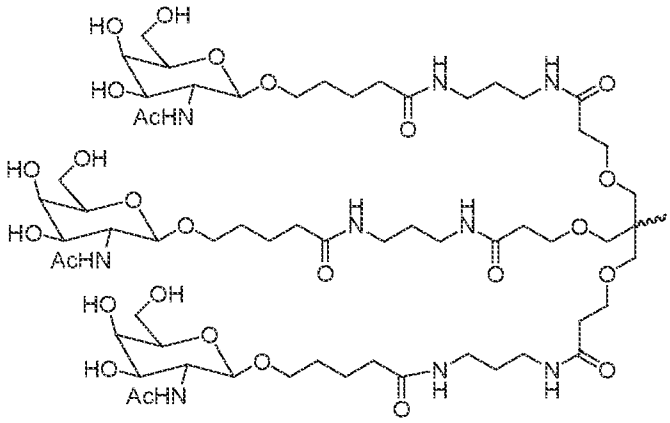
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate via a linker as shown in the following schematic, wherein X is O or S:



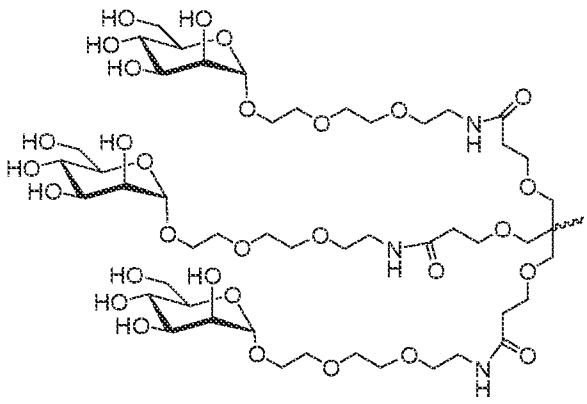
In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below:



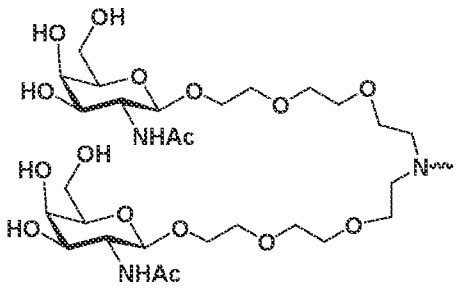
In some embodiments, a carbohydrate conjugate for use in the compositions and methods of the disclosure is selected from the group consisting of:



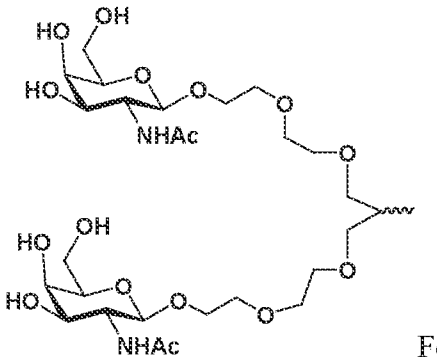
Formula II,



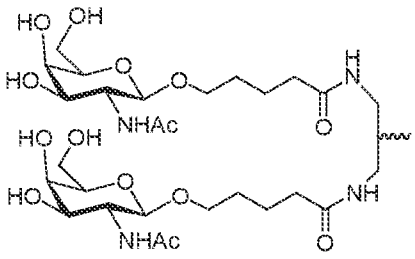
Formula III,



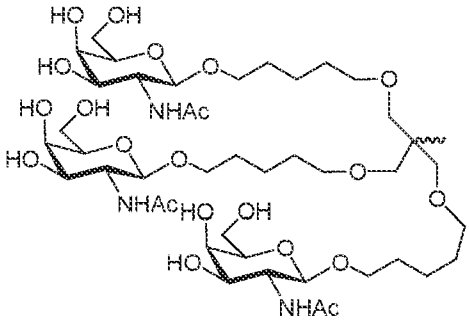
Formula IV,



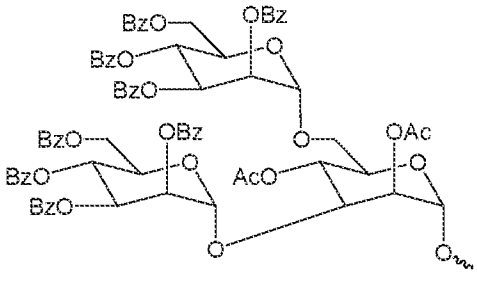
Formula V,



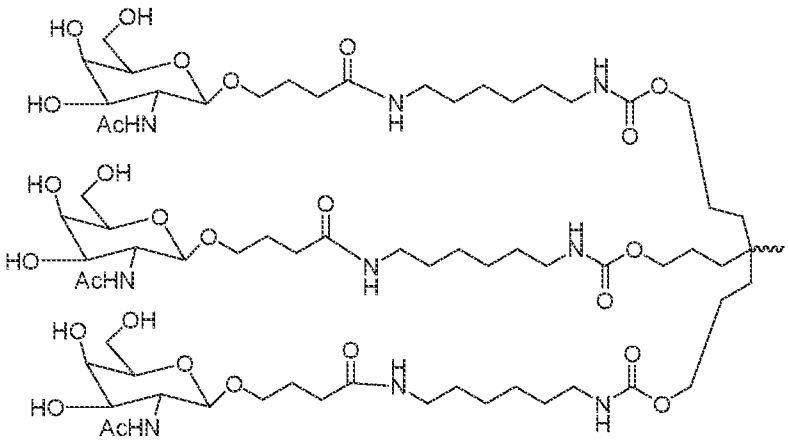
Formula VI,



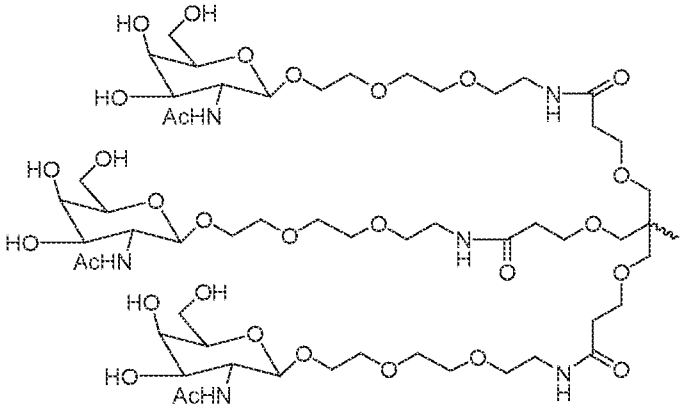
Formula VII,



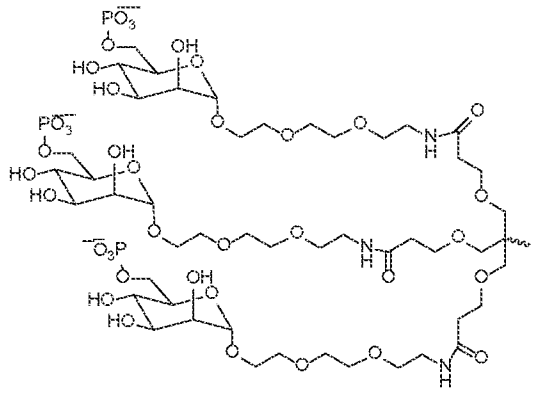
Formula VIII,



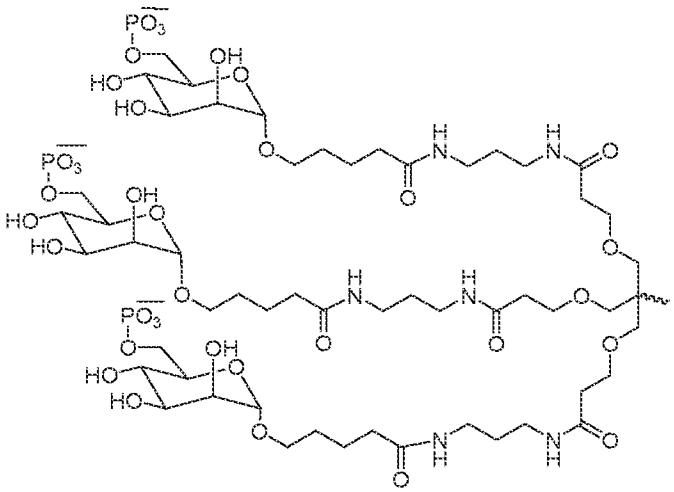
Formula IX,



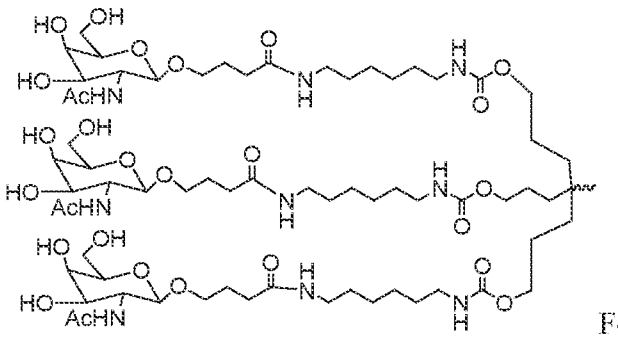
Formula X,



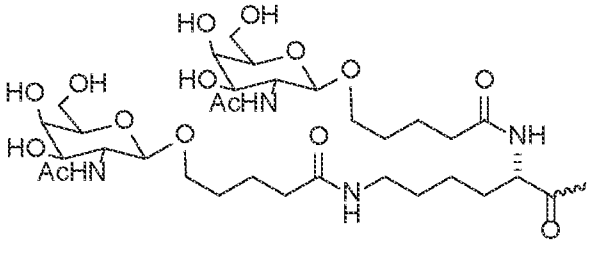
Formula XI,



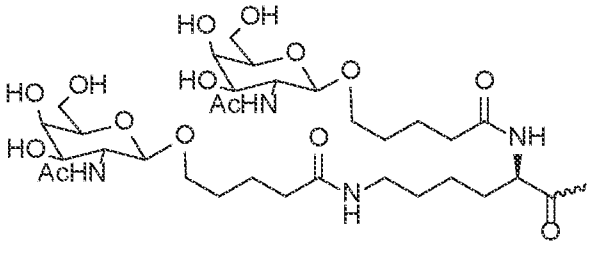
Formula XII,



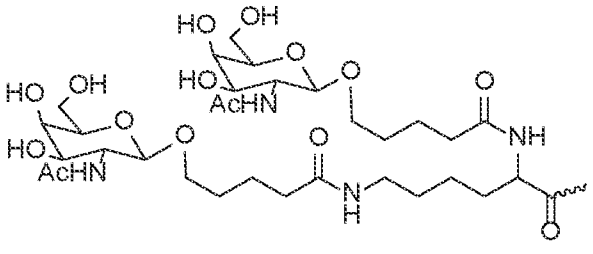
Formula XIII,



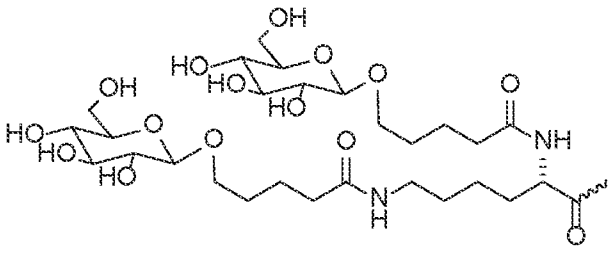
Formula XIV,



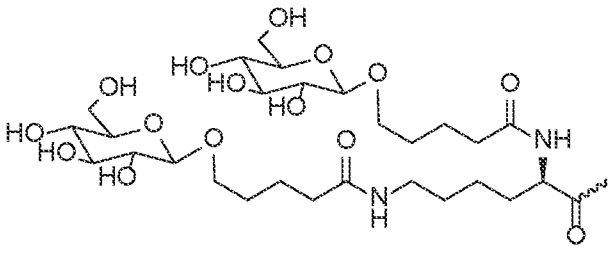
Formula XV,



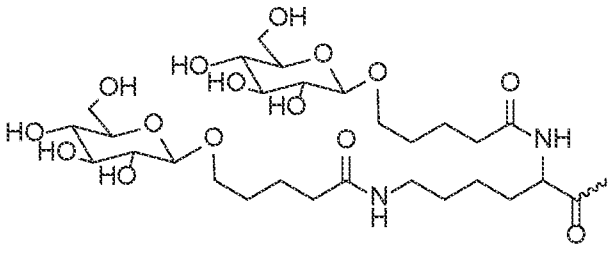
Formula XVI,



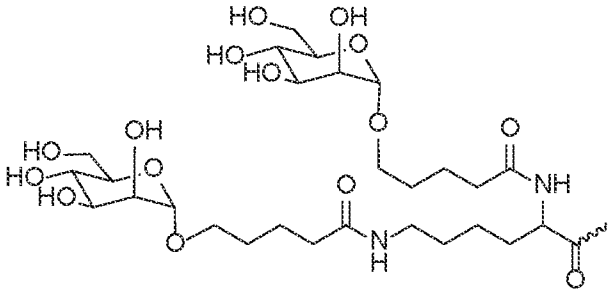
Formula XVII,



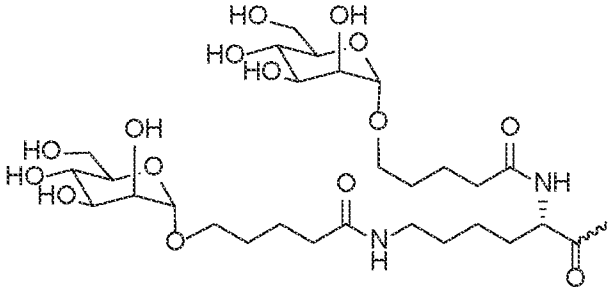
Formula XVIII,



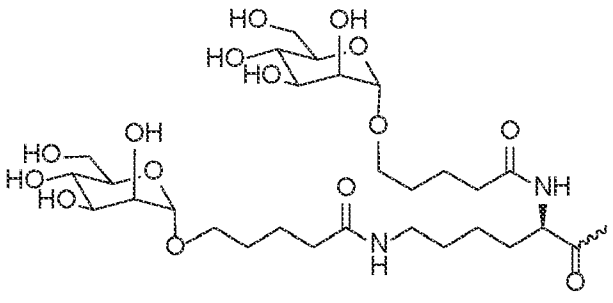
Formula XIX,



Formula XX,

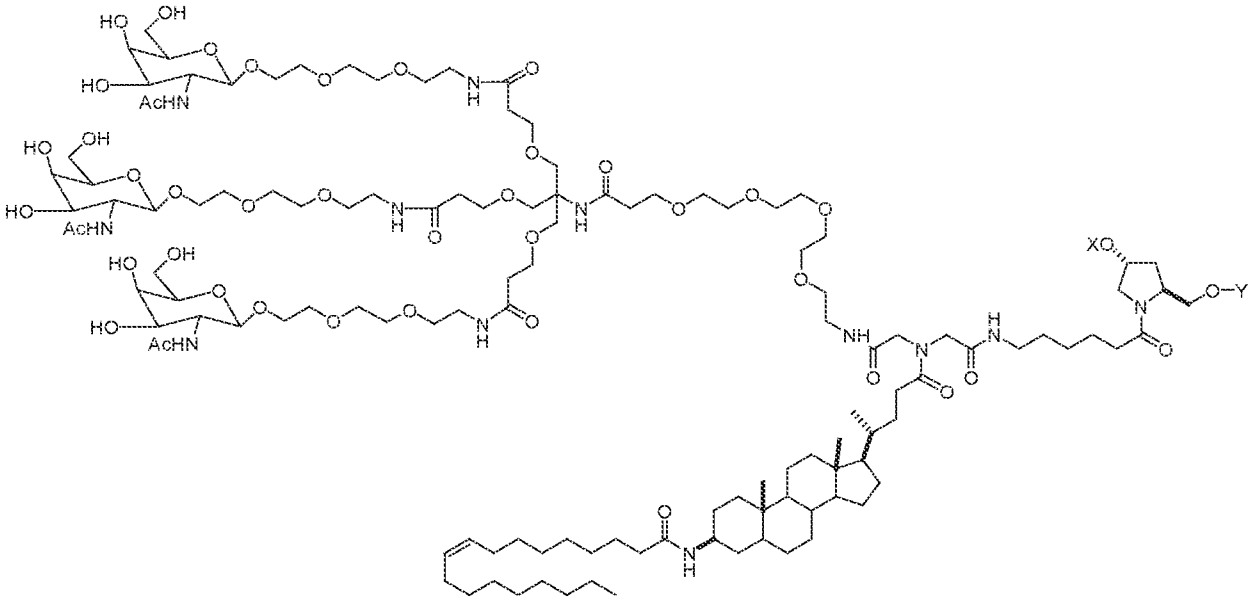


Formula XXI,



Formula XXII.

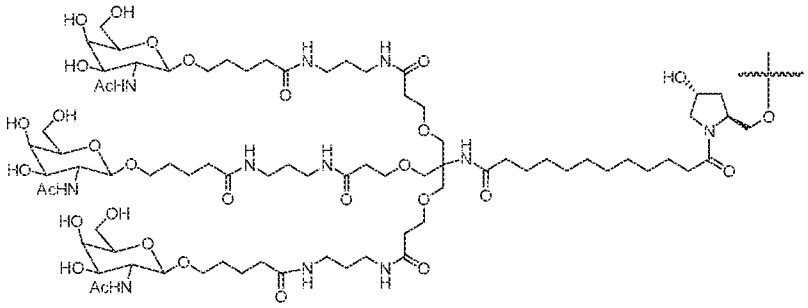
Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



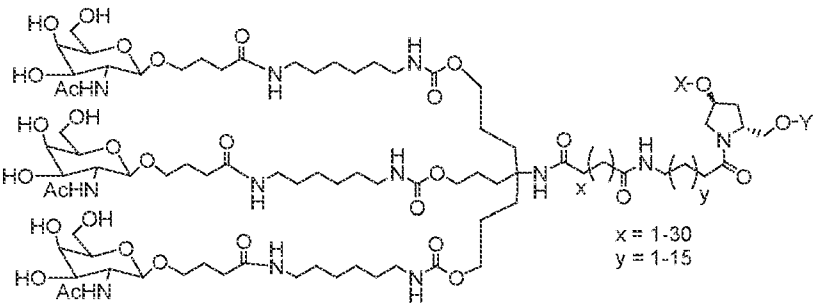
(Formula XXIII), when one of X or Y is an oligonucleotide, the other is a hydrogen.

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 and/or a cell permeation peptide.

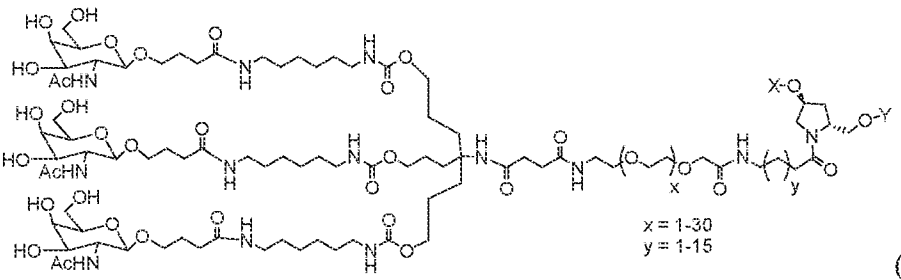
In some embodiments, an iRNA of the disclosure is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the disclosure include, but are not limited to,



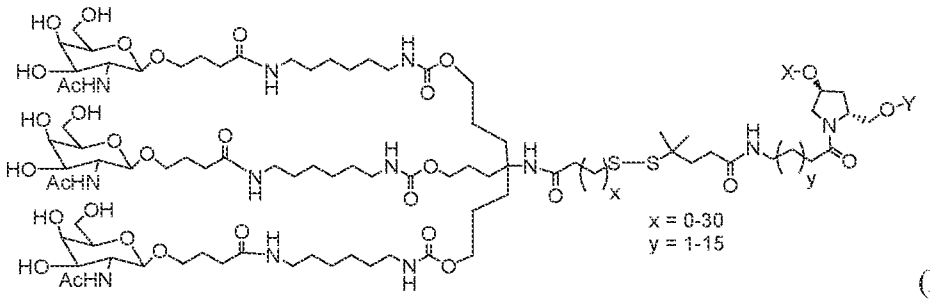
(Formula XXIV),



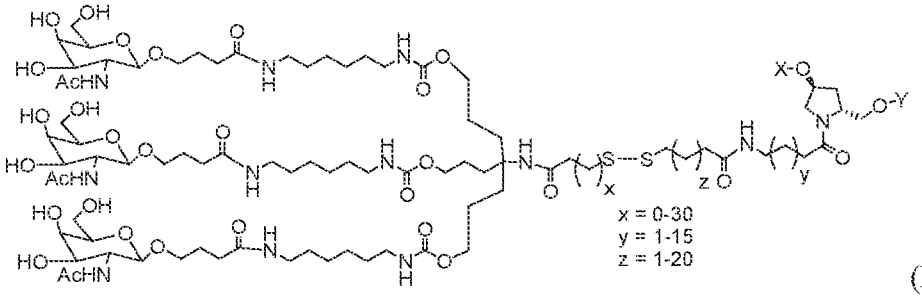
(Formula XXV),



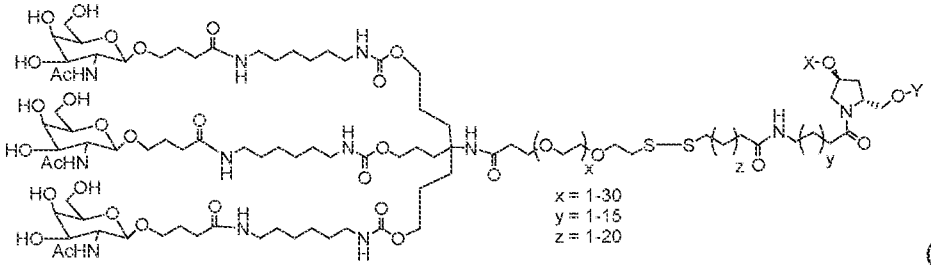
(Formula XXVI),



(Formula XXVII),

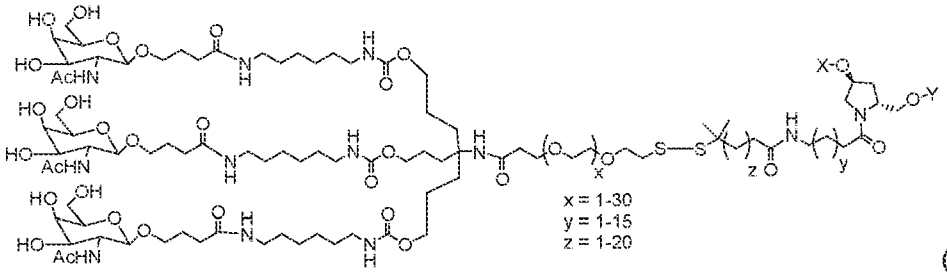


(Formula XXVIII),



(Formula XXIX),

and



(Formula XXX),

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

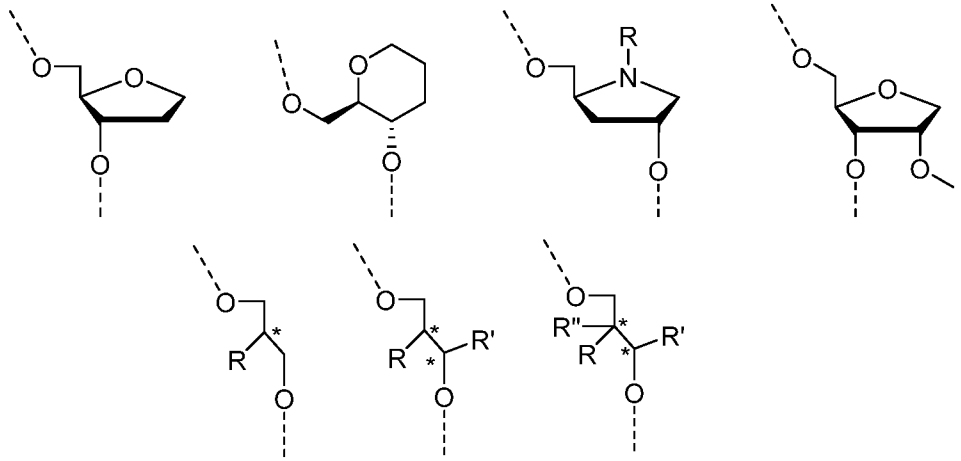
E. *Thermally Destabilizing Modifications*

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

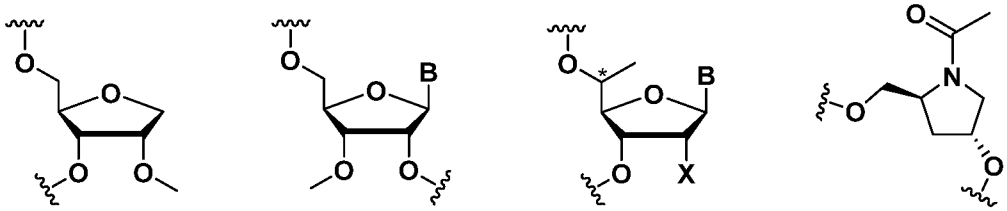
thermally destabilizing modification(s) of the duplex is/are located at position 6, 7, or 8 from the 5'-end of the antisense strand. In still some further embodiments, the thermally destabilizing modification of the duplex is located at position 7 from the 5'-end of the antisense strand. The term "thermally destabilizing modification(s)" includes modification(s) that would result with a dsRNA with a lower overall melting temperature (Tm) (preferably a Tm with one, two, three, or four degrees lower than the Tm 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.

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).

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



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



Mod2 (2'-OMe Abasic Spacer)

Mod3 (3'-OMe)

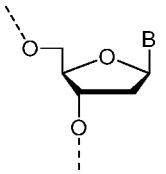
Mod4 (5'-Me)

X = OMe, F

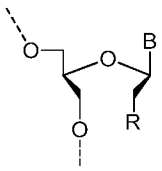
Mod5 (Hyp-spacer)

wherein B is a modified or unmodified nucleobase.

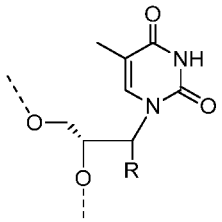
Exemplified sugar modifications include, but are not limited to the following:



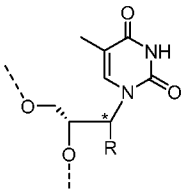
2'-deoxy



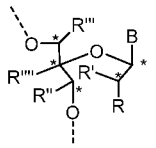
unlocked nucleic acid
R= H, OH, O-alkyl



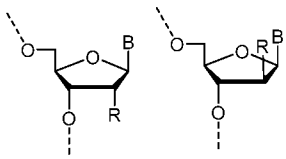
glycol nucleic acid
R= H, OH, O-alkyl



glycol nucleic acid
R= H, OH, O-alkyl



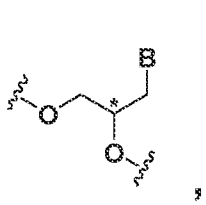
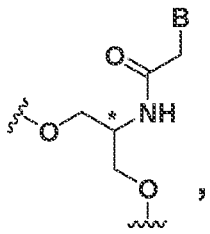
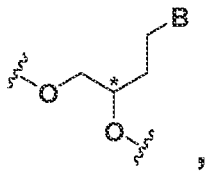
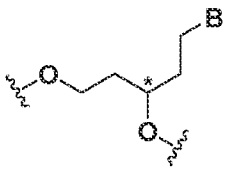
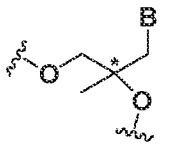
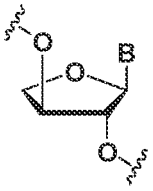
unlocked nucleic acid
R= H, OH, CH_3, CH_2CH_3, O-alkyl, NH_2, NHMe, NMe_2
R' = H, OH, CH_3, CH_2CH_3, O-alkyl, NH_2, NHMe, NMe_2
R'' = H, OH, CH_3, CH_2CH_3, O-alkyl, NH_2, NHMe, NMe_2
R''' = H, OH, CH_3, CH_2CH_3, O-alkyl, NH_2, NHMe, NMe_2
R'''' = H, OH, CH_3, CH_2CH_3, O-alkyl, NH_2, NHMe, NMe_2



R = H, methyl, ethyl

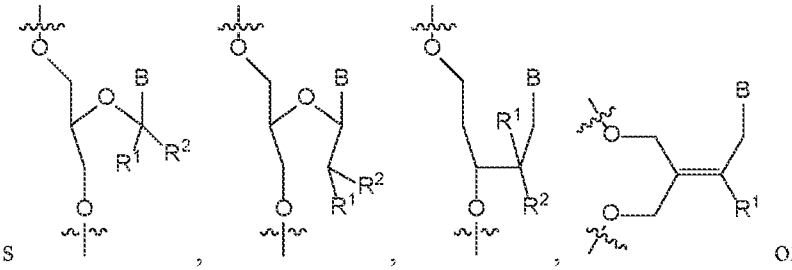
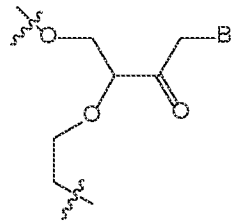
wherein B is a modified or unmodified nucleobase.

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

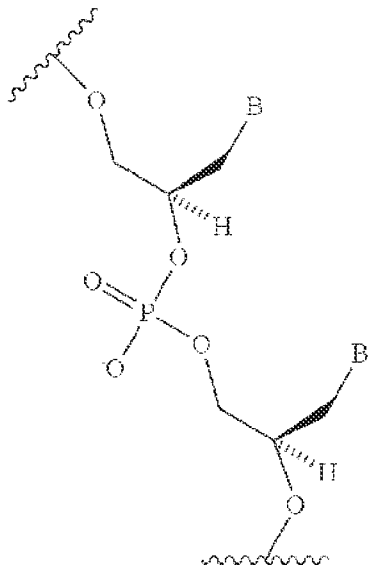

,

,

,

,

, and


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

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  ,  , wherein B is a modified or unmodified nucleobase, R^1 and R^2 independently are H, halogen, OR_3, or alkyl; and R_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.

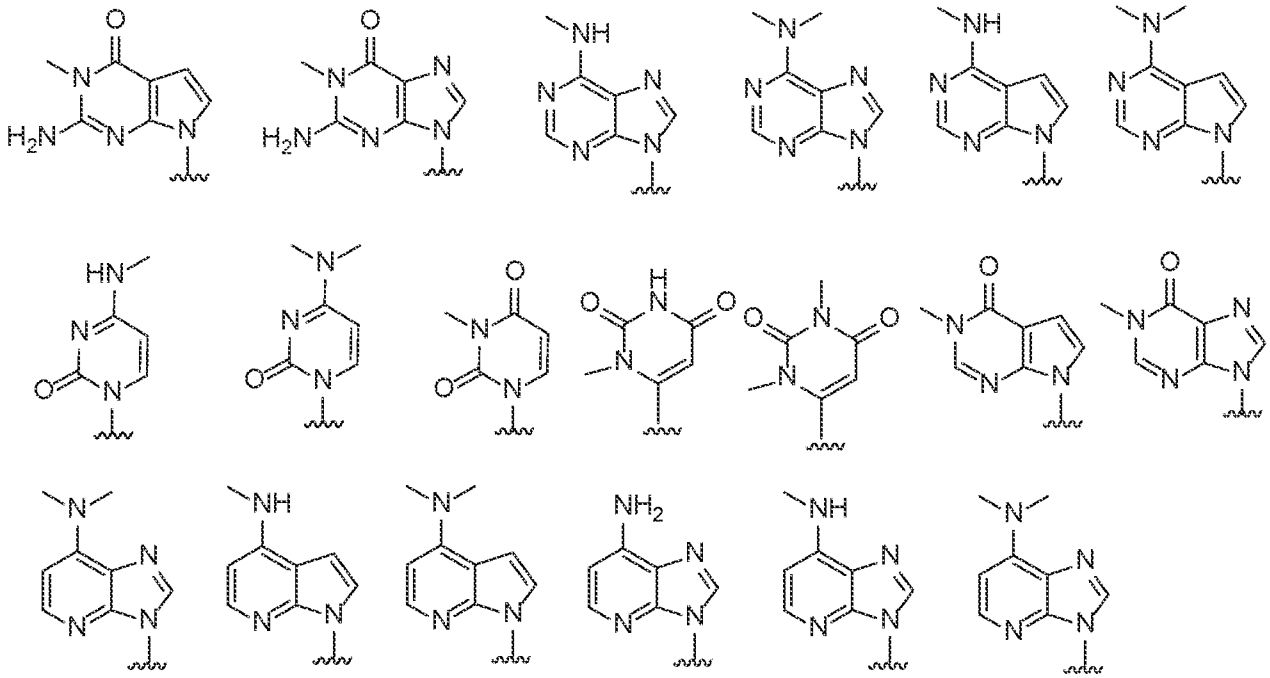
The term 'GNA' refers to glycol nucleic acid which is a polymer similar to DNA or RNA but differing in the composition of its "backbone" in that is composed of repeating glycerol units linked by phosphodiester bonds:



(R)-GNA

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.

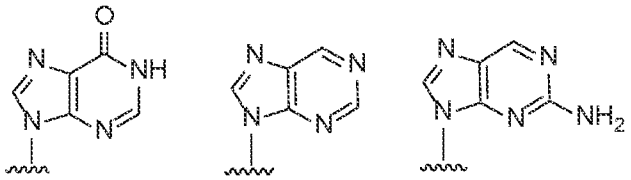
In some embodiments, the thermally destabilizing modification of the duplex in the seed region of the antisense strand includes nucleotides with impaired W-C H-bonding to complementary base on the target mRNA, such as:



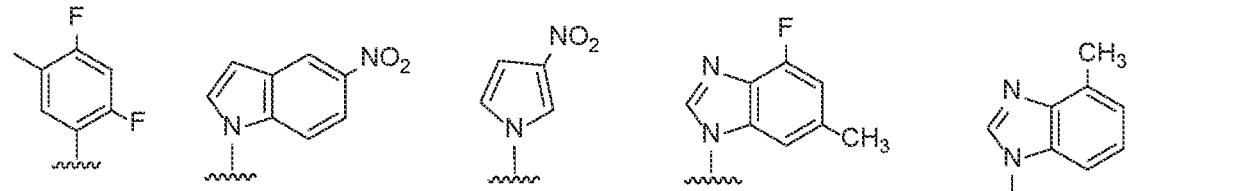
More examples of abasic nucleotide, acyclic nucleotide modifications (including UNA and GNA), and mismatch modifications have been described in detail in WO 2011/133876, which is herein incorporated by reference in its entirety.

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.

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:

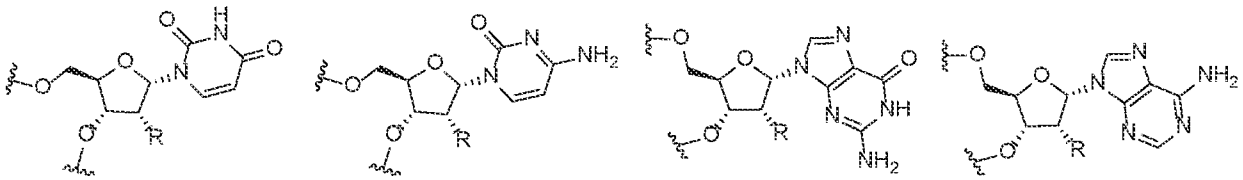


inosine nebularine 2-aminopurine



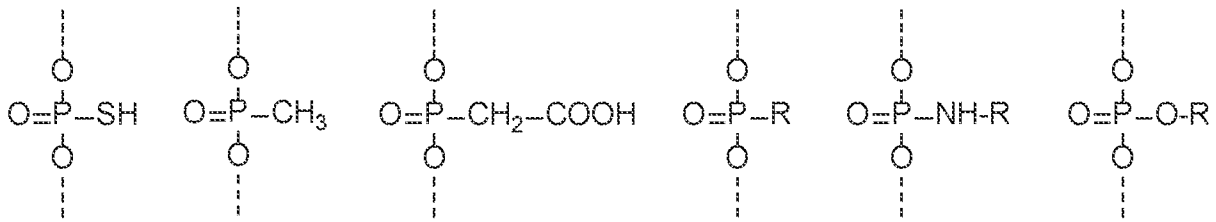
2,4-difluorotoluene 5-nitroindole 3-nitropyrrole 4-Fluoro-6-methylbenzimidazole 4-Methylbenzimidazole

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:



wherein R is H, OH, OCH_3, F, NH_2, NHMe, NMe_2 or O-alkyl.

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



R = alkyl

The alkyl for the R group can be a C_1-C_6alkyl. Specific alkyls for the R group include, but are not limited to methyl, ethyl, propyl, isopropyl, butyl, pentyl and hexyl.

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.

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.

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 strand comprises stabilizing modifications at positions 2, 6, 8, 9, 14, and 16 from the 5'-end. In some other embodiments, the antisense strand comprises stabilizing modifications at positions 2, 6, 14, and 16 from the 5'-end. In still some other embodiments, the antisense strand comprises stabilizing modifications at positions 2, 14, and 16 from the 5'-end.

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.

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.

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.

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.

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

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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 "ABABABABABAB...," "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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

In some embodiments, the dsRNA molecule of the disclosure further comprises one or more phosphorothioate or methylphosphonate internucleotide linkage modification within positions 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.

In some embodiments, the dsRNA molecule of the disclosure further comprises one or more phosphorothioate or methylphosphonate internucleotide linkage modification within positions 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 positions 1-10 of the termini position(s).

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).

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).

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).

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 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

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 2 and one phosphorothioate internucleotide linkage modification within positions 18-23 of the antisense strand (counting from the 5'-end).

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

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

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

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 (counting from the

5'-end) of the sense strand, 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).

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

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 two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

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).

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 20 and 21 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).

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).

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).

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).

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).

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).

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

Various publications describe multimeric siRNA which can all be used with the dsRNA of the disclosure. Such publications include WO2007/091269, US 7858769, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520 which are hereby incorporated by their entirely.

In some embodiments dsRNA molecules of the disclosure are 5' phosphorylated or include a phosphoryl analog at the 5' prime terminus. 5'-phosphate modifications include those which are compatible with RISC mediated gene silencing. Suitable modifications include: 5'-monophosphate ($(HO)_2(O)$P-O-5'); 5'-diphosphate ($(HO)_2(O)$P-O-P(HO)(O)-O-5'); 5'-triphosphate ($(HO)_2(O)$P-O-(HO)(O)P-O-P(HO)(O)-O-5'); 5'-guanosine cap (7-methylated or non-methylated) (7m-G-O-5'-(HO)(O)P-O-(HO)(O)P-O-P(HO)(O)-O-5'); 5'-adenosine cap (Appp), and any modified or unmodified nucleotide cap structure (N-O-5'-(HO)(O)P-O-(HO)(O)P-O-P(HO)(O)-O-5'); 5'-monothiophosphate (phosphorothioate; $(HO)_2(S)$P-O-5'); 5'-monodithiophosphate (phosphorodithioate; (HO)(HS)(S)P-O-5'), 5'-phosphorothiolate ((HO)2(O)P-S-5'); any additional combination of oxygen/sulfur replaced monophosphate, diphosphate and triphosphates (e.g. 5'-alpha-thiotriphosphate, 5'-gamma-thiotriphosphate, etc.), 5'-phosphoramidates ($(HO)_2(O)$P-NH-5', $(HO)(NH_2)(O)$P-O-5'), 5'-alkylphosphonates

(R=alkyl=methyl, ethyl, isopropyl, propyl, etc., e.g. RP(OH)(O)-O-5'-, 5'-alkenylphosphonates (i.e. vinyl, substituted vinyl), $(OH)_2(O)P$-5'-CH2-), 5'-alkyletherphosphonates (R=alkylether=methoxymethyl (MeOCH2-), ethoxymethyl, etc., e.g. RP(OH)(O)-O-5'-). In one example, the modification can in placed in the antisense strand of a dsRNA molecule.

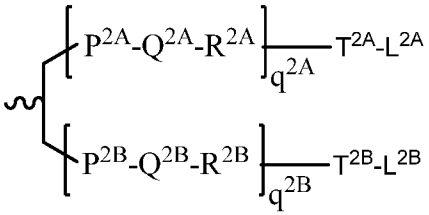
F. Linkers

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.

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_2, SO_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, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_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 some 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.

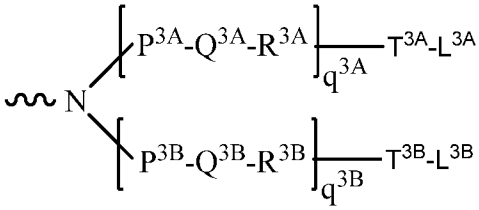
In some embodiments, a dsRNA of the disclosure is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XXXI) – (XXXIV):

Formula XXXI

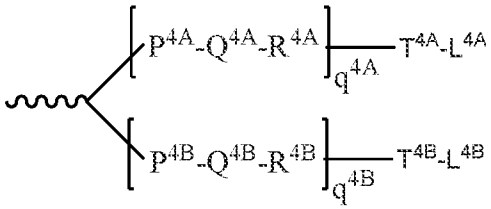


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Formula XXXII

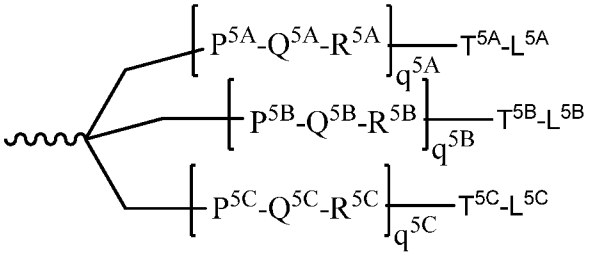


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Formula XXXIII



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Formula XXXIV

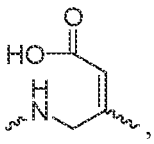
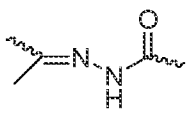
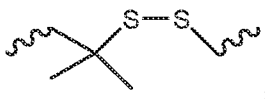
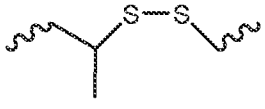
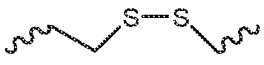
wherein:

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^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or CH_2O;

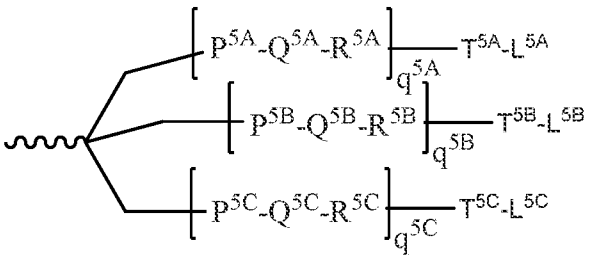
Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^N)$, C(R')=C(R''), C≡C or C(O);

R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, -C(O)-$CH(R^a)$-NH-, CO, CH=N-O,


,

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,

,

or heterocyclyl;

L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; and R^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 (XXXV):

Formula XXXV



,

wherein L^{5A}, L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

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.

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 some embodiments, 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).

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.

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 suitable pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

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.

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 some 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).

i. Redox cleavable linking groups

In some 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 know 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.

ii. Phosphate-based cleavable linking groups

In some 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-. In some embodiments, phosphate-based linking groups 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-. In some embodiments, a phosphate-based linking group is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

In some embodiments, a cleavable linker comprises an acid cleavable linking group. An *a*cid cleavable linking group is a linking group that is cleaved under acidic conditions. In some 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). In some embodiments, 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.

iv. Ester-based cleavable linking groups

In some 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.

v. Peptide-based cleavable linking groups

In some embodiments, 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 alkynelene. 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 –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. 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 and 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; 8,106,022, the entire contents of each of which is herein incorporated by reference.

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 may be incorporated in a single compound or even at a single nucleoside within an iRNA. The present disclosure also includes iRNA compounds that are chimeric compounds.

"Chimeric" iRNA compounds, or "chimeras," in the context of the present disclosure, are iRNA compounds, *e.g.*, dsRNAs, 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, and/or increased binding affinity for the target nucleic acid. An additional region of the iRNA may 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.

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. Let.*, 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 an 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 may 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 iRNA

The delivery of an iRNA to a subject in need thereof can be achieved in a number of different ways. *In vivo* delivery can be performed directly by administering a composition comprising an iRNA, *e.g.* a dsRNA, to a subject. Alternatively, delivery can be performed indirectly by administering one or more vectors that encode and direct the expression of the iRNA. These alternatives are discussed further below.

A. Direct delivery

In general, any method of delivering a nucleic acid molecule can be adapted for use with an iRNA (*see e.g.*, Akhtar S. and Julian RL. (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). However, there are three factors that are important to consider in order to successfully deliver an iRNA molecule *in vivo*: (a) biological stability of the delivered molecule, (2) preventing non-specific effects, and (3) accumulation of the delivered molecule in the target tissue. The non-specific effects of an iRNA can be minimized by local administration, for example by direct injection or implantation into a tissue (as a non-limiting example, the eye) 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 may otherwise be harmed by the agent or that may degrade the agent, and permits a lower total dose of the iRNA molecule to be administered. Several studies have shown successful knockdown of gene products when an iRNA is administered locally. For example, intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, MJ., *et al* (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, SJ., *et al* (2003) *Mol. Vis.* 9:210-216) were 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, WJ., *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, PH., *et al* (2005) *Gene Ther.* 12:59-66; Makimura, H., *et al* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al* (2004) *Neuroscience* 129:521-528; Thakker, ER., *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, KA., *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 an iRNA 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 iRNA composition to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to other groups, *e.g.*, a lipid or carbohydrate group as described herein. Such conjugates can be used to target iRNA to particular cells, *e.g.*, liver cells, *e.g.*, hepatocytes. For example, GalNAc conjugates or lipid (*e.g.*, LNP) formulations can be used to target iRNA to particular cells, *e.g.*, liver cells, *e.g.*, hepatocytes.

iRNA molecules can also be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA 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 iRNA to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, JO., *et al* (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the iRNA 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 an iRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (*see e.g.*, Kim SH., *et al* (2008) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA complexes are well within the abilities of one skilled in the art (*see e.g.*, Sorensen, DR., *et al* (2003) *J. Mol. Biol* 327:761-766; Verma, UN., *et al* (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS *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 iRNAs include DOTAP (Sorensen, DR., *et al* (2003), supra; Verma, UN., *et al* (2003), supra), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS., *et al* (2006) *Nature* 441:111-114), cardiolipin (Chien, PY., *et al* (2005) Cancer Gene Ther. 12:321-328; Pal, A., *et al* (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME., *et al* (2008) *Pharm. Res.* Aug 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, DA., *et al* (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H., *et al* (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be found in U.S. Patent No. 7,427,605, which is herein incorporated by reference in its entirety.

B. Vector encoded iRNAs

In another aspect, iRNA targeting MYOC can be expressed from transcription units inserted into DNA or RNA vectors (*see*, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), 12:5-10; Skillern, A., *et al.*, International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, U.S. Pat. No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to 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.*, *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

The individual strand or strands of an iRNA 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 some embodiments, a dsRNA is expressed as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

An iRNA expression vector is typically a DNA plasmid or viral vector. An expression vector compatible with eukaryotic cells, *e.g.*, with vertebrate cells, can be used to produce recombinant constructs for the expression of an iRNA as described herein. Eukaryotic cell expression vectors are well known in the art and are available from a number of commercial sources. Typically, such vectors contain convenient restriction sites for insertion of the desired nucleic acid segment. Delivery of iRNA 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.

An iRNA expression plasmid can be transfected into a target cell as a complex with a cationic lipid carrier (*e.g.*, Oligofectamine) or a non-cationic lipid-based carrier (*e.g.*, Transit-TKO™). Multiple lipid transfections for iRNA-mediated knockdowns targeting different regions of a target RNA over a period of a week or more are also contemplated by the disclosure. Successful introduction of vectors into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection of cells *ex vivo* can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (*e.g.*, antibiotics and drugs), such as hygromycin B resistance.

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) SV40 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 may be incorporated into vectors capable of episomal replication, *e.g* EPV and EBV vectors. Constructs for the recombinant expression of an iRNA will generally require regulatory elements, *e.g.*, promoters, enhancers, *etc.*, to ensure the expression of the iRNA in target cells. Other aspects to consider for vectors and constructs are further described below.

Vectors useful for the delivery of an iRNA will include regulatory elements (promoter, enhancer, *etc.*) sufficient for expression of the iRNA in the desired target cell or tissue. The regulatory elements can be chosen to provide either constitutive or regulated/inducible expression.

Expression of the iRNA can be precisely regulated, for example, by using an inducible regulatory sequence that is sensitive to certain physiological regulators, *e.g.*, circulating glucose levels, or hormones (Docherty *et al.*, 1994, *FASEB J.* 8:20-24). Such inducible expression

systems, suitable for the control of dsRNA expression in cells or in mammals include, for example, regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-β-D1-thiogalactopyranoside (IPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the iRNA transgene.

In a specific embodiment, viral vectors that contain nucleic acid sequences encoding an iRNA can be used. For example, a retroviral vector can be used (see Miller *et al.*, *Meth. Enzymol.* 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding an iRNA are cloned into one or more vectors, which facilitates delivery of the nucleic acid into a patient. More detail about retroviral vectors can be found, for example, in Boesen *et al.*, *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, *J. Clin. Invest.* 93:644-651 (1994); Kiem *et al.*, *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993). Lentiviral vectors contemplated for use include, for example, the HIV based vectors described in U.S. Patent Nos. 6,143,520; 5,665,557; and 5,981,276, which are herein incorporated by reference.

Adenoviruses are also contemplated for use in delivery of iRNAs. Adenoviruses are especially attractive vehicles, *e.g.*, for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout *et al.*, *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, *Science* 252:431-434 (1991); Rosenfeld *et al.*, *Cell* 68:143-155 (1992); Mastrangeli *et al.*, *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, *et al.*, *Gene Therapy* 2:775-783 (1995). A suitable AV vector for expressing an iRNA featured in the

disclosure, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H *et al.* (2002), *Nat. Biotech.* 20: 1006-1010.

Use of Adeno-associated virus (AAV) vectors is also contemplated (Walsh *et al., Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Pat. No. 5,436,146). In some embodiments, the iRNA can be expressed as two separate, complementary single-stranded RNA molecules from a recombinant AAV vector having, for example, either the U6 or H1 RNA promoters, or the cytomegalovirus (CMV) promoter. Suitable AAV vectors for expressing the dsRNA featured in the disclosure, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R *et al.* (1987), *J. Virol.* 61: 3096-3101; Fisher K J *et al.* (1996), *J. Virol.*, 70: 520-532; Samulski R *et al.* (1989), *J. Virol.* 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641, the entire disclosures of which are herein incorporated by reference.

Another typical viral vector is a pox virus such as a vaccinia virus, for example an attenuated vaccinia such as Modified Virus Ankara (MVA) or NYVAC, an avipox such as fowl pox or canary pox.

The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate. For example, lentiviral vectors can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors can be made to target different cells by engineering the vectors to express different capsid protein serotypes; *see*, *e.g.*, Rabinowitz J E *et al.* (2002), *J Virol* 76:791-801, the entire disclosure of which is herein incorporated by reference.

The pharmaceutical preparation of a vector can include the vector in an acceptable diluent, or can include a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

VI. Pharmaceutical compositions containing iRNA

In some embodiments, the disclosure provides pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical composition containing the iRNA is useful for treating a disease or disorder related to the expression or activity of MYOC (*e.g.*, glaucoma, *e.g.*, primary open angle glaucoma (POAG)). Such pharmaceutical compositions are formulated based on the mode of delivery. In some embodiments, compositions can be formulated for localized delivery, *e.g.*, by intraocular delivery (*e.g.*, intravitreal administration, e.g., intravitreal injection; transscleral administration, e.g., transscleral injection; subconjunctival administration, e.g., subconjunctival injection; retrobulbar administration, e.g., retrobulbar injection; intracameral administration, e.g., intracameral injection; or subretinal administration, e.g., subretinal injection). In other embodiments, compositions can be formulated for topical delivery. In another example, compositions can be formulated for systemic administration via parenteral delivery, *e.g.*, by intravenous (IV) delivery. In some embodiments, a composition provided herein (*e.g.*, a composition comprising a GalNAc conjugate or an LNP formulation) is formulated for intravenous delivery.

The pharmaceutical compositions featured herein are administered in a dosage sufficient to inhibit expression of MYOC. In general, a suitable dose of iRNA will be in the range of 0.01 to 200.0 milligrams per kilogram body weight of the recipient per day. The pharmaceutical composition may be administered once daily, or the iRNA may be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the iRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, *e.g.*, using a conventional sustained release formulation which provides sustained release of the iRNA over a several day period. Sustained release formulations are well known in the art and are particularly useful for delivery of agents at a particular site, such as can be used with the agents of the present disclosure. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

The effect of a single dose on MYOC levels can be long lasting, such that subsequent doses are administered at not more than 3, 4, or 5-day intervals, or at not more than 1, 2, 3, 4, 12, 24, or 36-week intervals.

The skilled artisan will appreciate that certain factors may 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 and/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. Estimates of effective dosages and *in vivo* half-lives for the individual iRNAs encompassed by the disclosure can be made using conventional methodologies or on the basis of *in vivo* testing using a suitable animal model.

A suitable animal model, *e.g.*, a mouse or a cynomolgus monkey, e.g., an animal containing a transgene expressing human MYOC, can be used to determine the therapeutically effective dose and/or an effective dosage regimen administration of MYOC siRNA.

The present disclosure also includes pharmaceutical compositions and formulations that include the iRNA compounds featured herein. 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 local (*e.g.*, by intraocular injection), topical (*e.g.*, by an eye drop solution), 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.

Pharmaceutical compositions and 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. Suitable topical formulations include those in which the iRNAs 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, distearolyphosphatidyl choline) negative (*e.g.*, dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.*, dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). iRNAs featured in the disclosure may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, iRNAs may 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, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C_{1-20} alkyl ester (*e.g.*, isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in U.S. Patent No. 6,747,014, which is incorporated herein by reference.

A. Liposomal formulations

There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present disclosure, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages *in vivo*.

In order to traverse 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. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

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 drugs in their internal compartments from metabolism and degradation (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., 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.

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 liposomes start to merge with the cellular membranes and as the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that 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 a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into the skin. Compounds including analgesics, antibodies, hormones and high-molecular weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/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.*, *Biochem. Biophys. Res. Commun.*, 1987, 147, 980-985).

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

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 and/or phosphatidylcholine and/or cholesterol.

Several studies have assessed the topical delivery of liposomal drug formulations to the skin. Application of liposomes containing interferon to guinea pig skin resulted in a reduction of skin herpes sores while delivery of interferon via other means (*e.g.*, as a solution or as an emulsion) were ineffective (Weiner *et al.*, *Journal of Drug Targeting*, 1992, 2, 405-410). Further, an additional study tested the efficacy of interferon administered as part of a liposomal formulation to the administration of interferon using an aqueous system, and concluded that the liposomal formulation was superior to aqueous administration (du Plessis *et al.*, *Antiviral Research*, 1992, 18, 259-265).

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 cyclosporin-A into different layers of the skin (Hu *et al.* S.T.P. *Pharma. Sci.*, 1994, 4, 6, 466).

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_{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.*, *FEBS Letters*, 1987, 223, 42; Wu *et al.*, *Cancer Research*, 1993, 53, 3765).

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_{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_{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*).

Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto *et al.* (Bull. *Chem. Soc. Jpn.*, 1980, 53, 2778) described liposomes comprising a nonionic detergent, $2C_{1215G}$, that contains a PEG moiety. Illum *et al.* (*FEBS Lett.*, 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (*e.g.*, PEG) are described by Sears (U.S. Pat. Nos. 4,426,330 and 4,534,899). Klibanov *et al.* (*FEBS Lett.*, 1990, 268, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume *et al.* (*Biochimica et Biophysica Acta*, 1990, 1029, 91) extended such observations to other PEG-derivatized phospholipids, *e.g.*, DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle *et al.* (U.S. Pat. Nos. 5,013,556 and 5,356,633) and Martin *et al.* (U.S. Pat. No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Pat. No. 5,225,212 (both to Martin *et al.*) and in WO 94/20073 (Zalipsky *et al.*). Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi *et al*). U.S. Pat. No. 5,540,935 (Miyazaki *et al.*) and U.S. Pat. No. 5,556,948 (Tagawa *et al.*) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

A number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry *et al.* discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Pat. No. 5,264,221 to Tagawa *et al.* discloses protein-bonded liposomes and asserts that the contents of such liposomes may include a dsRNA. U.S. Pat. No. 5,665,710 to Rahman *et al.* describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love *et al.* discloses liposomes comprising dsRNAs targeted to the raf gene.

Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes may 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.

Surfactants find wide application in formulations such as 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).

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.

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.

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.

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.

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).

B. Nucleic acid lipid particles

In some embodiments, a MYOC dsRNA featured in the disclosure is fully encapsulated in the lipid formulation, *e.g.*, to form a SPLP, pSPLP, SNALP, or other nucleic acid-lipid particle. SNALPs and SPLPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate). SNALPs and SPLPs 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). SPLPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. 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. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; and PCT Publication No. WO 96/40964.

In some embodiments, 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.

The cationic lipid may be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I -(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I -(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid may comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

In some embodiments, the compound 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane can be used to prepare lipid-siRNA nanoparticles. Synthesis of 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane is described in United States provisional patent application number 61/107,998 filed on October 23, 2008, which is herein incorporated by reference.

In some embodiments, the lipid-siRNA particle includes 40% 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0 ± 20 nm and a 0.027 siRNA/Lipid Ratio.

The non-cationic lipid may be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl- phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l- carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1 -trans PE, 1 -stearoyl-2-oleoyl- phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid may be from about 5 mol % to about 90 mol %, about 10 mol %, or about 58 mol % if cholesterol is included, of the total lipid present in the particle.

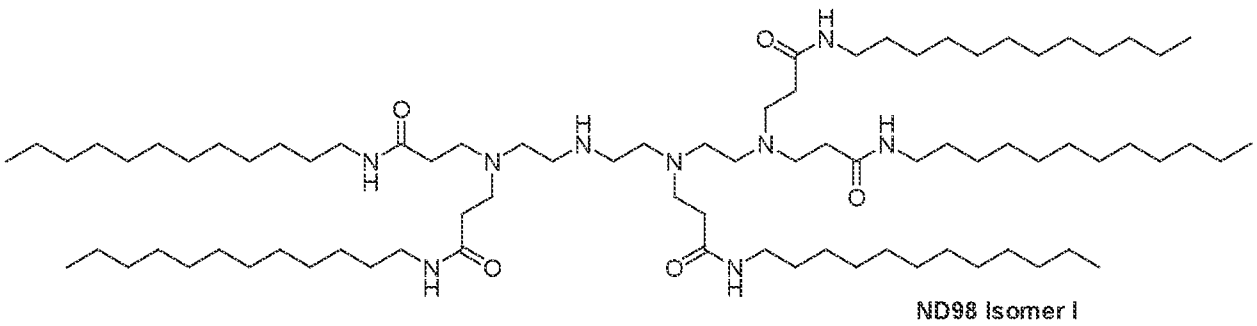
The conjugated lipid that inhibits aggregation of particles may be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate may be, for example, a PEG-dilauryloxypropyl (Ci_2), a PEG-dimyristyloxypropyl (Ci_4), a PEG-dipalmityloxypropyl (Ci_6), or a PEG- distearyloxypropyl ($C]_8$). The conjugated lipid that prevents aggregation of particles may be from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

In some embodiments, the nucleic acid-lipid particle further includes cholesterol at, *e.g.*, about 10 mol % to about 60 mol % or about 48 mol % of the total lipid present in the particle.

In some embodiments, the iRNA is formulated in a lipid nanoparticle (LNP).

LNP01

In some embodiments, the lipidoid ND98·4HCl (MW 1487) (see U.S. Patent Application No. 12/056,230, filed 3/26/2008, which is herein incorporated by reference), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid-dsRNA nanoparticles (*e.g.*, LNP01 particles). Stock solutions of each in ethanol can be prepared as follows: ND98, 133 mg/ml; Cholesterol, 25 mg/ml, PEG-Ceramide C16, 100 mg/ml. The ND98, Cholesterol, and PEG-Ceramide C16 stock solutions can then be combined in a, *e.g.*, 42:48:10 molar ratio. The combined lipid solution can be mixed with aqueous dsRNA (*e.g.*, in sodium acetate pH 5) such that the final ethanol concentration is about 35-45% and the final sodium acetate concentration is about 100-300 mM. Lipid-dsRNA nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (*e.g.*, 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion step can be omitted. Ethanol removal and simultaneous buffer exchange can be accomplished by, for example, dialysis or tangential flow filtration. Buffer can be exchanged with, for example, phosphate buffered saline (PBS) at about pH 7, *e.g.*, about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, or about pH 7.4.



Formula 1

LNP01 formulations are described, *e.g.*, in International Application Publication No. WO 2008/042973, which is hereby incorporated by reference.

Additional exemplary lipid-dsRNA formulations are provided in the following table.

Table 4: Exemplary lipid formulations

	Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio
SNALP	1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1
S-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA ~ 7:1
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1

LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (C12-200)	C12-200/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
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 (l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed April 15, 2009, which is hereby incorporated by reference.

XTC comprising formulations are described, *e.g.*, in U.S. Provisional Serial No. 61/148,366, filed January 29, 2009; U.S. Provisional Serial No. 61/156,851, filed March 2, 2009; U.S. Provisional Serial No. 61/185,712, filed June 10, 2009; U.S. Provisional Serial No. 61/228,373, filed July 24, 2009; U.S. Provisional Serial No. 61/239,686, filed September 3, 2009, and International Application No. PCT/US2010/022614, filed January 29, 2010, which are hereby incorporated by reference.

MC3 comprising formulations are described, *e.g.*, in U.S. Provisional Serial No. 61/244,834, filed September 22, 2009, U.S. Provisional Serial No. 61/185,800, filed June 10, 2009, and International Application No. PCT/US10/28224, filed June 10, 2010, which are hereby incorporated by reference.

ALNY-100 comprising formulations are described, *e.g.*, International patent application number PCT/US09/63933, filed on November 10, 2009, which is hereby incorporated by reference.

C12-200 comprising formulations are described in U.S. Provisional Serial No. 61/175,770, filed May 5, 2009 and International Application No. PCT/US10/33777, filed May 5, 2010, which are hereby incorporated by reference.

C. Synthesis of cationic lipids

Any of the compounds, *e.g.*, cationic lipids and the like, used in the nucleic acid-lipid particles featured in the disclosure may be prepared by known organic synthesis techniques. All substituents are as defined below unless indicated otherwise.

"Alkyl" means a straight chain or branched, noncyclic or cyclic, saturated aliphatic hydrocarbon containing from 1 to 24 carbon atoms. Representative saturated straight chain alkyls include methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, and the like; while saturated branched alkyls include isopropyl, *sec*-butyl, isobutyl, *tert*-butyl, isopentyl, and the like. Representative saturated cyclic alkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like; while unsaturated cyclic alkyls include cyclopentenyl and cyclohexenyl, and the like.

"Alkenyl" means an alkyl, as defined above, containing at least one double bond between adjacent carbon atoms. Alkenyls include both cis and trans isomers. Representative straight chain and branched alkenyls include ethylenyl, propylenyl, 1-butenyl, 2-butenyl, isobutylenyl, 1-pentenyl, 2-pentenyl, 3-methyl-1-butenyl, 2-methyl-2-butenyl, 2,3-dimethyl-2-butenyl, and the like.

"Alkynyl" means any alkyl or alkenyl, as defined above, which additionally contains at least one triple bond between adjacent carbons. Representative straight chain and branched alkynyls include acetylenyl, propynyl, 1-butynyl, 2-butynyl, 1-pentynyl, 2-pentynyl, 3-methyl-1 butynyl, and the like.

"Acyl" means any alkyl, alkenyl, or alkynyl wherein the carbon at the point of attachment is substituted with an oxo group, as defined below. For example, -C(=O)alkyl, -C(=O)alkenyl, and -C(=O)alkynyl are acyl groups.

"Heterocycle" means a 5- to 7-membered monocyclic, or 7- to 10-membered bicyclic, heterocyclic ring which is either saturated, unsaturated, or aromatic, and which contains from 1 or 2 heteroatoms independently selected from nitrogen, oxygen and sulfur, and wherein the nitrogen and sulfur heteroatoms may be optionally oxidized, and the nitrogen heteroatom may be optionally quaternized, including bicyclic rings in which any of the above heterocycles are fused to a benzene ring. The heterocycle may be attached via any heteroatom or carbon atom. Heterocycles include heteroaryls as defined below. Heterocycles include morpholinyl, pyrrolidinonyl, pyrrolidinyl, piperidinyl, piperizynyl, hydantoinyl, valerolactamyl, oxiranyl,

oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydroprimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, and the like.

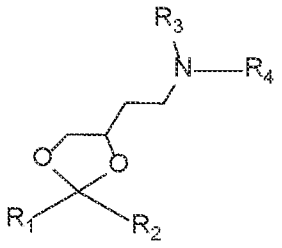
The terms "optionally substituted alkyl", "optionally substituted alkenyl", "optionally substituted alkynyl", "optionally substituted acyl", and "optionally substituted heterocycle" means that, when substituted, at least one hydrogen atom is replaced with a substituent. In the case of an oxo substituent (=O) two hydrogen atoms are replaced. In this regard, substituents include oxo, halogen, heterocycle, -CN, -OR^x, -NR^xR^y, -$NR^xC(=O)R^y$, -$NR^xSO_2R^y$, -$C(=O)R^x$, -$C(=O)OR^x$, -$C(=O)NR^xR^y$, –SO_nR^x and -$SO_nNR^xR^y$, wherein n is 0, 1 or 2, R^x and R^y are the same or different and independently hydrogen, alkyl or heterocycle, and each of said alkyl and heterocycle substituents may be further substituted with one or more of oxo, halogen, -OH, -CN, alkyl, -OR^x, heterocycle, -NR^xR^y, -$NR^xC(=O)R^y$, -$NR^xSO_2R^y$, -$C(=O)R^x$, -$C(=O)OR^x$, -$C(=O)NR^xR^y$, -SO_nR^x and -$SO_nNR^xR^y$.

"Halogen" means fluoro, chloro, bromo and iodo.

In some embodiments, the methods featured in the disclosure may require the use of protecting groups. Protecting group methodology is well known to those skilled in the art (*see, for example*, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, Green, T.W. *et al.*, Wiley-Interscience, New York City, 1999). Briefly, protecting groups within the context of this disclosure are any group that reduces or eliminates unwanted reactivity of a functional group. A protecting group can be added to a functional group to mask its reactivity during certain reactions and then removed to reveal the original functional group. In some embodiments an "alcohol protecting group" is used. An "alcohol protecting group" is any group which decreases or eliminates unwanted reactivity of an alcohol functional group. Protecting groups can be added and removed using techniques well known in the art.

Synthesis of Formula A

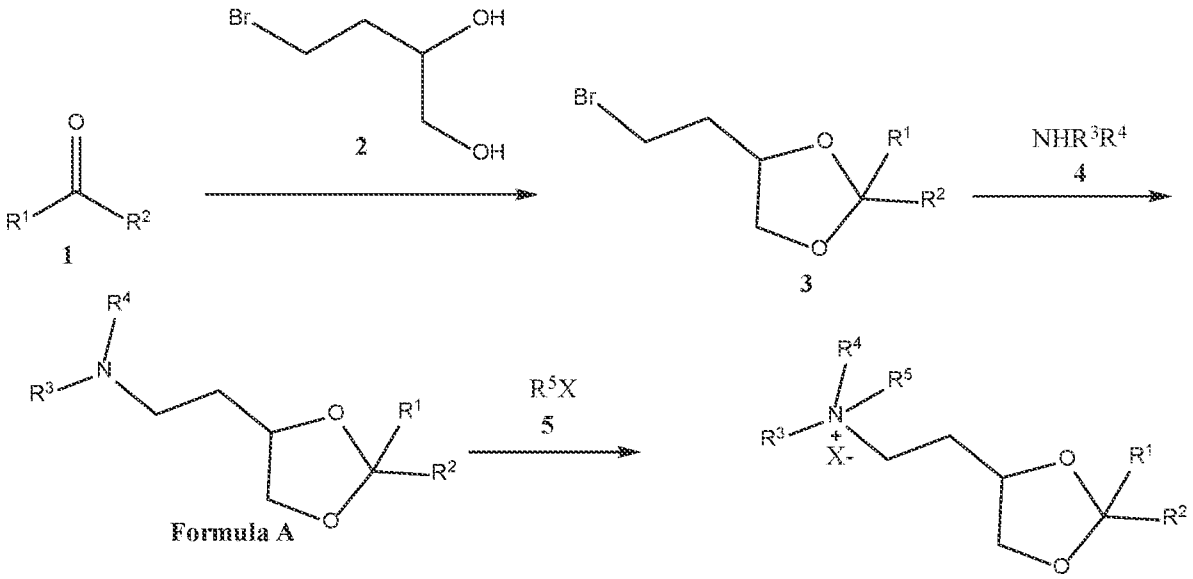
In some embodiments, nucleic acid-lipid particles featured in the disclosure are formulated using a cationic lipid of formula A:



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where R1 and R2 are independently alkyl, alkenyl or alkynyl, each can be optionally substituted, and R3 and R4 are independently lower alkyl or R3 and R4 can be taken together to form an optionally substituted heterocyclic ring. In some embodiments, the cationic lipid is XTC (2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane). In general, the lipid of formula A above may be made by the following Reaction Schemes 1 or 2, wherein all substituents are as defined above unless indicated otherwise.

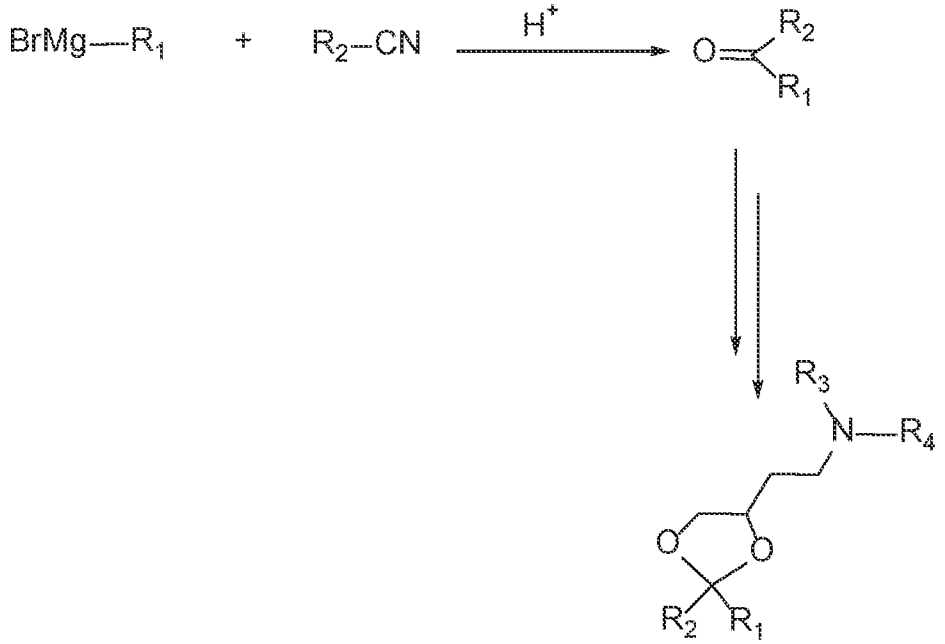
Scheme 1



Lipid A, where R_1 and R_2 are independently alkyl, alkenyl or alkynyl, each can be optionally substituted, and R_3 and R_4 are independently lower alkyl or R_3 and R_4 can be taken together to form an optionally substituted heterocyclic ring, can be prepared according to Scheme 1. Ketone 1 and bromide 2 can be purchased or prepared according to methods known to those of ordinary skill in the art. Reaction of 1 and 2 yields ketal 3. Treatment of ketal 3 with amine 4 yields lipids of formula A. The lipids of formula A can be converted to the

corresponding ammonium salt with an organic salt of formula 5, where X is anion counter ion selected from halogen, hydroxide, phosphate, sulfate, or the like.

Scheme 2



Alternatively, the ketone 1 starting material can be prepared according to Scheme 2. Grignard reagent 6 and cyanide 7 can be purchased or prepared according to methods known to those of ordinary skill in the art. Reaction of 6 and 7 yields ketone 1. Conversion of ketone 1 to the corresponding lipids of formula A is as described in Scheme 1.

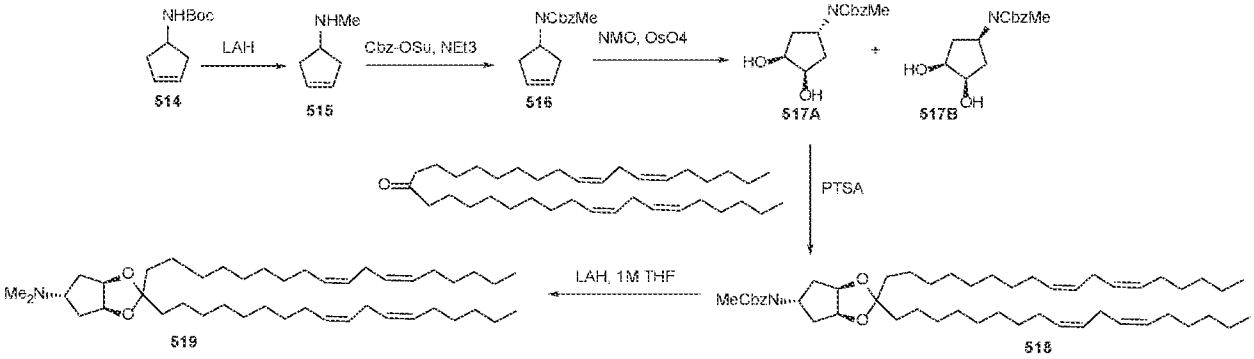
Synthesis of MC3

Preparation of DLin-M-C3-DMA (*i.e.*, (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate) was as follows. A solution of (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol (0.53 g), 4-N,N-dimethylaminobutyric acid hydrochloride (0.51 g), 4-N,N-dimethylaminopyridine (0.61g) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.53 g) in dichloromethane (5 mL) was stirred at room temperature overnight. The solution was washed with dilute hydrochloric acid followed by dilute aqueous sodium bicarbonate. The organic fractions were dried over anhydrous magnesium sulphate, filtered and the solvent removed on a rotovap. The residue was passed down a silica gel column (20 g) using a 1-5% methanol/dichloromethane elution gradient.

Fractions containing the purified product were combined and the solvent removed, yielding a colorless oil (0.54 g).

Synthesis of ALNY-100

Synthesis of ketal 519 [ALNY-100] was performed using the following scheme 3:



Synthesis of 515:

To a stirred suspension of LiAlH4 (3.74 g, 0.09852 mol) in 200 ml anhydrous THF in a two neck RBF (1L), was added a solution of 514 (10g, 0.04926mol) in 70 mL of THF slowly at 0 0C under nitrogen atmosphere. After complete addition, reaction mixture was warmed to room temperature and then heated to reflux for 4 h. Progress of the reaction was monitored by TLC. After completion of reaction (by TLC) the mixture was cooled to 0 °C and quenched with careful addition of saturated Na2SO4 solution. Reaction mixture was stirred for 4 h at room temperature and filtered off. Residue was washed well with THF. The filtrate and washings were mixed and diluted with 400 mL dioxane and 26 mL conc. HCl and stirred for 20 minutes at room temperature. The volatilities were stripped off under vacuum to furnish the hydrochloride salt of 515 as a white solid. Yield: 7.12 g 1H-NMR (DMSO, 400MHz): δ= 9.34 (broad, 2H), 5.68 (s, 2H), 3.74 (m, 1H), 2.66-2.60 (m, 2H), 2.50-2.45 (m, 5H).

Synthesis of 516:

To a stirred solution of compound 515 in 100 mL dry DCM in a 250 mL two neck RBF, was added NEt3 (37.2 mL, 0.2669 mol) and cooled to 0 °C under nitrogen atmosphere. After a slow addition of N-(benzyloxy-carbonyloxy)-succinimide (20 g, 0.08007 mol) in 50 mL dry DCM, reaction mixture was allowed to warm to room temperature. After completion of the

reaction (2-3 h by TLC) mixture was washed successively with 1N HCl solution (1 x 100 mL) and saturated NaHCO3 solution (1 x 50 mL). The organic layer was then dried over anhyd. Na2SO4 and the solvent was evaporated to give crude material which was purified by silica gel column chromatography to get 516 as sticky mass. Yield: 11g (89%). 1H-NMR (CDCl3, 400MHz): δ = 7.36-7.27(m, 5H), 5.69 (s, 2H), 5.12 (s, 2H), 4.96 (br., 1H) 2.74 (s, 3H), 2.60(m, 2H), 2.30-2.25(m, 2H). LC-MS [M+H] -232.3 (96.94%).

Synthesis of 517A and 517B:

The cyclopentene 516 (5 g, 0.02164 mol) was dissolved in a solution of 220 mL acetone and water (10:1) in a single neck 500 mL RBF and to it was added N-methyl morpholine-N-oxide (7.6 g, 0.06492 mol) followed by 4.2 mL of 7.6% solution of OsO4 (0.275 g, 0.00108 mol) in tert-butanol at room temperature. After completion of the reaction (~ 3 h), the mixture was quenched with addition of solid Na2SO3 and resulting mixture was stirred for 1.5 h at room temperature. Reaction mixture was diluted with DCM (300 mL) and washed with water (2 x 100 mL) followed by saturated NaHCO3 (1 x 50 mL) solution, water (1 x 30 mL) and finally with brine (1x 50 mL). Organic phase was dried over an.Na2SO4 and solvent was removed in vacuum. Silica gel column chromatographic purification of the crude material was afforded a mixture of diastereomers, which were separated by prep HPLC. Yield: - 6 g crude

517A - Peak-1 (white solid), 5.13 g (96%). 1H-NMR (DMSO, 400MHz): δ= 7.39-7.31(m, 5H), 5.04(s, 2H), 4.78-4.73 (m, 1H), 4.48-4.47(d, 2H), 3.94-3.93(m, 2H), 2.71(s, 3H), 1.72- 1.67(m, 4H). LC-MS - [M+H]-266.3, [M+NH4 +]-283.5 present, HPLC-97.86%. Stereochemistry confirmed by X-ray.

Synthesis of 518:

Using a procedure analogous to that described for the synthesis of compound 505, compound 518 (1.2 g, 41%) was obtained as a colorless oil. 1H-NMR (CDCl3, 400MHz): δ= 7.35-7.33(m, 4H), 7.30-7.27(m, 1H), 5.37-5.27(m, 8H), 5.12(s, 2H), 4.75(m,1H), 4.58-4.57(m,2H), 2.78-2.74(m,7H), 2.06-2.00(m,8H), 1.96-1.91(m, 2H), 1.62(m, 4H), 1.48(m, 2H), 1.37-1.25(br m, 36H), 0.87(m, 6H). HPLC-98.65%.

General Procedure for the Synthesis of Compound 519:

A solution of compound 518 (1 eq) in hexane (15 mL) was added in a drop-wise fashion to an ice-cold solution of LAH in THF (1 M, 2 eq). After complete addition, the mixture was heated at 40°C over 0.5 h then cooled again on an ice bath. The mixture was carefully hydrolyzed with saturated aqueous Na2SO4 then filtered through celite and reduced to an oil. Column chromatography provided the pure 519 (1.3 g, 68%) which was obtained as a colorless oil. 13C NMR = 130.2, 130.1 (x2), 127.9 (x3), 112.3, 79.3, 64.4, 44.7, 38.3, 35.4, 31.5, 29.9 (x2), 29.7, 29.6 (x2), 29.5 (x3), 29.3 (x2), 27.2 (x3), 25.6, 24.5, 23.3, 226, 14.1; Electrospray MS (+ve): Molecular weight for C44H80NO2 (M + H)+ Calc. 654.6, Found 654.6.

Formulations prepared by either the standard or extrusion-free method can be characterized in similar manners. For example, formulations are typically characterized by visual inspection. They should be whitish translucent solutions free from aggregates or sediment. Particle size and particle size distribution of lipid-nanoparticles can be measured by light scattering using, for example, a Malvern Zetasizer Nano ZS (Malvern, USA). Particles should be about 20-300 nm, such as 40-100 nm in size. The particle size distribution should be unimodal. The total dsRNA concentration in the formulation, as well as the entrapped fraction, is estimated using a dye exclusion assay. A sample of the formulated dsRNA can be incubated with an RNA-binding dye, such as Ribogreen (Molecular Probes) in the presence or absence of a formulation disrupting surfactant, *e.g.*, 0.5% Triton-X100. The total dsRNA in the formulation can be determined by the signal from the sample containing the surfactant, relative to a standard curve. The entrapped fraction is determined by subtracting the "free" dsRNA content (as measured by the signal in the absence of surfactant) from the total dsRNA content. Percent entrapped dsRNA is typically >85%. For SNALP formulation, the particle size is at least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm, at least 100 nm, at least 110 nm, and at least 120 nm. The suitable range is typically about at least 50 nm to about at least 110 nm, about at least 60 nm to about at least 100 nm, or about at least 80 nm to about at least 90 nm.

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 minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may 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 enhancers surfactants and chelators. Suitable surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic 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 may 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(isohexylcynaoacrylate), 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. Patent 6,887,906, US Publn. No. 20030027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

Compositions and formulations for parenteral, intraparenchymal (into the brain), intrathecal, intravitreal, subretinal, transscleral, subconjunctival, retrobulbar, intracameral,

intraventricular, or intrahepatic administration may include sterile aqueous solutions which may 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.

Pharmaceutical compositions of the present disclosure include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

The pharmaceutical formulations featured in the present disclosure, which may conveniently be presented in unit dosage form, may 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.

The compositions featured in the present disclosure may 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 may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

D. Additional Formulations

i. Emulsions

The compositions of the present disclosure may be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1μm in diameter (*see e.g.,* Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 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 may 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 may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants may also be present in emulsions as needed. Pharmaceutical emulsions may 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.

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 may 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 may be incorporated into either phase of the emulsion. Emulsifiers may 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 NG., and Ansel HC., 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).

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 NG., and Ansel HC., 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 may 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 NG., and Ansel HC., 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).

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.

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).

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.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that may 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 may 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.

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 NG., and Ansel HC., 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 NG., and Ansel HC., 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.

In some embodiments of the present disclosure, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion may 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 NG., and Ansel HC., 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, isotopically 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).

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 NG., and Ansel HC., 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.

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 (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), 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 may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may 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 may 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.

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. Patent 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. Patent 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 may form spontaneously when their components are brought together at ambient temperature. This may be particularly advantageous when formulating thermolabile drugs, peptides or iRNAs. 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 iRNAs and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of iRNAs and nucleic acids.

Microemulsions of the present disclosure may 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 iRNAs and nucleic acids of the present disclosure. Penetration enhancers used in the microemulsions of the present disclosure may 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.

ii. Penetration Enhancers

In some embodiments, the present disclosure employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly iRNAs, 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 may 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.

Penetration enhancers may 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.

Surfactants: In connection with the present disclosure, 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 iRNAs 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).

Fatty acids: 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, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C_{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).

Bile salts: 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), glucholic acid (sodium glucholate), 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).

Chelating Agents: 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 iRNAs 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, laureth-9 and N-amino acyl derivatives of β-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).

Non-chelating non-surfactants: 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 iRNAs through the alimentary mucosa (*see e.g.*, Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33). This class of penetration enhancers include, 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).

Agents that enhance uptake of iRNAs at the cellular level may 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 (Lollo *et al.*, PCT Application WO 97/30731), are also known to enhance the cellular uptake of dsRNAs. Examples of commercially available transfection reagents include, for example Lipofectamine™ (Invitrogen; Carlsbad, CA), Lipofectamine 2000™ (Invitrogen; Carlsbad, CA), 293fectin™ (Invitrogen; Carlsbad, CA), Cellfectin™ (Invitrogen; Carlsbad, CA), DMRIE-C™ (Invitrogen; Carlsbad, CA), FreeStyle™

MAX (Invitrogen; Carlsbad, CA), Lipofectamine™ 2000 CD (Invitrogen; Carlsbad, CA), Lipofectamine™ (Invitrogen; Carlsbad, CA), RNAiMAX (Invitrogen; Carlsbad, CA), Oligofectamine™ (Invitrogen; Carlsbad, CA), Optifect™ (Invitrogen; Carlsbad, CA), X-tremeGENE Q2 Transfection Reagent (Roche; Grenzacherstrasse, Switzerland), DOTAP Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), DOSPER Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), or Fugene (Grenzacherstrasse, Switzerland), Transfectam® Reagent (Promega; Madison, WI), TransFast™ Transfection Reagent (Promega; Madison, WI), Tfx™-20 Reagent (Promega; Madison, WI), Tfx™-50 Reagent (Promega; Madison, WI), DreamFect™ (OZ Biosciences; Marseille, France), EcoTransfect (OZ Biosciences; Marseille, France), TransPass[a] D1 Transfection Reagent (New England Biolabs; Ipswich, MA, USA), LyoVec™/LipoGen™ (Invivogen; San Diego, CA, USA), PerFectin Transfection Reagent (Genlantis; San Diego, CA, USA), NeuroPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), GenePORTER Transfection reagent (Genlantis; San Diego, CA, USA), GenePORTER 2 Transfection reagent (Genlantis; San Diego, CA, USA), Cytofectin Transfection Reagent (Genlantis; San Diego, CA, USA), BaculoPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), TroganPORTER™ transfection Reagent (Genlantis; San Diego, CA, USA), RiboFect (Bioline; Taunton, MA, USA), PlasFect (Bioline; Taunton, MA, USA), UniFECTOR (B-Bridge International; Mountain View, CA, USA), SureFECTOR (B-Bridge International; Mountain View, CA, USA), or HiFect™ (B-Bridge International, Mountain View, CA, USA), among others.

Other agents may 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.

iii. Carriers

Certain compositions of the present disclosure also incorporate carrier compounds in the formulation. As used herein, "carrier compound" can refer to a nucleic acid, or analog thereof, which is inert (*i.e.*, does not possess biological activity per se) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an

excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate dsRNA in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao *et al.*, *DsRNA Res. Dev.*, 1995, 5, 115-121; Takakura *et al.*, *DsRNA & Nucl. Acid Drug Dev.*, 1996, 6, 177-183).

iv. Excipients

In contrast to a carrier compound, a pharmaceutical carrier or excipient may comprise, *e.g.*, a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient may 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*).

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.

Formulations for topical administration of nucleic acids may 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 may 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.

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.

v. Other Components

The compositions of the present disclosure may additionally contain other adjunct components conventionally found in pharmaceutical compositions, *e.g.*, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may 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 and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions may contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the disclosure include (a) one or more iRNA compounds and (b) one or more biologic agents which function by a non-RNAi mechanism. Examples of such biologic agents include agents that interfere with an interaction of MYOC and at least one MYOC binding partner.

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 typical.

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 in the disclosure lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the disclosure, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may 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 may be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the disclosure can be administered in combination with other known agents effective in treatment of diseases or disorders related to MYOC expression (*e.g.*, glaucoma, *e.g.*, primary open angle glaucoma (POAG)). In any event, the administering physician can adjust the amount and timing of iRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VII. Methods of treating disorders related to expression of MYOC

The present disclosure relates to the use of an iRNA targeting MYOC to inhibit MYOC expression and/or to treat a disease, disorder, or pathological process that is related to MYOC expression (*e.g.*, glaucoma, *e.g.*, primary open angle glaucoma (POAG)).

In some aspects, a method of treatment of a disorder related to expression of MYOC is provided, the method comprising administering an iRNA (*e.g.*, a dsRNA) disclosed herein to a subject in need thereof. In some embodiments, the iRNA inhibits (decreases) MYOC expression.

In some embodiments, the subject is an animal that serves as a model for a disorder related to MYOC expression, *e.g.*, glaucoma, *e.g.*, primary open angle glaucoma (POAG). .

A. Glaucoma

In some embodiments, the disorder related to MYOC expression is glaucoma. A non-limiting example of glaucoma that is treatable using the method described herein includes primary open angle glaucoma (POAG).

Clinical and pathological features of glaucoma include, but are not limited to, vision loss, a reduction in visual acuity (*e.g.*, halos around lights and blurriness)) and decreased leakage of aqueous humor from the eye.

In some embodiments, the subject with glaucoma is less than 18 years old. In some embodiments, the subject with glaucoma is an adult. In some embodiments, the subject with glaucoma is more than 60 years old. In some embodiments, the subject has, or is identified as having, elevated levels of MYOC mRNA or protein relative to a reference level (e.g., a level of MYOC that is greater than a reference level).

In some embodiments, glaucoma is diagnosed using analysis of a sample from the subject (*e.g.*, an aqueous ocular fluid sample). In some embodiments, the sample is analyzed using a method selected from one or more of: fluorescent in situ hybridization (FISH), immunohistochemistry, MYOC immunoassay, electron microscopy, laser microdissection, and mass spectrometry. In some embodiments,glaucoma is diagnosed using any suitable diagnostic test or technique, *e.g.*, Goldmann Applanation Tonometry, measurement of central corneal thickness (CCT), automated static threshold perimetry (*e.g.* Humphrey field analysis), Van Herick technique, gonioscopy, ultrasound biomicroscopy and anterior segment optical coherence tomography (AS-OCT), angiography (*e.g.*, fluorescein angiography or indocyanine green angiography), electroretinography, ultrasonography, pachymetry, optical coherence tomography (OCT), computed tomography (CT) and magnetic resonance imaging (MRI), tonometry, color vision testing, visual field testing, slit-lamp examination, ophthalmoscopy, and physical examination (*e.g.*, to assess visual acuity (e.g., by fundoscopy or optical coherence tomography (OCT)).

B. Combination Therapies

In some embodiments, an iRNA (*e.g.*, a dsRNA) disclosed herein is administered in combination with a second therapy (*e.g.*, one or more additional therapies) known to be effective in treating a disorder related to MYOC expression (glaucoma, *e.g.*, primary open angle glaucoma (POAG)) or a symptom of such a disorder. The iRNA may be administered before, after, or concurrent with the second therapy. In some embodiments, the iRNA is administered before the second therapy. In some embodiments, the iRNA is administered after the second therapy. In some embodiments, the iRNA is administered concurrent with the second therapy.

The second therapy may be an additional therapeutic agent. The iRNA and the additional therapeutic agent can be administered in combination in the same composition or the additional therapeutic agent can be administered as part of a separate composition.

In some embodiments, the second therapy is a non-iRNA therapeutic agent that is effective to treat the disorder or symptoms of the disorder.

In some embodiments, the iRNA is administered in conjunction with a therapy.

Exemplary combination therapies include, but are not limited to, laser trabeculoplasty surgery, trabeculectomy surgery, a minimally invasive glaucoma surgery, placement of a drainage tube in the eye, oral medication or eye drops. .

Administration dosages, routes, and timing

A subject (*e.g.*, a human subject, *e.g.*, a patient) can be administered a therapeutic amount of iRNA. The therapeutic amount can be, *e.g.*, 0.05-50 mg/kg.

In some embodiments, the iRNA is formulated for delivery to a target organ, *e.g.*, to the eye.

In some embodiments, the iRNA is formulated as a lipid formulation, *e.g.*, an LNP formulation as described herein. In some such embodiments, the therapeutic amount is 0.05-5 mg/kg dsRNA. In some embodiments, the lipid formulation, *e.g.*, LNP formulation, is administered intravenously.

In some embodiments, the iRNA is in the form of a GalNAc conjugate *e.g.*, as described herein. In some such embodiments, the therapeutic amount is 0.5-50 mg dsRNA. In some embodiments, the *e.g.*, GalNAc conjugate is administered subcutaneously.

In some embodiments, the administration is repeated, for example, on a regular basis, such as, daily, biweekly (*i.e.*, every two weeks) for one month, two months, three months, four

months, six months or longer. After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration biweekly for three months, administration can be repeated once per month, for six months or a year or longer. In some embodiments, the iRNA agent is administered in two or more doses. In some embodiments, the number or amount of subsequent doses is dependent on the achievement of a desired effect, *e.g.*, to(a) inhibit or reduce the expression or activity of MYOC; (b) reduce the level of misfolded MYOC protein; (c) reduce trabecular meshwork cell death; (d) decrease intraocular pressure; or (e) increase visual acuity, or the achievement of a therapeutic or prophylactic effect, *e.g.*, reduction or prevention of one or more symptoms associated with the disorder.

In some embodiments, the iRNA agent is administered according to a schedule. For example, the iRNA agent may be administered once per week, twice per week, three times per week, four times per week, or five times per week. In some embodiments, the schedule involves regularly spaced administrations, *e.g.*, hourly, every four hours, every six hours, every eight hours, every twelve hours, daily, every 2 days, every 3 days, every 4 days, every 5 days, weekly, biweekly, or monthly. In some embodiments, the iRNA agent is administered at the frequency required to achieve a desired effect.

In some embodiments, the schedule involves closely spaced administrations followed by a longer period of time during which the agent is not administered. For example, the schedule may involve an initial set of doses that are administered in a relatively short period of time (*e.g.*, about every 6 hours, about every 12 hours, about every 24 hours, about every 48 hours, or about every 72 hours) followed by a longer time period (*e.g.*, about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, or about 8 weeks) during which the iRNA agent is not administered. In some embodiments, the iRNA agent is initially administered hourly and is later administered at a longer interval (*e.g.*, daily, weekly, biweekly, or monthly). In some embodiments, the iRNA agent is initially administered daily and is later administered at a longer interval (*e.g.*, weekly, biweekly, or monthly). In certain embodiments, the longer interval increases over time or is determined based on the achievement of a desired effect.

Before administration of a full dose of the iRNA, patients can be administered a smaller dose, such as a 5% infusion dose, and monitored for adverse effects, such as an allergic reaction,

or for elevated lipid levels or blood pressure. In another example, the patient can be monitored for unwanted effects.

VIII. Methods for modulating expression of MYOC

In some aspects, the disclosure provides a method for modulating (*e.g.*, inhibiting or activating) the expression of MYOC, *e.g.*, in a cell, in a tissue, or in a subject. In some embodiments, the cell or tissue is *ex vivo*, *in vitro*, or *in vivo*. In some embodiments, the cell or tissue is in the eye (*e.g.*, a trabecular meshwork tissue, a ciliary body, a retinal pigment epithelium (RPE), a retinal tissue, an astrocyte, a pericyte, a Müller cell, a ganglion cell, an endothelial cell, a photoreceptor cell, a retinal blood vessel (e.g., including endothelial cells and vascular smooth muscle cells), or choroid tissue, e.g., a choroid vessel). In some embodiments, the cell or tissue is in a subject (*e.g.*, a mammal, such as, for example, a human). In some embodiments, the subject (*e.g.*, the human) is at risk, or is diagnosed with a disorder related to expression of MYOC expression, as described herein.

In some embodiments, the method includes contacting the cell with an iRNA as described herein, in an amount effective to decrease the expression of MYOC in the cell. In some embodiments, 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. In some embodiments, the RNAi agent is put into physical contact with the cell by the individual performing the method, or the RNAi agent may be put into a situation that will permit or cause it to subsequently come into contact with the cell. 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, by injecting the RNAi agent into or near the tissue where the cell is located, or by injecting the RNAi agent into another area, *e.g.*, ocular tissue. 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/US2019/031170 which is incorporated herein by reference in its entirety, including the passages therein describing lipophilic moieties, that directs or otherwise stabilizes the RNAi agent at a site of interest. 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.

The expression of MYOC may be assessed based on the level of expression of MYOC mRNA, MYOC protein, or the level of another parameter functionally linked to the level of expression of MYOC. In some embodiments, the expression of MYOC is inhibited by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%. In some embodiments, the iRNA has an IC_{50} in the range of 0.001-0.01 nM, 0.001-0.10 nM, 0.001-1.0 nM, 0.001-10 nM, 0.01-0.05 nM, 0.01-0.50 nM, 0.02-0.60 nM, 0.01-1.0 nM, 0.01-1.5 nM, 0.01-10 nM. The IC_{50} value may be normalized relative to an appropriate control value, *e.g.*, the IC_{50} of a non-targeting iRNA.

In some embodiments, the method includes introducing into the cell or tissue an iRNA as described herein and maintaining the cell or tissue for a time sufficient to obtain degradation of the mRNA transcript of MYOC, thereby inhibiting the expression of MYOC in the cell or tissue.

In some embodiments, the method includes administering a composition described herein, *e.g.*, a composition comprising an iRNA that binds MYOC, to the mammal such that expression of the target MYOC is decreased, such as for an extended duration, *e.g.*, at least two, three, four days or more, *e.g.*, one week, two weeks, three weeks, or four weeks or longer. In some embodiments, the decrease in expression of MYOC is detectable within 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, or 24 hours of the first administration.

In some embodiments, the method includes administering a composition as described herein to a mammal such that expression of the target MYOC is increased by e.g., at least 10% compared to an untreated animal. In some embodiments, the activation of MYOC occurs over an extended duration, e.g., at least two, three, four days or more, e.g., one week, two weeks, three weeks, four weeks, or more. Without wishing to be bound by theory, an iRNA can activate MYOC expression by stabilizing the MYOC mRNA transcript, interacting with a promoter in the genome, or inhibiting an inhibitor of MYOC expression.

The iRNAs useful for the methods and compositions featured in the disclosure specifically target RNAs (primary or processed) of MYOC. Compositions and methods for inhibiting the expression of MYOC using iRNAs can be prepared and performed as described elsewhere herein.

In some embodiments, the method includes administering a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of MYOC of the subject, *e.g.*, the mammal, *e.g.*, the human, to be treated. The composition may be administered by any appropriate means known in the art including, but not limited to ocular (*e.g.*, intraocular), topical, and intravenous administration.

In certain embodiments, the composition is administered intraocularly (*e.g.*, by intravitreal administration, e.g., intravitreal injection; transscleral administration, e.g., transscleral injection; subconjunctival administration, e.g., subconjunctival injection; retrobulbar administration, e.g., retrobulbar injection; intracameral administration, e.g., intracameral injection; or subretinal administration, e.g., subretinal injection. In other embodiments, the composition is administered topically. In other embodiments, the composition is administered by intravenous infusion or injection.

In certain embodiments, the composition is administered by intravenous infusion or injection. In some such embodiments, the composition comprises a lipid formulated siRNA (*e.g.*, an LNP formulation, such as an LNP11 formulation) for intravenous infusion.

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 disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the iRNAs and methods featured in the disclosure, 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.

Specific Embodiments

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of myocilin (MYOC), 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 a coding strand of human MYOC 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 a non-coding strand of human MYOC such that the sense strand is complementary to the at least 15 contiguous nucleotides in the antisense strand.

2. The dsRNA agent of embodiment 1, wherein the coding strand of human MYOC comprises the sequence SEQ ID NO: 1.

3. The dsRNA agent of embodiment 1 or 2, wherein the non-coding strand of human MYOC comprises the sequence of SEQ ID NO: 2.

4. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of MYOC, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of nucleotide sequence of SEQ ID NO: 2 such that the sense strand is complementary to the at least 15 contiguous nucleotides in the antisense strand.

5. The dsRNA agent of embodiment 4, wherein the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, or 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO: 1.

5a. The dsRNA agent of any of the preceding embodiments, wherein the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1565804 and the sense

strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1565804.

5b. The dsRNA agent of any of the preceding embodiments, wherein the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1565837 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1565837.

6. The dsRNA of any of the preceding embodiments, wherein the dsRNA agent comprises a sense strand and an antisense strand, wherein the antisense strand comprises a nucleotide sequence comprising at least 17 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of nucleotide sequence of SEQ ID NO: 2 such that the sense strand is complementary to the at least 17 contiguous nucleotides in the antisense strand.

7. The dsRNA of embodiment 6, wherein the sense strand comprises a nucleotide sequence comprising at least 17 contiguous nucleotides, with 0, or 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO: 1.

8. The dsRNA of any of the preceding embodiments, wherein the dsRNA agent comprises a sense strand and an antisense strand, wherein the antisense strand comprises a nucleotide sequence comprising at least 19 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of nucleotide sequence of SEQ ID NO: 2 such that the sense strand is complementary to the at least 19 contiguous nucleotides in the antisense strand.

9. The dsRNA of embodiment 8, wherein the sense strand comprises a nucleotide sequence comprising at least 19 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO: 1.

10. The dsRNA of any of the preceding embodiments, wherein the dsRNA agent comprises a sense strand and an antisense strand, wherein the antisense strand comprises a nucleotide

sequence comprising at least 21 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, of a portion of nucleotide sequence of SEQ ID NO: 2 such that the sense strand is complementary to the at least 21 contiguous nucleotides in the antisense strand.

11. The dsRNA of embodiment 10, wherein the sense strand comprises a nucleotide sequence comprising at least 21 contiguous nucleotides, with 0, or 1, 2, or 3 mismatches, of the corresponding portion of the nucleotide sequence of SEQ ID NO: 1.

12. The dsRNA agent of any one of the preceding embodiments, wherein the portion of the sense strand is a portion within a sense strand in any one of Tables 2A and 2B.

13. The dsRNA agent of any one of the preceding embodiments, wherein the portion of the antisense strand is a portion within an antisense strand in any one of Tables 2A and 2B.

14. The dsRNA agent of any of the preceding embodiments, wherein the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from one of the antisense sequences listed in any one of Tables 2A and 2B.

15. The dsRNA agent of any of the preceding embodiments, wherein the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from a sense sequence listed in any one of Tables 2A and 2B that corresponds to the antisense sequence.

16. The dsRNA agent of any of the preceding embodiments, wherein the antisense strand comprises a nucleotide sequence comprising at least 17 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from one of the antisense sequences listed in any one of Tables 2A and 2B.

17. The dsRNA agent of any of the preceding embodiments, wherein the sense strand comprises a nucleotide sequence comprising at least 17 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from a sense sequence listed in any one of Tables 2A and 2B that corresponds to the antisense sequence.

18. The dsRNA agent of any of the preceding embodiments, wherein the antisense strand comprises a nucleotide sequence comprising at least 19 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from one of the antisense sequences listed in any one of Tables 2A and 2B.

19. The dsRNA agent of any of the preceding embodiments, wherein the sense strand comprises a nucleotide sequence comprising at least 19 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from a sense sequence listed in any one of Tables 2A and 2B that corresponds to the antisense sequence.

20. The dsRNA agent of any of the preceding embodiments, wherein the antisense strand comprises a nucleotide sequence comprising at least 21 contiguous nucleotides, with 0,1, 2, or 3 mismatches, from one of the antisense sequences listed in any one of Tables 2A and 2B.

21. The dsRNA agent of any of the preceding embodiments, wherein the sense strand comprises a nucleotide sequence comprising at least 21 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from a sense sequence listed in any one of Tables 2A and 2B that corresponds to the antisense sequence.

22. The dsRNA agent of any of the preceding embodiments, wherein the sense strand is at least 23 nucleotides in length, e.g., 23-30 nucleotides in length.

23. The dsRNA agent of any of the preceding embodiments, wherein at least one of the sense strand and the antisense strand is conjugated to one or more lipophilic moieties.

24. The dsRNA agent of embodiment 23, wherein the lipophilic moiety is conjugated to one or more positions in the double stranded region of the dsRNA agent.

25. The dsRNA agent of embodiment 23 or 24, wherein the lipophilic moiety is conjugated via a linker or carrier.

26. The dsRNA agent of any one of embodiments 23-25, wherein lipophilicity of the lipophilic moiety, measured by logKow, exceeds 0.

27. The dsRNA agent of any one of the preceding embodiments, wherein 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.

28. The dsRNA agent of embodiment 27, wherein the plasma protein binding assay is an electrophoretic mobility shift assay using human serum albumin protein.

29. The dsRNA agent of any of the preceding embodiments, wherein the dsRNA agent comprises at least one modified nucleotide.

30. The dsRNA agent of embodiment 29, wherein no more than five of the sense strand nucleotides and not more than five of the nucleotides of the antisense strand are unmodified nucleotides.

31. The dsRNA agent of embodiment 29, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

32. The dsRNA agent of any one of embodiments 29-31, wherein 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 phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a

glycol modified nucleotide, and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

33. The dsRNA agent of any of embodiments 29-31, wherein no more than five of the sense strand nucleotides and not more than five of the nucleotides of the antisense strand include modifications other than 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, unlocked nucleic acids (UNA) or glycerol nucleic acid (GNA).

34. The dsRNA agent of any of the preceding embodiments, which comprises a non-nucleotide spacer (wherein optionally the non-nucleotide spacer comprises a C3-C6 alkyl) between two of the contiguous nucleotides of the sense strand or between two of the contiguous nucleotides of the antisense strand.

35. The dsRNA agent of any of the preceding embodiments, wherein each strand is no more than 30 nucleotides in length.

36. The dsRNA agent of any of the preceding embodiments, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

37. The dsRNA agent of any of the preceding embodiments, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

38. The dsRNA agent of any of the preceding embodiments, wherein the double stranded region is 15-30 nucleotide pairs in length.

39. The dsRNA agent of embodiment 38, wherein the double stranded region is 17-23 nucleotide pairs in length.

40. The dsRNA agent of embodiment 38, wherein the double stranded region is 17-25 nucleotide pairs in length.

41. The dsRNA agent of embodiment 38, wherein the double stranded region is 23-27 nucleotide pairs in length.

42. The dsRNA agent of embodiment 38, wherein the double stranded region is 19-21 nucleotide pairs in length.

43. The dsRNA agent of embodiment 38, wherein the double stranded region is 21-23 nucleotide pairs in length.

44. The dsRNA agent of any of the preceding embodiments, wherein each strand has 19-30 nucleotides.

45. The dsRNA agent of any of the preceding embodiments, wherein each strand has 19-23 nucleotides.

46. The dsRNA agent of any of the preceding embodiments, wherein each strand has 21-23 nucleotides.

47. The dsRNA agent of any of the preceding embodiments, wherein the agent comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

48. The dsRNA agent of embodiment 47, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

49. The dsRNA agent of embodiment 48, wherein the strand is the antisense strand.

50. The dsRNA agent of embodiment 48, wherein the strand is the sense strand.

51. The dsRNA agent of embodiment 47, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

52. The dsRNA agent of embodiment 51, wherein the strand is the antisense strand.

53. The dsRNA agent of embodiment 51, wherein the strand is the sense strand.

54. The dsRNA agent of embodiment 47, wherein each of the 5'- and 3'-terminus of one strand comprises a phosphorothioate or methylphosphonate internucleotide linkage.

55. The dsRNA agent of embodiment 54, wherein the strand is the antisense strand.

56. The dsRNA agent of any of the preceding embodiments, wherein the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.

57. The dsRNA agent of embodiment 54, wherein the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

58. The dsRNA agent of any one of embodiments 23-57, wherein one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand.

59. The dsRNA agent of embodiment 58, wherein the one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand via a linker or carrier.

60. The dsRNA agent of embodiment 59, wherein the internal positions include all positions except the terminal two positions from each end of the at least one strand.

61. The dsRNA agent of embodiment 59, wherein the internal positions include all positions except the terminal three positions from each end of the at least one strand.

62. The dsRNA agent of any one of embodiments 59-61, wherein the internal positions exclude a cleavage site region of the sense strand.

63. The dsRNA agent of embodiment 62, wherein the internal positions include all positions except positions 9-12, counting from the 5'-end of the sense strand.

64. The dsRNA agent of embodiment 62, wherein the internal positions include all positions except positions 11-13, counting from the 3'-end of the sense strand.

65. The dsRNA agent of any one of embodiments 59-61, wherein the internal positions exclude a cleavage site region of the antisense strand.

66. The dsRNA agent of embodiment 65, wherein the internal positions include all positions except positions 12-14, counting from the 5'-end of the antisense strand.

67. The dsRNA agent of any one of embodiments 59-61, wherein 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.

68. The dsRNA agent of any one of embodiments 23-67, wherein 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.

69. The dsRNA agent of embodiment 68, wherein 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.

70. The dsRNA agent of embodiment 24, wherein the positions in the double stranded region exclude a cleavage site region of the sense strand.

71. The dsRNA agent of any one of embodiments 23-70, wherein 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.

72. The dsRNA agent of embodiment 71, wherein the lipophilic moiety is conjugated to position 21, position 20, position 15, position 1, or position 7 of the sense strand.

73. The dsRNA agent of embodiment 71, wherein the lipophilic moiety is conjugated to position 21, position 20, or position 15 of the sense strand.

74. The dsRNA agent of embodiment 71, wherein the lipophilic moiety is conjugated to position 20 or position 15 of the sense strand.

75. The dsRNA agent of embodiment 71, wherein the lipophilic moiety is conjugated to position 16 of the antisense strand.

76. The dsRNA agent of embodiment 71, wherein the lipophilic moiety is conjugated to position 6, counting from the 5'-end of the sense strand.

77. The dsRNA agent of any one of embodiments 23-76, wherein the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound.

78. The dsRNA agent of embodiment 77, wherein 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, geranyloxyhexyanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine.

79. The dsRNA agent of embodiment 78, wherein 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 alkyne.

80. The dsRNA agent of embodiment 79, wherein the lipophilic moiety contains a saturated or unsaturated C6-C18 hydrocarbon chain.

81. The dsRNA agent of embodiment 79, wherein the lipophilic moiety contains a saturated or unsaturated C16 hydrocarbon chain.

82. The dsRNA agent of any one of embodiments 23-81, wherein 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.

83. The dsRNA agent of embodiment 82, wherein 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.

84. The dsRNA agent of any one of embodiments 23-81, wherein 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.

85. The double-stranded iRNA agent of any one of embodiments 23-84, wherein the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

86. The dsRNA agent of any one of embodiments 23-85, wherein the lipophilic moiety 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.

87. The dsRNA agent of any one of embodiments 23-86, wherein 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.

88. The dsRNA agent of any one of embodiments 23-87, further comprising a targeting ligand, e.g., a ligand that targets an ocular tissue or a liver tissue.

89. The dsRNA agent of embodiment 88, wherein the ligand is conjugated to the sense strand.

90. The dsRNA agent of embodiment 88 or 89, wherein the ligand is conjugated to the 3' end or the 5' end of the sense strand.

91. The dsRNA agent of embodiment 88 or 89, wherein the ligand is conjugated to the 3' end of the sense strand.

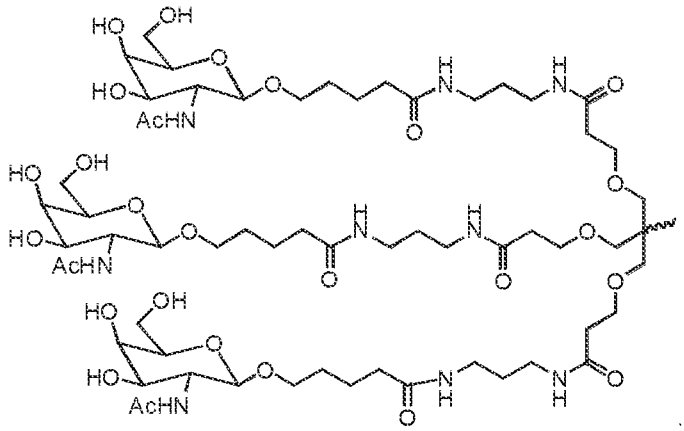
92. The dsRNA agent of any one of embodiments 88-91, wherein the ocular tissue is a trabecular meshwork tissue, a ciliary body, a retinal tissue, a retinal pigment epithelium (RPE) or choroid tissue, e.g., a choroid vessel.

93. The dsRNA agent of any one of embodiments 88-91, wherein the targeting ligand comprises N-acetylgalactosamine (GalNAc).

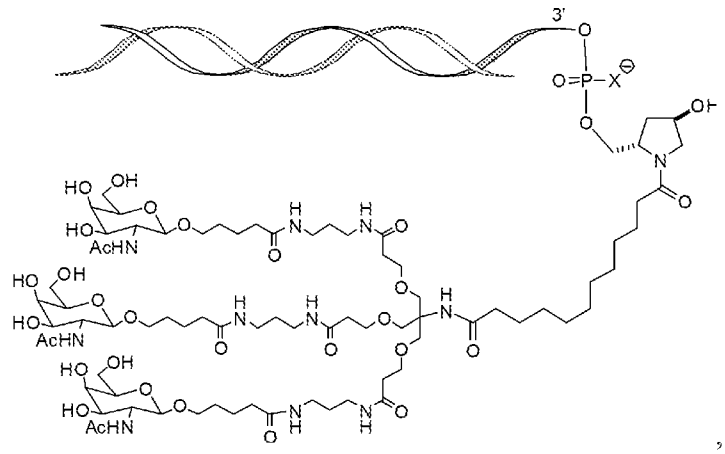
94. The dsRNA agent of any one of embodiments 88-91, wherein the targeting ligand is one or more GalNAc conjugates or one or more or GalNAc derivatives.

95. The dsRNA agent of embodiment 94, wherein the one or more GalNAc conjugates or one or more GalNAc derivatives are attached through a monovalent linker, or a bivalent, trivalent, or tetravalent branched linker.

96. The dsRNA agent of embodiment 94, wherein the ligand is



97. The dsRNA agent of embodiment 96, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

98. The dsRNA agent of embodiment 97, wherein the X is O.

99. The dsRNA agent of any one of embodiments 1-98, further comprising 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.

100. The dsRNA agent of any one of embodiments 1-98, further comprising

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.

101. The dsRNA agent of any one of embodiments 1-98, further comprising

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.

102. The dsRNA agent of any one of embodiments 1-98, further comprising

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.

103. The dsRNA agent of any one of embodiments 1-98, further comprising 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.

104. The dsRNA agent of any one of embodiments 1-103, further comprising a phosphate or phosphate mimic at the 5'-end of the antisense strand.

105. The dsRNA agent of embodiment 104, wherein the phosphate mimic is a 5'-vinyl phosphonate (VP).

106. A cell containing the dsRNA agent of any one of embodiments 1-105.

107. A human ocular cell, e.g., (a cell of the trabecular meshwork, a cell of the ciliary body, an RPE cell, an astrocyte, a pericyte, a Müller cell, a ganglion cell, an endothelial cell, or a photoreceptor cell) comprising a reduced level of MYOC mRNA or a level of MYOC protein as compared to an otherwise similar untreated cell, wherein optionally the level is reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%.

108. The human cell of embodiment 107, which was produced by a process comprising contacting a human cell with the dsRNA agent of any one of embodiments 1-94.

109. A pharmaceutical composition for inhibiting expression of MYOC, comprising the dsRNA agent of any one of embodiments 1-105.

110. A pharmaceutical composition comprising the dsRNA agent of any one of embodiments 1-105 and a lipid formulation.

111. A method of inhibiting expression of MYOC in a cell, the method comprising:

(a) contacting the cell with the dsRNA agent of any one of embodiments 1-105, or a pharmaceutical composition of embodiment 109 or 110; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of MYOC, thereby inhibiting expression of MYOC in the cell.

112. A method of inhibiting expression of MYOC in a cell, the method comprising:

(a) contacting the cell with the dsRNA agent of any one of embodiments 1-105, or a pharmaceutical composition of embodiment 109 or 110; and

(b) maintaining the cell produced in step (a) for a time sufficient to reduce levels of MYOC mRNA, MYOC protein, or both of MYOC mRNA and protein, thereby inhibiting expression of MYOC in the cell.

113. The method of embodiment 111 or 112, wherein the cell is within a subject.

114. The method of embodiment 113, wherein the subject is a human.

115. The method of any one of embodiments 111-114, wherein the level of MYOC mRNA is inhibited by at least 50%.

116. The method of any one of embodiments 111-114, wherein the level of MYOC protein is inhibited by at least 50%.

117. The method of embodiment 114-116, wherein inhibiting expression of MYOC decreases a MYOC protein level in a biological sample (*e.g.*, an aqueous ocular fluid sample) from the subject by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%.

118. The method of any one of embodiments 114-117, wherein the subject has been diagnosed with a MYOC-associated disorder, *e.g.*, glaucoma, *e.g.*, primary open angle glaucoma (POAG).

119. A method of inhibiting expression of MYOC in an ocular cell or tissue, the method comprising:

(a) contacting the cell or tissue with a dsRNA agent that binds MYOC; and

(b) maintaining the cell or tissue produced in step (a) for a time sufficient to reduce levels of MYOC mRNA, MYOC protein, or both of MYOC mRNA and protein, thereby inhibiting expression of MYOC in the cell or tissue.

120. The method of embodiment 119, wherein the ocular cell or tissue comprises a trabecular meshwork tissue, a ciliary body, an RPE cell, a retinal tissue, an astrocyte, a pericyte, a Müller cell, a ganglion cell, an endothelial cell, a photoreceptor cell, a retinal blood vessel (e.g., including endothelial cells and vascular smooth muscle cells), or choroid tissue, e.g., a choroid vessel.

120a. A method of reducing intraocular pressure in a subject, comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of embodiments 1-105 or a pharmaceutical composition of embodiment 109 or 110, thereby reducing intraocular pressure in the subject.

120b. A method of limiting an increase in intraocular pressure, or maintaining a constant intraocular pressure, in a subject, comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of embodiments 1-105 or a pharmaceutical composition of embodiment 109 or 110, thereby limiting the increase in intraocular pressure, or maintaining a constant intraocular pressure in the subject.

121. A method of treating a subject having, or diagnosed with having, a MYOC-associated disorder comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of embodiments 1-105 or a pharmaceutical composition of embodiment 109 or 110, thereby treating the disorder.

122. The method of embodiment 118 or 121, wherein the MYOC-associated disorder is glaucoma.

122a. A method of treating a subject having glaucoma, comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of embodiments 1-105 or a pharmaceutical composition of embodiment 109 or 110, thereby treating the glaucoma.

123. The method of embodiment 122 or 122a, wherein glaucoma is selected from the group consisting of primary open angle glaucoma (POAG).

124. The method of any one of embodiments 121-123, wherein treating comprises amelioration of at least one sign or symptom of the disorder.

125. The method of embodiment 124, wherein at least one sign or symptom of glaucoma comprises a measure of one or more of optic nerve damage, vision loss, tunnel vision, blurred vision, eye pain or presence, level, or activity of MYOC (*e.g.*, MYOC gene, MYOC mRNA, or MYOC protein).

126. The method of any one of embodiments 121-123, where treating comprises prevention of progression of the disorder.

127. The method of any one of embodiments 124-126, wherein the treating comprises one or more of (a) inhibiting or reducing the expression or activity of MYOC; (b) reducing the level of misfolded MYOC protein; (c) reducing trabecular meshwork cell death; (d) decreasing intraocular pressure; or (e) increasing visual acuity.

128. The method of embodiment 127, wherein the treating results in at least a 30% mean reduction from baseline of MYOC mRNA in the trabecular meshwork tissue, ciliary body, retina, RPE, a retinal blood vessel (e.g., including endothelial cells and vascular smooth muscle cells), or choroid tissue, e.g., a choroid vessel.

129. The method of embodiment 128 wherein the treating results in at least a 60% mean reduction from baseline of MYOC mRNA in the trabecular meshwork tissue, ciliary body, retina, RPE, a retinal blood vessel (e.g., including endothelial cells and vascular smooth muscle cells), or choroid tissue, e.g., a choroid vessel.

130. The method of embodiment 129, wherein the treating results in at least a 90% mean reduction from baseline of MYOC mRNA in the trabecular meshwork tissue, ciliary body, retina, RPE, a retinal blood vessel (e.g., including endothelial cells and vascular smooth muscle cells), or choroid tissue, e.g., a choroid vessel.

131. The method of any one of embodiments 124-129, wherein after treatment the subject experiences at least an 8-week duration of knockdown following a single dose of dsRNA as assessed by MYOC protein in the retina.

132. The method of embodiment 131, wherein treating results in at least a 12-week duration of knockdown following a single dose of dsRNA as assessed by MYOC protein in the retina.

133. The method of embodiment 132, wherein treating results in at least a 16-week duration of knockdown following a single dose of dsRNA as assessed by MYOC protein in the retina.

134. The method of any of embodiments 113-133, wherein the subject is human.

135. The method of any one of embodiments 114-134, wherein the dsRNA agent is administered at a dose of about 0.01 mg/kg to about 50 mg/kg.

136. The method of any one of embodiments 114-135, wherein the dsRNA agent is administered to the subject intraocularly, intravenously, or topically.

137. The method of embodiment 136, wherein the intraocular administration comprises intravitreal administration (e.g., intravitreal injection), transscleral administration (e.g., transscleral injection), subconjunctival administration (e.g., subconjunctival injection), retrobulbar administration (e.g., retrobulbar injection), intracameral administration (e.g., intracameral injection), or subretinal administration (e.g., subretinal injection).

138. The method of any one of embodiments 114-137, further comprising measuring level of MYOC (*e.g.*, MYOC gene, MYOC mRNA, or MYOC protein) in the subject.

139. The method of embodiment 138, where measuring the level of MYOC in the subject comprises measuring the level of MYOC gene, MYOC protein or MYOC mRNA in a biological sample from the subject (*e.g.*, an aqueous ocular fluid sample).

140. The method of any one of embodiments 114-139, further comprising performing a blood test, an imaging test, or an aqueous ocular fluid biopsy.

141. The method of any one of embodiments 138-140, wherein measuring level of MYOC (*e.g.*, MYOC gene, MYOC mRNA, or MYOC protein) in the subject is performed prior to treatment with the dsRNA agent or the pharmaceutical composition.

142. The method of embodiment 141, wherein, upon determination that a subject has a level of MYOC (*e.g.*, MYOC gene, MYOC mRNA, or MYOC protein) that is greater than a reference level, the dsRNA agent or the pharmaceutical composition is administered to the subject.

143. The method of any one of embodiments 139-142, wherein measuring level of MYOC (*e.g.*, MYOC gene, MYOC mRNA, or MYOC protein) in the subject is performed after treatment with the dsRNA agent or the pharmaceutical composition.

144. The method of any one of embodiments 121-143, further comprising administering to the subject an additional agent and/or therapy suitable for treatment or prevention of an MYOC-associated disorder.

145. The method of embodiment 144, wherein the additional agent and/or therapy comprises one or more of a photodynamic therapy, photocoagulation therapy, a steroid, a non-steroidal anti-inflammatory agent, an anti-MYOC agent, and/or a vitrectomy.

EXAMPLES

Example 1. MYOC siRNA

Nucleic acid sequences provided herein are represented using standard nomenclature. *See* the abbreviations of Table 1.

Table 1. Abbreviations of nucleotide monomers used in nucleic acid sequence representation

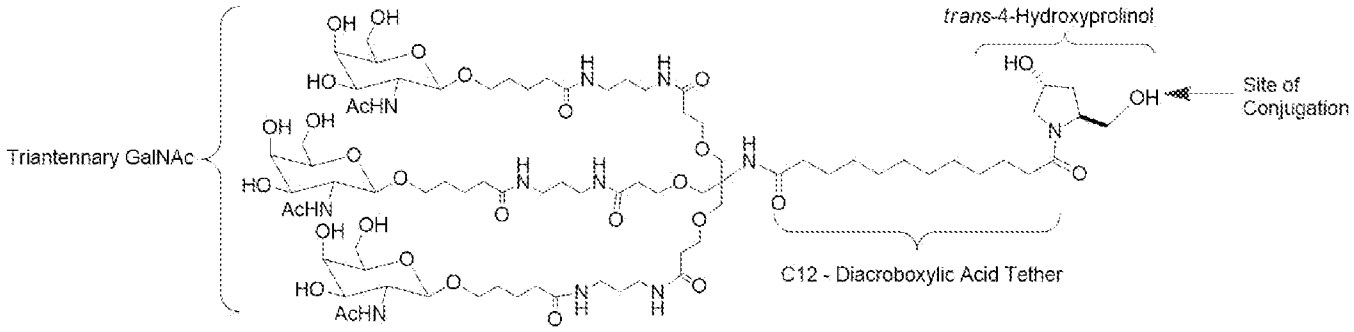
It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation	Nucleotide(s)
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3'-phosphate
Abs	beta-L-adenosine-3'-phosphorothioate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
(Ahd)	2'-O-hexadecyl-adenosine-3'-phosphate
(Ahds)	2'-O-hexadecyl-adenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
(A2p)	adenosine 2'-phosphate
(A2ps)	adenosine-2'-phosphorothioate
C	cytidine-3'-phosphate
Cb	beta-L-cytidine-3'-phosphate
Cbs	beta-L-cytidine-3'-phosphorothioate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Chds)	2'-O-hexadecyl-cytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
(C2p)	cytosine 2'-phosphate
(C2ps)	cytidine-2'-phosphorothioate
G	guanosine-3'-phosphate
Gb	beta-L-guanosine-3'-phosphate
Gbs	beta-L-guanosine-3'-phosphorothioate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
(Ghd)	2'-O-hexadecyl-guanosine-3'-phosphate
(Ghds)	2'-O-hexadecyl-guanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
(G2p)	guanosine 2'-phosphate
(G2ps)	guanosine-2'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tb	beta-L-thymidine-3'-phosphate

Tbs	beta-L-thymidine-3'-phosphorothioate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Tgn	thymidine-glycol nucleic acid (GNA) S-Isomer
Agn	adenosine- glycol nucleic acid (GNA) S-Isomer
Cgn	cytidine-glycol nucleic acid (GNA) S-Isomer
Ggn	guanosine-glycol nucleic acid (GNA) S-Isomer
Ts	5-methyluridine-3'-phosphorothioate
(T2p)	thymidine 2'-phosphate
U	Uridine-3'-phosphate
Ub	beta-L-uridine-3'-phosphate
Ubs	beta-L-uridine-3'-phosphorothioate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine -3'-phosphorothioate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate
(Uhds)	2'-O-hexadecyl-uridine-3'-phosphorothioate
Us	uridine -3'-phosphorothioate
(U2p)	uracil 2'-phosphate
(U2ps)	uridine-2'-phosphorothioate
N	any nucleotide (G, A, C, T or U)
VP	Vinyl phosphonate
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
dA	2'-deoxyadenosine-3'-phosphate
dAs	2'-deoxyadenosine-3'-phosphorothioate
dC	2'-deoxycytidine-3'-phosphate
dCs	2'-deoxycytidine-3'-phosphorothioate
dG	2'-deoxyguanosine-3'-phosphate
dGs	2'-deoxyguanosine-3'-phosphorothioate
dT	2'-deoxythymidine
dTs	2'-deoxythymidine-3'-phosphorothioate
dU	2'-deoxyuridine
s	phosphorothioate linkage
L96[1]	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3
(Aeo)	2'-O-methoxyethyladenosine-3'-phosphate
(Aeos)	2'-O-methoxyethyladenosine-3'-phosphorothioate

(Geo)	2'-O-methoxyethylguanosine-3'-phosphate
(Geos)	2'-O-methoxyethylguanosine-3'- phosphorothioate
(Teo)	2'-O-methoxyethyl-5-methyluridine-3'-phosphate
(Teos)	2'-O-methoxyethyl-5-methyluridine-3'- phosphorothioate
(m5Ceo)	2'-O-methoxyethyl-5-methylcytidine-3'-phosphate
(m5Ceos)	2'-O-methoxyethyl-5-methylcytidine-3'- phosphorothioate

[1]The chemical structure of L96 is as follows:



Experimental Methods

Bioinformatics

Transcripts

Four sets of siRNAs targeting the human MYOC, "myocilin" (human: NCBI refseqID NM_000261.2 ; NCBI GeneID: 4653) were generated. The human NM_000261.2 REFSEQ mRNA has a length of 2100 bases. Pairs of oligos were generated using bioinformatic methods and ranked, and exemplary pairs of oligos are shown in Table 2A and Table 2B. Modified sequences are presented in Table 2A. Unmodified sequences are presented in Table 2B.

Table 2A. Exemplary Human MYOC siRNA Modified Single Strands and Duplex Sequences

Column 1 indicates duplex name and the number following the decimal point in a duplex name merely refers to a batch production number. Column 2 indicates the name of the sense sequence. Column 3 indicates the sequence ID for the sequence of column 4. Column 4 provides the modified sequence of a sense strand suitable for use in a duplex described herein. Column 5 indicates the antisense sequence name. Column 6 indicates the sequence ID for the sequence of column 7. Column 7 provides the sequence of a modified antisense strand suitable for use in a duplex described herein, e.g., a duplex comprising the sense sequence in the same row of the table. Column 8 indicates the position in the target mRNA (NM_000261.2) that is complementary to the antisense strand of Column 7. Column 9 indicated the sequence ID for the sequence of column 8.

Duplex Name	Sense sequence name	Seq ID NO: (sense)	Sense sequence (5'-3')	Antisense sequence name	Seq ID NO: (anti sense)	Antisense sequence (5'-3')	mRNA target sequence in NM_000261.2	SEQ ID NO: (mRNA target)
AD-1565444.1	A-2893965.1	7	csuscuc(Ahd)GfcAfCfAfgcagagcususa	A-2893966.1	451	VPusAfsadGc(Tgn)cugcuguGfcUfgagagsgsu	ACCUCUCAGCACAGCAGAGCUUU	895
AD-1565445.1	A-2893965.1	8	csuscuc(Ahd)GfcAfCfAfgcagagcususa	A-2893967.1	452	VPusAfsagcdTc(Tgn)gcuguGfcUfgagagsgsu	ACCUCUCAGCACAGCAGAGCUUU	896
AD-1565692.1	A-2893965.1	9	csuscuc(Ahd)GfcAfCfAfgcagagcususa	A-2894431.1	453	VPusAfsagdCu(C2p)ugcuguGfcUfgagagsgsu	ACCUCUCAGCACAGCAGAGCUUU	897
AD-1565446.1	A-2893968.1	10	uscsucag(Chd)aCfAfGfcagagcuususa	A-2893969.1	454	VPusAfsaadGc(Tgn)cugcugUfgCfugagasgsg	CCUCUCAGCACAGCAGAGCUUUC	898
AD-1565447.1	A-2893970.1	11	csasgcag(Ahd)gCfUfUfuccagaggsasa	A-2893971.1	455	VPusUfsccdTc(Tgn)ggaaagCfuCfugcugsusg	CACAGCAGAGCUUUCCAGAGGAA	899

AD-1565448.1	A-2893972.1	12	asasug(Ahd)gGfuUfCfUfucugugcascsa	A-2893973.1	456	VPusGfsudGc(Agn)cagaagaAfcCfucauusgsc	GCAAUGAGGUUCUUCUGUGCACG	900
AD-1565693.1	A-2893972.1	13	asasug(Ahd)gGfuUfCfUfucugugcascsa	A-2894432.1	457	VPusGfsugdCa(C2p)agaagaAfcCfucauusgsc	GCAAUGAGGUUCUUCUGUGCACG	901
AD-1565449.1	A-2893974.1	14	asusgagg(Uhd)uCfUfUfcugugcacsgsa	A-2893975.1	458	VPusCfsgudGc(Agn)cagaagAfaCfcucaususg	CAAUGAGGUUCUUCUGUGCACGU	902
AD-1565450.1	A-2893976.1	15	usgsagg(Uhd)UfcUfUfCfugugcacgsusa	A-2893977.1	459	VPusAfscgudGc(Agn)cagaaGfaAfccucasusu	AAUGAGGUUCUUCUGUGCACGUU	903
AD-1565694.1	A-2893976.1	16	usgsagg(Uhd)UfcUfUfCfugugcacgsusa	A-2894433.1	460	VPusAfscgdTg(C2p)acagaaGfaAfccucasusu	AAUGAGGUUCUUCUGUGCACGUU	904
AD-1565695.1	A-2894434.1	17	gsasggu(Uhd)CfuUfCfUfgugcacgususa	A-2894435.1	461	VPusAfsacdGu(G2p)cacagaAfgAfaccucsasu	AUGAGGUUCUUCUGUGCACGUUG	905
AD-1193175.5	A-2058874.1	18	asgsguu(Chd)UfuCfUfGfugcacguusgsa	A-1801665.1	462	VPusCfsaacGfuGfCfacagAfaGfaaccuscsa	UGAGGUUCUUCUGUGCACGUUGC	906
AD-1565793.1	A-2893978.1	19	asgsguu(Chd)uuCfUfGfugcacguusgsa	A-2894582.1	463	VPusdCsaadCgdTgcacdAgAfagaaccuscsg	UGAGGUUCUUCUGUGCACGUUGC	907
AD-1565795.1	A-2058874.1	20	asgsguu(Chd)UfuCfUfGfugcacguusgsa	A-2894585.1	464	VPusCfsaadCg(Tgn)gcacagAfaGfaaccuscsg	UGAGGUUCUUCUGUGCACGUUGC	908
AD-1565796.1	A-2058874.1	21	asgsguu(Chd)UfuCfUfGfugcacguusgsa	A-2894586.1	465	VPudCsaadCg(Tgn)gcacdAgAfagaaccuscsg	UGAGGUUCUUCUGUGCACGUUGC	909
AD-1565797.1	A-2894587.1	22	asgsgua(Chd)UfuCfUfGfugcacguusgsa	A-2894588.1	466	VPusCfsaadCg(Tgn)gcacagAfaGfuaccuscsg	UGAGGUUCUUCUGUGCACGUUGC	910

AD-1565798.1	A-2894589.1	23	asgsgau(Chd)UfuCfUfGfugcacguusgsa	A-2894590.1	467	VPusCfsaadCg(Tgn)gcacagAfaGfauccuscsg	UGAGGUUCUUCUGUGCACGUUGC	911
AD-1565799.1	A-2894591.1	24	asgscuu(Chd)UfuCfUfGfugcacguusgsa	A-2894592.1	468	VPusCfsaadCg(Tgn)gcacagAfaGfaagcuscsg	UGAGGUUCUUCUGUGCACGUUGC	912
AD-1565451.1	A-2893978.1	25	asgsguu(Chd)uuCfUfGfugcacguusgsa	A-2893979.1	469	VPusdCsaadCgdTgcacdAgAfagaaccuscsa	UGAGGUUCUUCUGUGCACGUUGC	913
AD-1565452.1	A-2893980.1	26	gsgsuuc(Uhd)ucUfGfUfgcacguugscsa	A-2893981.1	470	VPusdGscadAcdGugcadCaGfaagaaccsusc	GAGGUUCUUCUGUGCACGUUGCU	914
AD-1565696.1	A-2894436.1	27	gsgsuuc(Uhd)UfcUfGfUfgcacguugscsa	A-2894437.1	471	VPusGfscadAc(G2p)ugcacaGfaAfgaaccsusc	GAGGUUCUUCUGUGCACGUUGCU	915
AD-1565794.1	A-2894583.1	28	gsusucu(Uhd)CfUfGfugcacguusgsa	A-2894584.1	472	VPusdCsaadCgdTgcacdAgAfagaacscsu	AGGUUCUUCUGUGCACGUUGC	916
AD-1565453.1	A-2893982.1	29	gsusucu(Uhd)cuGfUfGfcacguugcsusa	A-2893983.1	473	VPusdAsgcdAadCgugcdAcAfgaagaacscsu	AGGUUCUUCUGUGCACGUUGCUG	917
AD-1565454.1	A-2893984.1	30	ususcuu(Chd)ugUfGfCfacguugcusgsa	A-2893985.1	474	VPusdCsagdCadAcgugdCaCfagaagaascsc	GGUUCUUCUGUGCACGUUGCUGC	918
AD-1565455.1	A-2893986.1	31	uscsuuc(Uhd)GfuGfCfAfcguugcugscsa	A-2893987.1	475	VPusGfscadGc(Agn)acgugcAfcAfgaagasasc	GUUCUUCUGUGCACGUUGCUGCA	919
AD-1565456.1	A-2893988.1	32	uscsuuc(Uhd)guGfCfAfcguugcugscsa	A-2893989.1	476	VPusdGscadGcdAacgudGcAfcagaagasasc	GUUCUUCUGUGCACGUUGCUGCA	920
AD-1565457.1	A-2893990.1	33	csusucug(Uhd)gCfAfCfguugcugcsasa	A-2893991.1	477	VPusUfsgcadGc(Agn)acgugCfaCfagaagsasa	UUCUUCUGUGCACGUUGCUGCAG	921

AD-1565697.1	A-2893990.1	34	csusucug(Uhd)gCfAfCfguugcugcsasa	A-2894438.1	478	VPusUfsgcdAg(C2p)aacgugCfaCfagaagsasa	UUCUUCUGUGCACGUUGCUGCAG	922
AD-1565698.1	A-2894439.1	35	ususcug(Uhd)GfcAfCfGfuugcugcasgsa	A-2894440.1	479	VPusCfsugdCa(G2p)caacguGfcAfcagaasgsa	UCUUCUGUGCACGUUGCUGCAGC	923
AD-1565458.1	A-2893992.1	36	uscsugug(Chd)aCfGfUfugcugcagscsa	A-2893993.1	480	VPusGfscudGc(Agn)gcaacgUfgCfacagasasg	CUUCUGUGCACGUUGCUGCAGCU	924
AD-1565459.1	A-2893994.1	37	csusgug(Chd)AfcGfUfUfgcugcagcsusa	A-2893995.1	481	VPusAfsgcudGc(Agn)gcaacGfuGfcacagsasa	UUCUGUGCACGUUGCUGCAGCUU	925
AD-1565699.1	A-2893994.1	38	csusgug(Chd)AfcGfUfUfgcugcagcsusa	A-2894441.1	482	VPusAfsgcdTg(C2p)agcaacGfuGfcacagsasa	UUCUGUGCACGUUGCUGCAGCUU	926
AD-1565700.1	A-2894442.1	39	usgsugc(Ahd)CfgUfUfGfcugcagcususa	A-2894443.1	483	VPusAfsagdCu(G2p)cagcaaCfgUfgcacasgsa	UCUGUGCACGUUGCUGCAGCUUU	927
AD-1565460.1	A-2893996.1	40	gsusgca(Chd)GfuUfGfCfugcagcuususa	A-2893997.1	484	VPusAfsaadGc(Tgn)gcagcaAfcGfugcacsasg	CUGUGCACGUUGCUGCAGCUUUG	928
AD-1565461.1	A-2893998.1	41	gscsacg(Uhd)ugCfUfGfcagcuuugsgsa	A-2893999.1	485	VPusdCscadAadGcugcdAgCfaacgugcsasc	GUGCACGUUGCUGCAGCUUUGGG	929
AD-1565462.1	A-2894000.1	42	csascgu(Uhd)gcUfGfCfagcuuuggsgsa	A-2894001.1	486	VPusdCsccdAadAgcugdCaGfcaacgugscsa	UGCACGUUGCUGCAGCUUUGGGC	930
AD-1565701.1	A-2894444.1	43	gsasgaug(Chd)cAfGfCfuguccagcsusa	A-2894445.1	487	VPusAfsgcdTg(G2p)acagcuGfgCfaucucsasg	CUGAGAUGCCAGCUGUCCAGCUG	931
AD-1565463.1	A-2894002.1	44	gsasugc(Chd)AfgCfUfGfuccagcugscsa	A-2894003.1	488	VPusGfscadGc(Tgn)ggacagCfuGfgcaucsusc	GAGAUGCCAGCUGUCCAGCUGCU	932

AD-1565464.1	A-2894004.1	45	csusguc(Chd)agCfUfGfcugcuucusgsa	A-2894005.1	489	VPusdCsagdAadGcagcdAgCfuggacagscsu	AGCUGUCCAGCUGCUGCUUCUGG	933
AD-1565465.1	A-2894006.1	46	cscsagc(Uhd)GfcUfGfCfuucuggccsusa	A-2894007.1	490	VPusAfsggdCc(Agn)gaagcaGfcAfgcuggsasc	GUCCAGCUGCUGCUUCUGGCCUG	934
AD-1565466.1	A-2894008.1	47	csusggc(Chd)UfgCfCfUfgggugugggsasa	A-2894009.1	491	VPusUfscdCc(Agn)caccaggCfaGfgccagsasa	UUCUGGCCUGCCUGGUGUGGGAU	935
AD-1565467.1	A-2894008.1	48	csusggc(Chd)UfgCfCfUfgggugugggsasa	A-2894010.1	492	VPusUfsccdAc(Agn)ccaggCfaGfgccagsasa	UUCUGGCCUGCCUGGUGUGGGAU	936
AD-1565702.1	A-2894008.1	49	csusggc(Chd)UfgCfCfUfgggugugggsasa	A-2894446.1	493	VPusUfsccdCa(C2p)accaggCfaGfgccagsasa	UUCUGGCCUGCCUGGUGUGGGAU	937
AD-1565468.1	A-2894011.1	50	usgsgcc(Uhd)GfcCfUfGfgugugggasusa	A-2894012.1	494	VPusAfsucdCc(Agn)caccagGfcAfggccasgsa	UCUGGCCUGCCUGGUGUGGGAUG	938
AD-1565469.1	A-2894013.1	51	gscscug(Chd)CfuGfGfUfgugugaugsusa	A-2894014.1	495	VPusAfscadTcdAcacaccAfgGfcaggcscsa	UGGCCUGCCUGGUGUGGGAUGUG	939
AD-1565703.1	A-2894447.1	52	gscscug(Chd)CfuGfGfUfgugggaugsusa	A-2894448.1	496	VPusAfscadTc(C2p)caccaccAfgGfcaggcscsa	UGGCCUGCCUGGUGUGGGAUGUG	940
AD-1565704.1	A-2894449.1	53	asgsag(Uhd)gGfcCfGfAfugccaguasusa	A-2894450.1	497	VPusAfsuadCu(G2p)gcaucgGfcCfacucusgsg	CCAGAGUGGCCGAUGCCAGUAUA	941
AD-1565705.1	A-2894451.1	54	asgsug(Uhd)gGfcCfAfGfucccaaugsasa	A-2894452.1	498	VPusUfscadTu(G2p)ggacugGfcCfacacusgsa	UCAGUGUGGCCAGUCCCAAUGAA	942
AD-1565470.1	A-2894015.1	55	gsusgugg(Chd)cAfGfUfcccaaugasasa	A-2894016.1	499	VPusUfsucdAu(Tgn)gggacuGfgCfcacacsusg	CAGUGUGGCCAGUCCCAAUGAAU	943

AD-1565471.1	A-2894015.1	56	gsusgugg(Chd)cAfGfUfcccaaugasasa	A-2894017.1	500	VPusdTsucdAudTgggadCuGfgccacacsusg	CAGUGUGGCCAGUCCCAAUGAAU	944
AD-1565472.1	A-2894018.1	57	gscscagg(Chd)cAfUfGfucagucauscsa	A-2894019.1	501	VPusGfsadTg(Agn)cugacauGfgCfcuggcsusc	GAGCCAGGCCAUGUCAGUCAUCC	945
AD-1565473.1	A-2894018.1	58	gscscagg(Chd)cAfUfGfucagucauscsa	A-2894020.1	502	VPusGfsaugdAc(Tgn)gacauGfgCfcuggcsusc	GAGCCAGGCCAUGUCAGUCAUCC	946
AD-1565706.1	A-2894018.1	59	gscscagg(Chd)cAfUfGfucagucauscsa	A-2894453.1	503	VPusGfsaudGa(C2p)ugacauGfgCfcuggcsusc	GAGCCAGGCCAUGUCAGUCAUCC	947
AD-1565474.1	A-2894021.1	60	gsgscca(Uhd)GfuCfAfGfucauccausasa	A-2894022.1	504	VPusUfsaudGg(Agn)ugacugAfcAfuggccsusg	CAGGCCAUGUCAGUCAUCCAUAA	948
AD-1565707.1	A-2894454.1	61	gsascc(Uhd)gGfaGfGfCfcaccaaagscsa	A-2894455.1	505	VPusGfscudTu(G2p)guggccUfcCfaggucsusa	UAGACCUGGAGGCCACCAAAGCU	949
AD-1565475.1	A-2894023.1	62	csusgg(Ahd)gGfcCfAfCfcaaagcucsgsa	A-2894024.1	506	VPusCfsgadGc(Tgn)uuggugGfcCfuccagsgsu	ACCUGGAGGCCACCAAAGCUCGA	950
AD-1565476.1	A-2894025.1	63	gsasaac(Chd)CfaAfAfCfcagagagususa	A-2894026.1	507	VPusAfsacdTc(Tgn)cugguuUfgGfguuucscsa	UGGAAACCCAAACCAGAGAGUUG	951
AD-1565477.1	A-2894027.1	64	csasgca(Ahd)CfcUfCfCfcuccgagacsasa	A-2894028.1	508	VPusUfsgdTc(Tgn)cggaggaGfgUfugcugsusa	UACAGCAACCUCCUCCGAGACAA	952
AD-1565708.1	A-2894027.1	65	csasgca(Ahd)CfcUfCfCfcuccgagacsasa	A-2894456.1	509	VPusUfsgudCu(C2p)ggaggaGfgUfugcugsusa	UACAGCAACCUCCUCCGAGACAA	953
AD-1565478.1	A-2894029.1	66	gscsaac(Chd)UfcCfUfCfcgagacaasgsa	A-2894030.1	510	VPusCfsudTg(Tgn)cucggagGfaGfguugcsusg	CAGCAACCUCCUCCGAGACAAGU	954

AD-1565479.1	A-2894029.1	67	gscsaac(Chd)UfcCfUfCfcgagacaasgsa	A-2894031.1	511	VPusCfsuugdTc(Tgn)cggagGfaGfguugcsusg	CAGCAACCUCCUCCGAGACAAGU	955
AD-1565709.1	A-2894029.1	68	gscsaac(Chd)UfcCfUfCfcgagacaasgsa	A-2894457.1	512	VPusCfsuudGu(C2p)ucggagGfaGfguugcsusg	CAGCAACCUCCUCCGAGACAAGU	956
AD-1565480.1	A-2894032.1	69	asgsaca(Ahd)guCfAfGfuucuggagsgsa	A-2894033.1	513	VPusdCscudCcdAgaacdTgAfcuugucuscsg	CGAGACAAGUCAGUUCUGGAGGA	957
AD-1565481.1	A-2894034.1	70	csasagu(Chd)AfgUfUfCfuggaggaasgsa	A-2894035.1	514	VPusCfsuudCc(Tgn)ccagaaCfuGfacuugsusc	GACAAGUCAGUUCUGGAGGAAGA	958
AD-1565482.1	A-2894036.1	71	gsuscag(Uhd)UfcUfGfGfaggaagagsasa	A-2894037.1	515	VPusUfscudCu(Tgn)ccuccaGfaAfcugacsusu	AAGUCAGUUCUGGAGGAAGAGAA	959
AD-1565483.1	A-2894038.1	72	asgsaau(Chd)ugGfCfCfaggagguusgsa	A-2894039.1	516	VPusdCsaadCcdTccugdGcCfagauucuscsa	UGAGAAUCUGGCCAGGAGGUUGG	960
AD-1565484.1	A-2894040.1	73	usgsgcc(Ahd)GfgAfGfGfuuggaaagscsa	A-2894041.1	517	VPusGfscdTu(Tgn)ccaaccuCfcUfggccasgsa	UCUGGCCAGGAGGUUGGAAAGCA	961
AD-1565710.1	A-2894040.1	74	usgsgcc(Ahd)GfgAfGfGfuuggaaagscsa	A-2894458.1	518	VPusGfscudTu(C2p)caaccuCfcUfggccasgsa	UCUGGCCAGGAGGUUGGAAAGCA	962
AD-1565711.1	A-2894459.1	75	csasgcc(Ahd)GfgAfGfGfuagcaaggscsa	A-2894460.1	519	VPusGfsccdTu(G2p)cuaccuCfcUfggcugscsu	AGCAGCCAGGAGGUAGCAAGGCU	963
AD-1565485.1	A-2894042.1	76	usgscca(Chd)CfaGfGfCfuccagagasasa	A-2894043.1	520	VPusUfsucdTc(Tgn)ggagccUfgGfuggcascsa	UGUGCCACCAGGCUCCAGAGAAG	964
AD-1565486.1	A-2894044.1	77	asasuu(Uhd)gGfaCfAfCfuuuggccususa	A-2894045.1	521	VPusAfsaggdCc(Agn)aagugUfcCfaaauuscsc	GGAAUUUGGACACUUUGGCCUUC	965

AD-1565712.1	A-2894044.1	78	asasuu(Uhd)gGfaCfAfCfuuuggccususa	A-2894461.1	522	VPusAfsagdGc(C2p)aaagugUfcCfaaauuscsc	GGAAUUUGGACACUUUGGCCUUC	966
AD-1565713.1	A-2894462.1	79	ascsacu(Uhd)UfgGfCfCfuuccaggasasa	A-2894463.1	523	VPusUfsucdCu(G2p)gaaggcCfaAfaguguscsc	GGACACUUUGGCCUUCCAGGAAC	967
AD-1565487.1	A-2894046.1	80	csusuugg(Chd)cUfUfCfcaggaacusgsa	A-2894047.1	524	VPusdCsagdTudCcuggdAaGfgccaaagsusg	CACUUUGGCCUUCCAGGAACUGA	968
AD-1565714.1	A-2894048.1	81	asgsgaa(Chd)UfgAfAfGfuccgagcusasa	A-2894464.1	525	VPusUfsagdCu(C2p)ggacuuCfaGfuuccusgsg	CCAGGAACUGAAGUCCGAGCUAA	969
AD-1565488.1	A-2894048.1	82	asgsgaa(Chd)UfgAfAfGfuccgagcusasa	A-2894049.1	526	VPusUfsadGc(Tgn)cggacuuCfaGfuuccusgsg	CCAGGAACUGAAGUCCGAGCUAA	970
AD-1565715.1	A-2894465.1	83	gsasagu(Chd)CfgAfGfCfuaacugaasgsa	A-2894466.1	527	VPusCfsuudCa(G2p)uuagcuCfgGfacuucsasg	CUGAAGUCCGAGCUAACUGAAGU	971
AD-1565716.1	A-2894467.1	84	gscsuaa(Chd)UfgAfAfGfuuccugcususa	A-2894468.1	528	VPusAfsagdCa(G2p)gaacuuCfaGfuuagcsusc	GAGCUAACUGAAGUUCCUGCUUC	972
AD-1565489.1	A-2894050.1	85	csusaac(Uhd)GfaAfGfUfuccugcuuscsa	A-2894051.1	529	VPusGfsaadGc(Agn)ggaacuUfcAfguuagscsu	AGCUAACUGAAGUUCCUGCUUCC	973
AD-1565717.1	A-2894469.1	86	gsasagu(Uhd)CfcUfGfCfuucccgaasusa	A-2894470.1	530	VPusAfsuudCg(G2p)gaagcaGfgAfacuucsasg	CUGAAGUUCCUGCUUCCCGAAUU	974
AD-1565718.1	A-2894052.1	87	gsasgaa(Chd)UfaGfUfUfuggguaggsasa	A-2894471.1	531	VPusUfsccdTa(C2p)ccaaacUfaGfuucucscsa	UGGAGAACUAGUUUGGGUAGGAG	975
AD-1565490.1	A-2894052.1	88	gsasgaa(Chd)UfaGfUfUfuggguaggsasa	A-2894053.1	532	VPusUfscdCu(Agn)cccaaacUfaGfuucucscsa	UGGAGAACUAGUUUGGGUAGGAG	976

AD-1565719.1	A-2894054.1	89	ascsuag(Uhd)UfuGfGfGfuaggagagscsa	A-2894472.1	533	VPusGfscudCu(C2p)cuacccAfaAfcuagususc	GAACUAGUUUGGGUAGGAGAGCC	977
AD-1565491.1	A-2894054.1	90	ascsuag(Uhd)UfuGfGfGfuaggagagscsa	A-2894055.1	534	VPusGfscdTc(Tgn)cuaccAfaAfcuagususc	GAACUAGUUUGGGUAGGAGAGC	978
AD-1565720.1	A-2894473.1	91	gsgsgu(Ahd)gGfaGfAfGfccucucacsgsa	A-2894474.1	535	VPusCfsgudGa(G2p)aggcucUfcCfuacccsasa	UUGGGUAGGAGAGCCUCUCACGC	979
AD-1565721.1	A-2894475.1	92	gsusagg(Ahd)GfaGfCfCfucucacgcsusa	A-2894476.1	536	VPusAfsgcdGu(G2p)agaggcUfcUfccuacscsc	GGGUAGGAGAGCCUCUCACGCUG	980
AD-1565492.1	A-2894056.1	93	gsusagg(Ahd)gaGfCfCfucucacgcsusa	A-2894057.1	537	VPusdAsgcdGudGagaggdGcUfcuccuacscsc	GGGUAGGAGAGCCUCUCACGCUG	981
AD-1565493.1	A-2894058.1	94	usasggag(Ahd)gCfCfUfcucacgcusgsa	A-2894059.1	538	VPusdCsagdCgdTgagagdGgCfucuccuascsc	GGUAGGAGAGCCUCUCACGCUGA	982
AD-1565722.1	A-2894477.1	95	asgsgag(Ahd)GfcCfUfCfucacgcugsasa	A-2894478.1	539	VPusUfscadGc(G2p)ugagagGfcUfcuccusasc	GUAGGAGAGCCUCUCACGCUGAG	983
AD-1565723.1	A-2894060.1	96	gscscuc(Uhd)CfaCfGfCfugagaacasgsa	A-2894479.1	540	VPusCfsugdTu(C2p)ucagcgUfgAfgaggcsusc	GAGCCUCUCACGCUGAGAACAGC	984
AD-1565494.1	A-2894060.1	97	gscscuc(Uhd)CfaCfGfCfugagaacasgsa	A-2894061.1	541	VPusCfsudGu(Tgn)cucagcgUfgAfgaggcsusc	GAGCCUCUCACGCUGAGAACAGC	985
AD-1565495.1	A-2894060.1	98	gscscuc(Uhd)CfaCfGfCfugagaacasgsa	A-2894062.1	542	VPusCfsugudTc(Tgn)cagcgUfgAfgaggcsusc	GAGCCUCUCACGCUGAGAACAGC	986
AD-1565724.1	A-2894480.1	99	csascgc(Uhd)GfaGfAfAfcagcagaasasa	A-2894481.1	543	VPusUfsuudCu(G2p)cuguucUfcAfgcgugsasg	CUCACGCUGAGAACAGCAGAAAC	987

AD-1565496.1	A-2894063.1	100	ascsgcug(Ahd)gAfAfCfagcagaaascsa	A-2894064.1	544	VPusGfsuudTc(Tgn)gcuguuCfuCfagcgusgsa	UCACGCUGAGAACAGCAGAAACA	988
AD-1565725.1	A-2894065.1	101	csgscug(Ahd)GfaAfCfAfgcagaaacsasa	A-2894482.1	545	VPusUfsgudTu(C2p)ugcuguUfcUfcagcgsusg	CACGCUGAGAACAGCAGAAACAA	989
AD-1565497.1	A-2894065.1	102	csgscug(Ahd)GfaAfCfAfgcagaaacsasa	A-2894066.1	546	VPusUfsgdTu(Tgn)cugcuguUfcUfcagcgsusg	CACGCUGAGAACAGCAGAAACAA	990
AD-1565498.1	A-2894067.1	103	gscsugag(Ahd)aCfAfGfcagaaacasasa	A-2894068.1	547	VPusUfsugdTu(Tgn)cugcugUfuCfucagcsgsu	ACGCUGAGAACAGCAGAAACAAU	991
AD-1565499.1	A-2894069.1	104	csusgag(Ahd)AfcAfGfCfagaaacaasusa	A-2894070.1	548	VPusAfsuudGu(Tgn)ucugcuGfuUfcucagscsg	CGCUGAGAACAGCAGAAACAAUU	992
AD-1565500.1	A-2894071.1	105	usgsaga(Ahd)CfaGfCfAfgaaacaaususa	A-2894072.1	549	VPusAfsaudTg(Tgn)uucugcUfgUfucucasgsc	GCUGAGAACAGCAGAAACAAUUA	993
AD-1565726.1	A-2894483.1	106	gsasgaa(Chd)AfgCfAfGfaaacaauusasa	A-2894484.1	550	VPusUfsaadTu(G2p)uuucugCfuGfuucucsasg	CUGAGAACAGCAGAAACAAUUAC	994
AD-1565501.1	A-2894073.1	107	asgsaac(Ahd)GfcAfGfAfaacaauuascsa	A-2894074.1	551	VPusGfsuadAu(Tgn)guuucuGfcUfguucuscsa	UGAGAACAGCAGAAACAAUUACU	995
AD-1565502.1	A-2894075.1	108	asgsaac(Ahd)gcAfGfAfaacaauuascsa	A-2894076.1	552	VPusdGsuadAudTguudCuGfcuguucuscsa	UGAGAACAGCAGAAACAAUUACU	996
AD-1565503.1	A-2894077.1	109	gsasacag(Chd)aGfAfAfacaauuacsusa	A-2894078.1	553	VPusdAsgudAadTuguudTcUfgcuguucsusc	GAGAACAGCAGAAACAAUUACUG	997
AD-1565801.1	A-1991726.1	110	asascag(Chd)AfgAfAfcAfcaauuacusgsa	A-2894595.1	554	VPusCfsagdTa(Agn)uuguuuCfuGfcuguuscsu	AGAACAGCAGAAACAAUUACUGG	998

AD-1565802.1	A-1991726.1	111	asascag(Chd)AfgAfAfAfcaauuacusgsa	A-2894596.1	555	VPusCfsagdTa(A2p)uuguuuCfuGfcuguuscsu	AGAACAGCAGAAACAAUUACUGG	999
AD-1565803.1	A-2894597.1	112	asascac(Chd)AfgAfAfAfcaauuacusgsa	A-2894598.1	556	VPusdCsagdTadAuugudTuCfugguguuscsu	AGAACAGCAGAAACAAUUACUGG	1000
AD-1565804.1	A-2894599.1	113	asascug(Chd)AfgAfAfAfcaauuacusgsa	A-2894600.1	557	VPusdCsagdTadAuugudTuCfugcaguuscsu	AGAACAGCAGAAACAAUUACUGG	1001
AD-1565805.1	A-2894601.1	114	asasgag(Chd)AfgAfAfAfcaauuacusgsa	A-2894602.1	558	VPusdCsagdTadAuugudTuCfugcucuuscsu	AGAACAGCAGAAACAAUUACUGG	1002
AD-1073418.5	A-1991726.1	115	asascag(Chd)AfgAfAfAfcaauuacusgsa	A-1802980.1	559	VPusCfsaguAfaUfUfguuuCfuGfcuguuscsu	AGAACAGCAGAAACAAUUACUGG	1003
AD-1565504.1	A-2894079.1	116	asascag(Chd)agAfAfAfcaauuacusgsa	A-2894080.1	560	VPusdCsagdTadAuugudTuCfugcuguuscsu	AGAACAGCAGAAACAAUUACUGG	1004
AD-1565504.2	A-2894079.1	117	asascag(Chd)agAfAfAfcaauuacusgsa	A-2894080.1	561	VPusdCsagdTadAuugudTuCfugcuguuscsu	AGAACAGCAGAAACAAUUACUGG	1005
AD-1565505.1	A-2894081.1	118	ascsagc(Ahd)gaAfAfCfaauuacugsgsa	A-2894082.1	562	VPusdCscadGudAauugdTuUfcugcugususc	GAACAGCAGAAACAAUUACUGGC	1006
AD-1565800.1	A-2894593.1	119	csasgc(Ahd)gAfAfAfcaauuacusgsa	A-2894594.1	563	VPusdCsagdTadAuugudTuCfugcugsusu	AACAGCAGAAACAAUUACUGG	1007
AD-1565506.1	A-2894083.1	120	csasgcag(Ahd)aAfCfAfauuacuggscsa	A-2894084.1	564	VPusGfsccdAg(Tgn)aauuguUfuCfugcugsusu	AACAGCAGAAACAAUUACUGGCA	1008
AD-1565727.1	A-2894485.1	121	asgscag(Ahd)AfaCfAfAfuuacuggcsasa	A-2894486.1	565	VPusUfsgcdCa(G2p)uaauugUfuUfcugcusgsu	ACAGCAGAAACAAUUACUGGCAA	1009

AD-1565507.1	A-2894085.1	122	gscsaga(Ahd)AfcAfAfUfuacuggcasasa	A-2894086.1	566	VPusUfsugdCc(Agn)guaauuGfuUfucugcsusg	CAGCAGAAACAAUUACUGGCAAG	1010
AD-1565728.1	A-1577510.1	123	csasgaa(Ahd)CfaAfUfUfacuggcaasgsa	A-2894487.1	567	VPusCfsuudGc(C2p)aguaauUfgUfuucugscsu	AGCAGAAACAAUUACUGGCAAGU	1011
AD-1565508.1	A-1577510.1	124	csasgaa(Ahd)CfaAfUfUfacuggcaasgsa	A-2894087.1	568	VPusCfsuugdCc(Agn)guaauUfgUfuucugscsu	AGCAGAAACAAUUACUGGCAAGU	1012
AD-1565509.1	A-2894088.1	125	asgsaaa(Chd)AfaUfUfAfcugucaagsusa	A-2894089.1	569	VPusAfscudTgdAcaguaaUfuGfuuucusgsc	GCAGAAACAAUUACUGGCAAGUA	1013
AD-1565730.1	A-2894490.1	126	gsasaac(Ahd)AfuUfAfCfuggcaagusasa	A-2894491.1	570	VPusUfsacdTu(G2p)ccaguaAfuUfguuucsusg	CAGAAACAAUUACUGGCAAGUAU	1014
AD-1565510.1	A-2894090.1	127	asasaca(Ahd)UfuAfCfUfggcaaguasusa	A-2894091.1	571	VPusAfsuadCu(Tgn)gccaguAfaUfuguuuscsu	AGAAACAAUUACUGGCAAGUAUG	1015
AD-1565511.1	A-2894092.1	128	asascaa(Uhd)UfaCfUfGfgcaaguausgsa	A-2894093.1	572	VPusCfsaudAc(Tgn)ugccagUfaAfuuguususc	GAAACAAUUACUGGCAAGUAUGG	1016
AD-1565512.1	A-2894094.1	129	asascaa(Uhd)uaCfUfGfgcaaguausgsa	A-2894095.1	573	VPusdCsaudAcdTugccdAgUfaauuguususc	GAAACAAUUACUGGCAAGUAUGG	1017
AD-1565731.1	A-2894096.1	130	gsgscaag(Uhd)aUfGfGfuguguggasusa	A-2894492.1	574	VPusAfsucdCa(C2p)acaccaUfaCfuugccsasg	CUGGCAAGUAUGGUGUGUGGAUG	1018
AD-1565513.1	A-2894096.1	131	gsgscaag(Uhd)aUfGfGfuguguggasusa	A-2894097.1	575	VPusAfsudCc(Agn)cacaccaUfaCfuugccsasg	CUGGCAAGUAUGGUGUGUGGAUG	1019
AD-1565514.1	A-2894096.1	132	gsgscaag(Uhd)aUfGfGfuguguggasusa	A-2894098.1	576	VPusAfsuccdAc(Agn)caccaUfaCfuugccsasg	CUGGCAAGUAUGGUGUGUGGAUG	1020

AD-1565732.1	A-2894099.1	133	csasagu(Ahd)UfgGfUfGfuguggaugscsa	A-2894493.1	577	VPusGfscadTc(C2p)acacacCfaUfacuugscsc	GGCAAGUAUGGUGUGUGGAUGCG	1021
AD-1565515.1	A-2894099.1	134	csasagu(Ahd)UfgGfUfGfuguggaugscsa	A-2894100.1	578	VPusGfscaudCc(Agn)cacacCfaUfacuugscsc	GGCAAGUAUGGUGUGUGGAUGCG	1022
AD-1565516.1	A-2894101.1	135	usgsagu(Ahd)UfgAfCfCfucaucagcscsa	A-2894102.1	579	VPusGfsgcdTg(Agn)ugagguCfaUfacucasasa	UUUGAGUAUGACCUCAUCAGCCA	1023
AD-1565733.1	A-2894494.1	136	gsasgua(Uhd)GfaCfCfUfcaucagccsasa	A-2894495.1	580	VPusUfsggdCu(G2p)augaggUfcAfuacucsasa	UUGAGUAUGACCUCAUCAGCCAG	1024
AD-1565517.1	A-2894103.1	137	asgsuaug(Ahd)cCfUfCfaucagccasgsa	A-2894104.1	581	VPusCfsugdGc(Tgn)gaugagGfuCfauacuscsa	UGAGUAUGACCUCAUCAGCCAGU	1025
AD-1565734.1	A-2894105.1	138	gsusaug(Ahd)CfcUfCfAfucagccagsusa	A-2894496.1	582	VPusAfscudGg(C2p)ugaugaGfgUfcauacsusc	GAGUAUGACCUCAUCAGCCAGUU	1026
AD-1565518.1	A-2894105.1	139	gsusaug(Ahd)CfcUfCfAfucagccagsusa	A-2894106.1	583	VPusAfscugdGc(Tgn)gaugaGfgUfcauacsusc	GAGUAUGACCUCAUCAGCCAGUU	1027
AD-1565735.1	A-2894497.1	140	usasuga(Chd)CfuCfAfUfcagccagususa	A-2894498.1	584	VPusAfsacdTg(G2p)cugaugAfgGfucauascsu	AGUAUGACCUCAUCAGCCAGUUU	1028
AD-1565736.1	A-2894499.1	141	asusgac(Chd)UfcAfUfCfagccaguususa	A-2894500.1	585	VPusAfsaadCu(G2p)gcugauGfaGfgucausasc	GUAUGACCUCAUCAGCCAGUUUA	1029
AD-1565519.1	A-2894107.1	142	usgsacc(Uhd)CfaUfCfAfgccaguuusasa	A-2894108.1	586	VPusUfsaadAc(Tgn)ggcugaUfgAfggucasusa	UAUGACCUCAUCAGCCAGUUUAU	1030
AD-1565520.1	A-2894109.1	143	gsasccu(Chd)auCfAfGfccaguuuasusa	A-2894110.1	587	VPusdAsuadAadCuggcdTgAfugaggucsasu	AUGACCUCAUCAGCCAGUUUAUG	1031

AD-1565521.1	A-2894111.1	144	ascscuc(Ahd)ucAfGfCfcaguuuausgsa	A-2894112.1	588	VPusdCsaudAadAcuggdCuGfaugagguscsa	UGACCUCAUCAGCCAGUUUAUGC	1032
AD-1244360.3	A-2298063.1	145	cscsuca(Uhd)CfaGfCfCfaguuuaugscsa	A-1803173.1	589	VPusGfscauAfaAfCfuggcUfgAfugaggsusc	GACCUCAUCAGCCAGUUUAUGCA	1033
AD-1565522.1	A-2894113.1	146	cscsuca(Uhd)caGfCfCfaguuuaugscsa	A-2894114.1	590	VPusdGscadTadAacugdGcUfgaugaggsusc	GACCUCAUCAGCCAGUUUAUGCA	1034
AD-1565806.1	A-2298063.1	147	cscsuca(Uhd)CfaGfCfCfaguuuaugscsa	A-2894603.1	591	VPusGfscadTa(Agn)acuggcUfgAfugaggsusc	GACCUCAUCAGCCAGUUUAUGCA	1035
AD-1565807.1	A-2298063.1	148	cscsuca(Uhd)CfaGfCfCfaguuuaugscsa	A-2894604.1	592	VPusGfscadTa(A2p)acuggcUfgAfugaggsusc	GACCUCAUCAGCCAGUUUAUGCA	1036
AD-1565808.1	A-2894113.1	149	cscsuca(Uhd)caGfCfCfaguuuaugscsa	A-2894605.1	593	VPusdGscadTa(Agn)acugdGcUfgaugaggsusc	GACCUCAUCAGCCAGUUUAUGCA	1037
AD-1565809.1	A-2894606.1	150	cscsuca(Uhd)cagCfCfAfguuuaugscsa	A-2894603.1	594	VPusGfscadTa(Agn)acuggcUfgAfugaggsusc	GACCUCAUCAGCCAGUUUAUGCA	1038
AD-1565810.1	A-2894607.1	151	cscsuca(Uhd)cagCfCfdAguuuaugscsa	A-2894608.1	595	VPusdGscadTa(Agn)acugdGcUfgAfugaggsusc	GACCUCAUCAGCCAGUUUAUGCA	1039
AD-1565812.1	A-2894611.1	152	cscsucu(Uhd)CfaGfCfCfaguuuaugscsa	A-2894612.1	596	VPusGfscadTa(Agn)acuggcUfgAfagaggsusc	GACCUCAUCAGCCAGUUUAUGCA	1040
AD-1565813.1	A-2894613.1	153	cscsuga(Uhd)CfaGfCfCfaguuuaugscsa	A-2894614.1	597	VPusGfscadTa(Agn)acuggcUfgAfucaggsusc	GACCUCAUCAGCCAGUUUAUGCA	1041
AD-1565814.1	A-2894615.1	154	cscsaca(Uhd)CfaGfCfCfaguuuaugscsa	A-2894616.1	598	VPusGfscadTa(Agn)acuggcUfgAfuguggsusc	GACCUCAUCAGCCAGUUUAUGCA	1042

AD-1565522.2	A-2894113.1	155	cscsuca(Uhd)caGfCfCfaguuuaugscsa	A-2894114.1	599	VPusdGscadTadAacugdGcUfgaugaggsusc	GACCUCAUCAGCCAGUUUAUGCA	1043
AD-1565523.1	A-2894115.1	156	csuscau(Chd)agCfCfAfguuuaugcsasa	A-2894116.1	600	VPusdTsgcdAudAaacudGgCfugaugagsgsu	ACCUCAUCAGCCAGUUUAUGCAG	1044
AD-1565811.1	A-2894609.1	157	uscsauc(Ahd)GfCfCfaguuuaugscsa	A-2894610.1	601	VPusGfscadTa(Agn)acuggcUfgAfugasgsg	CCUCAUCAGCCAGUUUAUGCA	1045
AD-1565524.1	A-2894117.1	158	uscsauc(Ahd)GfcCfAfGfuuuaugcasgsa	A-2894118.1	602	VPusCfsugdCa(Tgn)aaacugGfcUfgaugasgsg	CCUCAUCAGCCAGUUUAUGCAGG	1046
AD-1565525.1	A-2894119.1	159	uscsauc(Ahd)gcCfAfGfuuuaugcasgsa	A-2894120.1	603	VPusdCsugdCadTaaacdTgGfcugaugasgsg	CCUCAUCAGCCAGUUUAUGCAGG	1047
AD-1565526.1	A-2894121.1	160	csasucag(Chd)cAfGfUfuuaugcagsgsa	A-2894122.1	604	VPusCfscudGc(Agn)uaaacuGfgCfugaugsasg	CUCAUCAGCCAGUUUAUGCAGGG	1048
AD-1565737.1	A-2894123.1	161	asuscag(Chd)CfaGfUfUfuaugcaggsgsa	A-2894501.1	605	VPusCfsccdTg(C2p)auaaacUfgGfcugausgsa	UCAUCAGCCAGUUUAUGCAGGGC	1049
AD-1565527.1	A-2894123.1	162	asuscag(Chd)CfaGfUfUfuaugcaggsgsa	A-2894124.1	606	VPusCfsccudGc(Agn)uaaacUfgGfcugausgsa	UCAUCAGCCAGUUUAUGCAGGGC	1050
AD-1565738.1	A-2894502.1	163	uscsagc(Chd)AfgUfUfUfaugcagggscsa	A-2894503.1	607	VPusGfsccdCu(G2p)cauaaaCfuGfgcugasusg	CAUCAGCCAGUUUAUGCAGGGCU	1051
AD-1565528.1	A-2894125.1	164	csasgcc(Ahd)GfuUfUfAfugcagggcsusa	A-2894126.1	608	VPusAfsgcdCc(Tgn)gcauaaAfcUfggcugsasu	AUCAGCCAGUUUAUGCAGGGCUA	1052
AD-1565739.1	A-2894127.1	165	asgsccag(Uhd)uUfAfUfgcagggcusasa	A-2894504.1	609	VPusUfsagdCc(C2p)ugcauaAfaCfuggcusgsa	UCAGCCAGUUUAUGCAGGGCUAC	1053

AD-1565529.1	A-2894127.1	166	asgsccag(Uhd)uUfAfUfgcagggcusasa	A-2894128.1	610	VPusUfsagcdCc(Tgn)gcauaAfaCfuggcusgsa	UCAGCCAGUUUAUGCAGGGCUAC	1054
AD-1565740.1	A-2894505.1	167	gscscag(Uhd)UfuAfUfGfcagggcuascsa	A-2894506.1	611	VPusGfsuadGc(C2p)cugcauAfaAfcuggcsusg	CAGCCAGUUUAUGCAGGGCUACC	1055
AD-1565530.1	A-2894129.1	168	gscscag(Uhd)UfuAfUfGfcagugcuascsa	A-2894130.1	612	VPusGfsuadGcdAcugcauAfaAfcuggcsusg	CAGCCAGUUUAUGCAGGGCUACC	1056
AD-1565741.1	A-2894507.1	169	cscsagu(Uhd)UfaUfGfCfagggcuacscsa	A-2894508.1	613	VPusGfsgudAg(C2p)ccugcaUfaAfacuggcscsu	AGCCAGUUUAUGCAGGGCUACCC	1057
AD-1565531.1	A-2894131.1	170	cscsagu(Uhd)UfaUfGfCfaggucuacscsa	A-2894132.1	614	VPusGfsgudAgdAccugcaUfaAfacuggcscsu	AGCCAGUUUAUGCAGGGCUACCC	1058
AD-1565532.1	A-2894133.1	171	usgscagggcUfAfCfccuu(Chd)uaasgsa	A-2894134.1	615	VPusdCsuudAgdAagggdTaGfcccugcasusa	UAUGCAGGGCUACCCUUCUAAGG	1059
AD-1565533.1	A-2894135.1	172	gsgsgug(Chd)UfgUfGfGfuguacucgsgsa	A-2894136.1	616	VPusCfscgdAg(Tgn)acaccaCfaGfcacccsgsu	ACGGGUGCUGUGGUGUACUCGGG	1060
AD-1565534.1	A-2894137.1	173	gsgsgug(Chd)ugUfGfGfuguacucgsgsa	A-2894138.1	617	VPusdCscgdAgdTacacdCaCfagcacccsgsu	ACGGGUGCUGUGGUGUACUCGGG	1061
AD-1565535.1	A-2894139.1	174	gsasgcc(Uhd)CfuAfUfUfuccagggcsgsa	A-2894140.1	618	VPusCfsgcdCc(Tgn)ggaaauAfgAfggcucscsc	GGGAGCCUCUAUUUCCAGGGCGC	1062
AD-1565536.1	A-2894141.1	175	gsasgcc(Uhd)cuAfUfUfuccagggcsgsa	A-2894142.1	619	VPusdCsgcdCcdTggaadAuAfgaggcucscsc	GGGAGCCUCUAUUUCCAGGGCGC	1063
AD-1565537.1	A-2894143.1	176	cscsucu(Ahd)uuUfCfCfagggcgcusgsa	A-2894144.1	620	VPusdCsagdCgdCccugdGaAfauagaggscsu	AGCCUCUAUUUCCAGGGCGCUGA	1064

AD-1565742.1	A-2894145.1	177	ususcc(Ahd)gGfgCfGfCfugaguccasgsa	A-2894509.1	621	VPusCfsugdGa(C2p)ucagcgCfcCfuggaasasu	AUUUCCAGGGCGCUGAGUCCAGA	1065
AD-1565538.1	A-2894145.1	178	ususcc(Ahd)gGfgCfGfCfugaguccasgsa	A-2894146.1	622	VPusCfsudGg(Agn)cucagcgCfcCfuggaasasu	AUUUCCAGGGCGCUGAGUCCAGA	1066
AD-1565539.1	A-2894145.1	179	ususcc(Ahd)gGfgCfGfCfugaguccasgsa	A-2894147.1	623	VPusCfsuggdAc(Tgn)cagcgCfcCfuggaasasu	AUUUCCAGGGCGCUGAGUCCAGA	1067
AD-1565540.1	A-2894148.1	180	asgsggcg(Chd)uGfAfGfuccagaacsusa	A-2894149.1	624	VPusAfsgudTc(Tgn)ggacucAfgCfgcccusgsg	CCAGGGCGCUGAGUCCAGAACUG	1068
AD-1565743.1	A-2894150.1	181	gsgsgcg(Chd)UfgAfGfUfccagaacusgsa	A-2894510.1	625	VPusCfsagdTu(C2p)uggacuCfaGfcgcccsusg	CAGGGCGCUGAGUCCAGAACUGU	1069
AD-1565541.1	A-2894150.1	182	gsgsgcg(Chd)UfgAfGfUfccagaacusgsa	A-2894151.1	626	VPusCfsadGu(Tgn)cuggacuCfaGfcgcccsusg	CAGGGCGCUGAGUCCAGAACUGU	1070
AD-1565542.1	A-2894152.1	183	gsgsgcg(Chd)ugAfGfUfccagaacusgsa	A-2894153.1	627	VPusdCsagdTudCuggadCuCfagcgcccsusg	CAGGGCGCUGAGUCCAGAACUGU	1071
AD-1565543.1	A-2894154.1	184	gsgscgc(Uhd)GfaGfUfCfcagaacugsusa	A-2894155.1	628	VPusAfscadGu(Tgn)cuggacUfcAfgcgccscsu	AGGGCGCUGAGUCCAGAACUGUC	1072
AD-1565544.1	A-2894156.1	185	gscsgcug(Ahd)gUfCfCfagaacuguscsa	A-2894157.1	629	VPusGfsacdAg(Tgn)ucuggaCfuCfagcgcscsc	GGGCGCUGAGUCCAGAACUGUCA	1073
AD-1565744.1	A-2894511.1	186	csgscug(Ahd)GfuCfCfAfgaacugucsasa	A-2894512.1	630	VPusUfsgadCa(G2p)uucuggAfcUfcagcgscsc	GGCGCUGAGUCCAGAACUGUCAU	1074
AD-1565545.1	A-2894158.1	187	gscsugag(Uhd)cCfAfGfaacugucasusa	A-2894159.1	631	VPusAfsugdAc(Agn)gucugGfaCfucagcsgsc	GCGCUGAGUCCAGAACUGUCAUA	1075

AD-1565745.1	A-1577552.1	188	csusgag(Uhd)CfcAfGfAfacugucausasa	A-2894513.1	632	VPusUfsaudGa(C2p)aguucuGfgAfcucagscsg	CGCUGAGUCCAGAACUGUCAUAA	1076
AD-1565546.1	A-1577552.1	189	csusgag(Uhd)CfcAfGfAfacugucausasa	A-2894160.1	633	VPusUfsadTg(Agn)caguucuGfgAfcucagscsg	CGCUGAGUCCAGAACUGUCAUAA	1077
AD-1565547.1	A-1577552.1	190	csusgag(Uhd)CfcAfGfAfacugucausasa	A-2894161.1	634	VPusUfsaugdAc(Agn)guucuGfgAfcucagscsg	CGCUGAGUCCAGAACUGUCAUAA	1078
AD-1565548.1	A-2894162.1	191	usgsagu(Chd)CfaGfAfAfcugucauasasa	A-2894163.1	635	VPusUfsuadTg(Agn)caguucUfgGfacucasgsc	GCUGAGUCCAGAACUGUCAUAAG	1079
AD-1565746.1	A-1577518.1	192	gsasguc(Chd)AfgAfAfCfugucauaasgsa	A-2894514.1	636	VPusCfsuudAu(G2p)acaguuCfuGfgacucsasg	CUGAGUCCAGAACUGUCAUAAGA	1080
AD-1565549.1	A-2894164.1	193	gsasguc(Chd)agAfAfCfugucauaasgsa	A-2894165.1	637	VPusdCsuudAudGacagdTuCfuggacucsasg	CUGAGUCCAGAACUGUCAUAAGA	1081
AD-1565550.1	A-2894166.1	194	asgsucc(Ahd)GfaAfCfUfgucauaagsasa	A-2894167.1	638	VPusUfscudTa(Tgn)gacaguUfcUfggacuscsa	UGAGUCCAGAACUGUCAUAAGAU	1082
AD-1565551.1	A-2894168.1	195	asgsucc(Ahd)gaAfCfUfgucauaagsasa	A-2894169.1	639	VPusdTscudTadTgacadGuUfcuggacuscsa	UGAGUCCAGAACUGUCAUAAGAU	1083
AD-1244365.3	A-2324726.1	196	gsuscca(Ghd)AfaCfUfGfucauaagasusa	A-1803353.1	640	VPusAfsucuUfaUfGfacagUfuCfuggacsusc	GAGUCCAGAACUGUCAUAAGAUA	1084
AD-1565552.1	A-2894170.1	197	gsusccag(Ahd)aCfUfGfucauaagasusa	A-2894171.1	641	VPusAfsucdTu(Agn)ugacagUfuCfuggacsusc	GAGUCCAGAACUGUCAUAAGAUA	1085
AD-1565553.1	A-2894170.1	198	gsusccag(Ahd)aCfUfGfucauaagasusa	A-2894172.1	642	VPusdAsucdTudAugacdAgUfucuggacsusc	GAGUCCAGAACUGUCAUAAGAUA	1086

AD-1565815.1	A-2894170.1	199	gsusccag(Ahd)aCfUfGfucauaagasusa	A-2894617.1	643	VPusdAsucdTu(Agn)ugacdAgUfucuggacsusc	GAGUCCAGAACUGUCAUAAGAUA	1087
AD-1565816.1	A-2894170.1	200	gsusccag(Ahd)aCfUfGfucauaagasusa	A-2894618.1	644	VPusAfsucdTu(A2p)ugacagUfuCfuggacsusc	GAGUCCAGAACUGUCAUAAGAUA	1088
AD-1565818.1	A-2894621.1	201	gsusccug(Ahd)aCfUfGfucauaagasusa	A-2894622.1	645	VPusdAsucdTu(Agn)ugacagUfuCfaggacsusc	GAGUCCAGAACUGUCAUAAGAUA	1089
AD-1565819.1	A-2894623.1	202	gsuscgag(Ahd)aCfUfGfucauaagasusa	A-2894624.1	646	VPusdAsucdTu(Agn)ugacagUfuCfucgacsusc	GAGUCCAGAACUGUCAUAAGAUA	1090
AD-1565820.1	A-2894625.1	203	gsusgcag(Ahd)aCfUfGfucauaagasusa	A-2894626.1	647	VPusdAsucdTu(Agn)ugacagUfuCfuggacsusc	GAGUCCAGAACUGUCAUAAGAUA	1091
AD-1565552.2	A-2894170.1	204	gsusccag(Ahd)aCfUfGfucauaagasusa	A-2894171.1	648	VPusAfsucdTu(Agn)ugacagUfuCfuggacsusc	GAGUCCAGAACUGUCAUAAGAUA	1092
AD-1565553.2	A-2894170.1	205	gsusccag(Ahd)aCfUfGfucauaagasusa	A-2894172.1	649	VPusdAsucdTudAugacdAgUfucuggacsusc	GAGUCCAGAACUGUCAUAAGAUA	1093
AD-1565554.1	A-2894173.1	206	uscscag(Ahd)AfcUfGfUfcauaagausasa	A-2894174.1	650	VPusUfsaudCu(Tgn)augacaGfuUfcuggascsu	AGUCCAGAACUGUCAUAAGAUAU	1094
AD-1565817.1	A-2894619.1	207	cscsag(Ahd)aCfUfGfucauaagasusa	A-2894620.1	651	VPusdAsucdTu(Agn)ugacagUfuCfuggsgsc	GUCCAGAACUGUCAUAAGAUA	1095
AD-1565555.1	A-2894175.1	208	cscsaga(Ahd)CfuGfUfCfauaagauasusa	A-2894176.1	652	VPusAfsuadTc(Tgn)uaugacAfgUfucuggsasc	GUCCAGAACUGUCAUAAGAUAUG	1096
AD-1565747.1	A-2894177.1	209	csasgaa(Chd)UfgUfCfAfuaagauausgsa	A-2894515.1	653	VPusCfsaudAu(C2p)uuaugaCfaGfuucugsgsa	UCCAGAACUGUCAUAAGAUAUGA	1097

AD-1565556.1	A-2894177.1	210	csasgaa(Chd)UfgUfCfAfuaagauausgsa	A-2894178.1	654	VPusCfsadTa(Tgn)cuuaugaCfaGfuucugsgsa	UCCAGAACUGUCAUAAGAUAUGA	1098
AD-1565557.1	A-2894179.1	211	csasgaa(Chd)ugUfCfAfuaagauausgsa	A-2894180.1	655	VPusdCsaudAudCuuaudGaCfaguucugsgsa	UCCAGAACUGUCAUAAGAUAUGA	1099
AD-1565558.1	A-2894181.1	212	asgsaac(Uhd)GfuCfAfUfaagauaugsasa	A-2894182.1	656	VPusUfscadTa(Tgn)cuaugAfcAfguucusgsg	CCAGAACUGUCAUAAGAUAUGAG	1100
AD-1565559.1	A-2894183.1	213	asgsaac(Uhd)guCfAfUfaagauaugsasa	A-2894184.1	657	VPusdTscadTadTcuuadTgAfcaguucusgsg	CCAGAACUGUCAUAAGAUAUGAG	1101
AD-1565560.1	A-2894185.1	214	gsasacug(Uhd)cAfUfAfagauaugasgsa	A-2894186.1	658	VPusdCsucdAudAucuudAuGfacaguucsusg	CAGAACUGUCAUAAGAUAUGAGC	1102
AD-1565561.1	A-2894187.1	215	asascug(Uhd)CfaUfAfAfgauaugagscsa	A-2894188.1	659	VPusGfscudCa(Tgn)aucuuaUfgAfcaguuscsu	AGAACUGUCAUAAGAUAUGAGCU	1103
AD-1565562.1	A-2894189.1	216	ascsugu(Chd)AfuAfAfGfauaugagcsusa	A-2894190.1	660	VPusAfsgcdTc(Agn)uaucuuAfuGfacagususc	GAACUGUCAUAAGAUAUGAGCUG	1104
AD-1565748.1	A-2894191.1	217	csusguc(Ahd)UfaAfGfAfuaugagcusgsa	A-2894516.1	661	VPusCfsagdCu(C2p)auaucuUfaUfgacagsusu	AACUGUCAUAAGAUAUGAGCUGA	1105
AD-1565563.1	A-2894191.1	218	csusguc(Ahd)UfaAfGfAfuaugagcusgsa	A-2894192.1	662	VPusCfsadGc(Tgn)cauaucuUfaUfgacagsusu	AACUGUCAUAAGAUAUGAGCUGA	1106
AD-1565564.1	A-2894191.1	219	csusguc(Ahd)UfaAfGfAfuaugagcusgsa	A-2894193.1	663	VPusCfsagcdTc(Agn)uaucuUfaUfgacagsusu	AACUGUCAUAAGAUAUGAGCUGA	1107
AD-1565565.1	A-2894194.1	220	usgsuca(Uhd)AfaGfAfUfaugagcugsasa	A-2894195.1	664	VPusUfscadGc(Tgn)cauaucUfuAfugacasgsu	ACUGUCAUAAGAUAUGAGCUGAA	1108

AD-1565566.1	A-2894196.1	221	csasuaag(Ahd)uAfUfGfagcugaausasa	A-2894197.1	665	VPusUfsaudTc(Agn)gcucauAfuCfuuaugsasc	GUCAUAAGAUAUGAGCUGAAUAC	1109
AD-1565567.1	A-2894198.1	222	csusgaa(Uhd)AfcCfGfAfgacagugasasa	A-2894199.1	666	VPusUfsucdAc(Tgn)gucucgGfuAfuucagscsu	AGCUGAAUACCGAGACAGUGAAG	1110
AD-1565568.1	A-2894200.1	223	usasccg(Ahd)GfaCfAfGfugaaggcusgsa	A-2894201.1	667	VPusCfsagdCc(Tgn)ucacugUfcUfcgguasusu	AAUACCGAGACAGUGAAGGCUGA	1111
AD-1565569.1	A-2894202.1	224	usasccg(Ahd)gaCfAfGfugaaggcusgsa	A-2894203.1	668	VPusdCsagdCcdTucacdTgUfcucgguasusu	AAUACCGAGACAGUGAAGGCUGA	1112
AD-1565749.1	A-2894204.1	225	ascsagug(Ahd)aGfGfCfugagaaggsasa	A-2894517.1	669	VPusUfsccdTu(C2p)ucagccUfuCfacuguscsu	AGACAGUGAAGGCUGAGAAGGAA	1113
AD-1565570.1	A-2894204.1	226	ascsagug(Ahd)aGfGfCfugagaaggsasa	A-2894205.1	670	VPusUfscdCu(Tgn)cucagccUfuCfacuguscsu	AGACAGUGAAGGCUGAGAAGGAA	1114
AD-1565571.1	A-2894204.1	227	ascsagug(Ahd)aGfGfCfugagaaggsasa	A-2894206.1	671	VPusUfsccudTc(Tgn)cagccUfuCfacuguscsu	AGACAGUGAAGGCUGAGAAGGAA	1115
AD-1565750.1	A-2894518.1	228	usgsaga(Ahd)GfgAfAfAfucccuggasgsa	A-2894519.1	672	VPusCfsucdCa(G2p)ggauuuCfcUfucucasgsc	GCUGAGAAGGAAAUCCCUGGAGC	1116
AD-1565573.1	A-2894209.1	229	gscscaa(Ahd)ggUfGfCfcauuguccsusa	A-2894210.1	673	VPusdAsggdAcdAauggdCaCfcuuuggcscsu	AGGCCAAAGGUGCCAUUGUCCUC	1117
AD-1565751.1	A-2894211.1	230	cscsaaa(Chd)UfgAfAfCfccagagaasusa	A-2894520.1	674	VPusAfsuudCu(C2p)uggguuCfaGfuuuggsasg	CUCCAAACUGAACCCAGAGAAUC	1118
AD-1565574.1	A-2894211.1	231	cscsaaa(Chd)UfgAfAfCfccagagaasusa	A-2894212.1	675	VPusAfsudTc(Tgn)cuggguuCfaGfuuuggsasg	CUCCAAACUGAACCCAGAGAAUC	1119

AD-1565575.1	A-2894211.1	232	cscsaaa(Chd)UfgAfAfCfccagagaasusa	A-2894213.1	676	VPusAfsuucdTc(Tgn)ggguuCfaGfuuuggsasg	CUCCAAACUGAACCCAGAGAAUC	1120
AD-1565576.1	A-2894214.1	233	csasaac(Uhd)GfaAfCfCfcagagaauscsa	A-2894215.1	677	VPusGfsaudTc(Tgn)cuggguUfcAfguuugsgsa	UCCAAACUGAACCCAGAGAAUCU	1121
AD-1565752.1	A-2894216.1	234	uscscgu(Ahd)AfgCfAfGfucagucgcscsa	A-2894521.1	678	VPusGfsgcdGa(C2p)ugacugCfuUfacggasusg	CAUCCGUAAGCAGUCAGUCGCCA	1122
AD-1565577.1	A-2894216.1	235	uscscgu(Ahd)AfgCfAfGfucagucgcscsa	A-2894217.1	679	VPusGfsgdCg(Agn)cugacugCfuUfacggasusg	CAUCCGUAAGCAGUCAGUCGCCA	1123
AD-1565578.1	A-2894216.1	236	uscscgu(Ahd)AfgCfAfGfucagucgcscsa	A-2894218.1	680	VPusGfsgcgdAc(Tgn)gacugCfuUfacggasusg	CAUCCGUAAGCAGUCAGUCGCCA	1124
AD-1565579.1	A-2894219.1	237	uscscgu(Ahd)agCfAfGfucagucgcscsa	A-2894220.1	681	VPusdGsgcdGadCugacdTgCfuuacggasusg	CAUCCGUAAGCAGUCAGUCGCCA	1125
AD-1565753.1	A-2894522.1	238	usasagc(Ahd)GfuCfAfGfucgccaausgsa	A-2894523.1	682	VPusCfsaudTg(G2p)cgacugAfcUfgcuuascsg	CGUAAGCAGUCAGUCGCCAAUGC	1126
AD-1565580.1	A-2894221.1	239	csasguc(Ahd)GfuCfGfCfcaaugccususa	A-2894222.1	683	VPusAfsagdGc(Agn)uuggcgAfcUfgacugscsu	AGCAGUCAGUCGCCAAUGCCUUC	1127
AD-1565754.1	A-2894524.1	240	uscsagu(Chd)GfcCfAfAfugccuucasusa	A-2894525.1	684	VPusAfsugdAa(G2p)gcauugGfcGfacugascsu	AGUCAGUCGCCAAUGCCUUCAUC	1128
AD-1565581.1	A-2894223.1	241	asgsucg(Chd)CfaAfUfGfccuucaucsasa	A-2894224.1	685	VPusUfsgadTg(Agn)aggcauUfgGfcgacusgsa	UCAGUCGCCAAUGCCUUCAUCAU	1129
AD-1565582.1	A-2894225.1	242	gscscaa(Uhd)gcCfUfUfcaucaucusgsa	A-2894226.1	686	VPusdCsagdAudGaugadAgGfcauuggcsgsa	UCGCCAAUGCCUUCAUCAUCUGU	1130

AD-1565755.1	A-2894227.1	243	cscsuuc(Ahd)UfcAfUfCfuguggcacscsa	A-2894526.1	687	VPusGfsgudGc(C2p)acagauGfaUfgaaggscsa	UGCCUUCAUCAUCUGUGGCACCU	1131
AD-1565583.1	A-2894227.1	244	cscsuuc(Ahd)UfcAfUfCfuguggcacscsa	A-2894228.1	688	VPusGfsgugdCc(Agn)cagauGfaUfgaaggscsa	UGCCUUCAUCAUCUGUGGCACCU	1132
AD-1565584.1	A-2894229.1	245	csusgugg(Chd)aCfCfUfuguacaccsgsa	A-2894230.1	689	VPusCfsggdTg(Tgn)acaaggUfgCfcacagsasu	AUCUGUGGCACCUUGUACACCGU	1133
AD-1565756.1	A-2894231.1	246	csusaccg(Uhd)cAfAfCfuuugcuuasusa	A-2894527.1	690	VPusAfsuadAg(C2p)aaaguuGfaCfgguagscsa	UGCUACCGUCAACUUUGCUUAUG	1134
AD-1565585.1	A-2894231.1	247	csusaccg(Uhd)cAfAfCfuuugcuuasusa	A-2894232.1	691	VPusAfsuaadGc(Agn)aaguuGfaCfgguagscsa	UGCUACCGUCAACUUUGCUUAUG	1135
AD-1073420.3	A-1991728.1	248	usasccg(Uhd)CfaAfCfUfuugcuuausgsa	A-1806295.1	692	VPusCfsauaa(Ggn)caaaguUfgAfcgguasgsc	GCUACCGUCAACUUUGCUUAUGA	1136
AD-1244366.3	A-1991728.1	249	usasccg(Uhd)CfaAfCfUfuugcuuausgsa	A-1803954.1	693	VPusCfsauaAfgCfAfaaguUfgAfcgguasgsc	GCUACCGUCAACUUUGCUUAUGA	1137
AD-1565757.1	A-1991728.1	250	usasccg(Uhd)CfaAfCfUfuugcuuausgsa	A-2894528.1	694	VPusCfsaudAa(G2p)caaaguUfgAfcgguasgsc	GCUACCGUCAACUUUGCUUAUGA	1138
AD-1565821.1	A-1991728.1	251	usasccg(Uhd)CfaAfCfUfuugcuuausgsa	A-2894627.1	695	VPusCfsadTa(Agn)gcaaaguUfgAfcgguasgsc	GCUACCGUCAACUUUGCUUAUGA	1139
AD-1565822.1	A-1991728.1	252	usasccg(Uhd)CfaAfCfUfuugcuuausgsa	A-2894628.1	696	VPusdCsaudAa(G2p)caaadGuUfgAfcgguasgsc	GCUACCGUCAACUUUGCUUAUGA	1140
AD-1565824.1	A-2894631.1	253	usascccc(Uhd)CfaAfCfUfuugcuuausgsa	A-2894632.1	697	VPusCfsaudAa(G2p)caaaguUfgAfggguasgsc	GCUACCGUCAACUUUGCUUAUGA	1141

AD-1565825.1	A-2894633.1	254	usascgg(Uhd)CfaAfCfUfuugcuuausgsa	A-2894634.1	698	VPusCfsaudAa(G2p)caaaguUfgAfccguasgsc	GCUACCGUCAACUUUGCUUAUGA	1142
AD-1565826.1	A-2894635.1	255	usasgcg(Uhd)CfaAfCfUfuugcuuausgsa	A-2894636.1	699	VPusCfsaudAa(G2p)caaaguUfgAfcgcuasgsc	GCUACCGUCAACUUUGCUUAUGA	1143
AD-1565757.2	A-1991728.1	256	usasccg(Uhd)CfaAfCfUfuugcuuausgsa	A-2894528.1	700	VPusCfsaudAa(G2p)caaaguUfgAfcgguasgsc	GCUACCGUCAACUUUGCUUAUGA	1144
AD-1565586.1	A-2894233.1	257	usasccg(Uhd)caAfCfUfuugcuuausgsa	A-2894234.1	701	VPusdCsaudAadGcaaadGuUfgacgguasgsc	GCUACCGUCAACUUUGCUUAUGA	1145
AD-1565587.1	A-2324727.1	258	ascscgu(Chd)AfaCfUfUfugcuuaugsasa	A-2894235.1	702	VPusUfscadTa(Agn)gcaaagUfuGfacggusasg	CUACCGUCAACUUUGCUUAUGAC	1146
AD-1565588.1	A-2894236.1	259	ascscgu(Chd)aaCfUfUfugcuuaugsasa	A-2894237.1	703	VPusdTscadTadAgcaadAgUfugacggusasg	CUACCGUCAACUUUGCUUAUGAC	1147
AD-1565823.1	A-2894629.1	260	cscsguc(Ahd)AfCfUfuugcuuausgsa	A-2894630.1	704	VPusCfsaudAa(G2p)caaaguUfgAfcggsusg	UACCGUCAACUUUGCUUAUGA	1148
AD-1565589.1	A-2894238.1	261	cscsguc(Ahd)acUfUfUfgcuuaugascsa	A-2894239.1	705	VPusdGsucdAudAagcadAaGfuugacggsusa	UACCGUCAACUUUGCUUAUGACA	1149
AD-1565590.1	A-2894240.1	262	csgsuca(Ahd)CfuUfUfGfcuuaugacsasa	A-2894241.1	706	VPusUfsgudCa(Tgn)aagcaaAfgUfugacgsgsu	ACCGUCAACUUUGCUUAUGACAC	1150
AD-1565591.1	A-2894242.1	263	gsuscaa(Chd)UfuUfGfCfuuaugacascsa	A-2894243.1	707	VPusGfsugdTc(Agn)uaagcaAfaGfuugacsgsg	CCGUCAACUUUGCUUAUGACACA	1151
AD-1565758.1	A-2894244.1	264	uscsaac(Uhd)UfuGfCfUfuaugacacsasa	A-2894529.1	708	VPusUfsgudGu(C2p)auaagcAfaAfguugascsg	CGUCAACUUUGCUUAUGACACAG	1152

AD-1565592.1	A-2894244.1	265	uscsaac(Uhd)UfuGfCfUfuaugacacsasa	A-2894245.1	709	VPusUfsgdTg(Tgn)cauaagcAfaAfguugascsg	CGUCAACUUUGCUUAUGACACAG	1153
AD-1565594.1	A-2894247.1	266	csasacu(Uhd)UfgCfUfUfaugacacasgsa	A-2894248.1	710	VPusCfsugdTg(Tgn)cauaagCfaAfaguugsasc	GUCAACUUUGCUUAUGACACAGG	1154
AD-1565759.1	A-2894530.1	267	asascuu(Uhd)GfcUfUfAfugacacagsgsa	A-2894531.1	711	VPusCfscudGu(G2p)ucauaaGfcAfaaguusgsa	UCAACUUUGCUUAUGACACAGGC	1155
AD-1565595.1	A-2894249.1	268	ascsuuug(Chd)uUfAfUfgacacaggscsa	A-2894250.1	712	VPusGfsccdTg(Tgn)gucauaAfgCfaaagususg	CAACUUUGCUUAUGACACAGGCA	1156
AD-1565760.1	A-2894532.1	269	csusuug(Chd)UfuAfUfGfacacaggcsasa	A-2894533.1	713	VPusUfsgcdCu(G2p)ugucauAfaGfcaaagsusu	AACUUUGCUUAUGACACAGGCAC	1157
AD-1565761.1	A-2894534.1	270	ascsagg(Chd)AfcAfGfGfuaucagcasasa	A-2894535.1	714	VPusUfsugdCu(G2p)auaccuGfuGfccugusgsu	ACACAGGCACAGGUAUCAGCAAG	1158
AD-1565596.1	A-2894251.1	271	asusaag(Uhd)AfcAfGfCfagcaugaususa	A-2894252.1	715	VPusAfsaudCa(Tgn)gcugcuGfuAfcuuausasg	CUAUAAGUACAGCAGCAUGAUUG	1159
AD-1565597.1	A-2894253.1	272	usasagu(Ahd)CfaGfCfAfgcaugauusgsa	A-2894254.1	716	VPusCfsaadTc(Agn)ugcugcUfgUfacuuasusa	UAUAAGUACAGCAGCAUGAUUGA	1160
AD-1565762.1	A-2894255.1	273	asasgua(Chd)AfgCfAfGfcaugauugsasa	A-2894536.1	717	VPusUfscadAu(C2p)augcugCfuGfuacuusasu	AUAAGUACAGCAGCAUGAUUGAC	1161
AD-1565598.1	A-2894255.1	274	asasgua(Chd)AfgCfAfGfcaugauugsasa	A-2894256.1	718	VPusUfscdAa(Tgn)caugcugCfuGfuacuusasu	AUAAGUACAGCAGCAUGAUUGAC	1162
AD-1565599.1	A-2894255.1	275	asasgua(Chd)AfgCfAfGfcaugauugsasa	A-2894257.1	719	VPusUfscaadTc(Agn)ugcugCfuGfuacuusasu	AUAAGUACAGCAGCAUGAUUGAC	1163

AD-1565600.1	A-2894258.1	276	asgsuac(Ahd)GfcAfGfCfaugauugascsa	A-2894259.1	720	VPusGfsucdAa(Tgn)caugcuGfcUfguacususa	UAAGUACAGCAGCAUGAUUGACU	1164
AD-1565601.1	A-2894260.1	277	asgsuac(Ahd)gcAfGfCfaugauugascsa	A-2894261.1	721	VPusdGsucdAadTcaugdCuGfcuguacususa	UAAGUACAGCAGCAUGAUUGACU	1165
AD-1565602.1	A-2894262.1	278	gsusacag(Chd)aGfCfAfugauugacsusa	A-2894263.1	722	VPusAfsgudCa(Agn)ucaugcUfgCfuguacsusu	AAGUACAGCAGCAUGAUUGACUA	1166
AD-1565827.1	A-2894637.1	279	gsusaca(Ghd)CfaGfCfAfugauugacsusa	A-1804098.1	723	VPusAfsgucAfaUfCfaugcUfgCfuguacsusu	AAGUACAGCAGCAUGAUUGACUA	1167
AD-1565828.1	A-2894262.1	280	gsusacag(Chd)aGfCfAfugauugacsusa	A-2894638.1	724	VPusAfsgudCa(A2p)ucaugcUfgCfuguacsusu	AAGUACAGCAGCAUGAUUGACUA	1168
AD-1565829.1	A-2894262.1	281	gsusacag(Chd)aGfCfAfugauugacsusa	A-2894639.1	725	VPusdAsgudCadAucaudGcUfgcuguacsusu	AAGUACAGCAGCAUGAUUGACUA	1169
AD-1565830.1	A-2894262.1	282	gsusacag(Chd)aGfCfAfugauugacsusa	A-2894640.1	726	VPusdAsgudCa(Agn)ucaugcUfgCfuguacsusu	AAGUACAGCAGCAUGAUUGACUA	1170
AD-1565831.1	A-2894262.1	283	gsusacag(Chd)aGfCfAfugauugacsusa	A-2894641.1	727	VPusdAsgudCa(Agn)ucaudGcUfgcuguacsusu	AAGUACAGCAGCAUGAUUGACUA	1171
AD-1565833.1	A-2894644.1	284	gsusacug(Chd)aGfCfAfugauugacsusa	A-2894645.1	728	VPusdAsgudCa(Agn)ucaugcUfgCfaguacsusu	AAGUACAGCAGCAUGAUUGACUA	1172
AD-1565834.1	A-2894646.1	285	gsusagag(Chd)aGfCfAfugauugacsusa	A-2894647.1	729	VPusdAsgudCa(Agn)ucaugcUfgCfucuacsusu	AAGUACAGCAGCAUGAUUGACUA	1173
AD-1565835.1	A-2894648.1	286	gsusucag(Chd)aGfCfAfugauugacsusa	A-2894649.1	730	VPusdAsgudCa(Agn)ucaugcUfgCfugaacsusu	AAGUACAGCAGCAUGAUUGACUA	1174

AD-1565602.2	A-2894262.1	287	gsusacag(Chd)aGfCfAfugauugacsusa	A-2894263.1	731	VPusAfsgudCa(Agn)ucaugcUfgCfuguacsusu	AAGUACAGCAGCAUGAUUGACUA	1175
AD-1565603.1	A-2894264.1	288	usascag(Chd)AfgCfAfUfgauugacusasa	A-2894265.1	732	VPusUfsagdTc(Agn)aucaugCfuGfcuguascsu	AGUACAGCAGCAUGAUUGACUAC	1176
AD-1565832.1	A-2894642.1	289	ascsag(Chd)aGfCfAfugauugacsusa	A-2894643.1	733	VPusdAsgudCa(Agn)ucaugcUfgCfugusgsc	GUACAGCAGCAUGAUUGACUA	1177
AD-1565763.1	A-2894266.1	290	ascsagc(Ahd)GfcAfUfGfauugacuascsa	A-2894537.1	734	VPusGfsuadGu(C2p)aaucauGfcUfgcugusasc	GUACAGCAGCAUGAUUGACUACA	1178
AD-1565604.1	A-2894266.1	291	ascsagc(Ahd)GfcAfUfGfauugacuascsa	A-2894267.1	735	VPusGfsudAg(Tgn)caaucauGfcUfgcugusasc	GUACAGCAGCAUGAUUGACUACA	1179
AD-1565605.1	A-2894266.1	292	ascsagc(Ahd)GfcAfUfGfauugacuascsa	A-2894268.1	736	VPusGfsuagdTc(Agn)aucauGfcUfgcugusasc	GUACAGCAGCAUGAUUGACUACA	1180
AD-1565606.1	A-2894269.1	293	csasgcag(Chd)aUfGfAfuugacuacsasa	A-2894270.1	737	VPusUfsgudAg(Tgn)caaucaUfgCfugcugsusa	UACAGCAGCAUGAUUGACUACAA	1181
AD-1565764.1	A-2894538.1	294	asgscag(Chd)AfuGfAfUfugacuacasasa	A-2894539.1	738	VPusUfsugdTa(G2p)ucaaucAfuGfcugcusgsu	ACAGCAGCAUGAUUGACUACAAC	1182
AD-1565607.1	A-2894271.1	295	gscsagc(Ahd)UfgAfUfUfgacuacaascsa	A-2894272.1	739	VPusGfsuudGu(Agn)gucaauCfaUfgcugcsusg	CAGCAGCAUGAUUGACUACAACC	1183
AD-1565608.1	A-2894273.1	296	csasgca(Uhd)GfaUfUfGfacuacaacscsa	A-2894274.1	740	VPusGfsgudTg(Tgn)agucaaUfcAfugcugscsu	AGCAGCAUGAUUGACUACAACCC	1184
AD-1565609.1	A-2894275.1	297	gscsaug(Ahd)UfuGfAfCfuacaacccscsa	A-2894276.1	741	VPusGfsggdGu(Tgn)guagucAfaUfcaugcsusg	CAGCAUGAUUGACUACAACCCCC	1185

AD-1565766.1	A-2894279.1	298	asgscuc(Uhd)UfuGfCfCfugggacaascsa	A-2894542.1	742	VPusGfsuudGu(C2p)ccaggcAfaAfgagcususc	GAAGCUCUUUGCCUGGGACAACU	1186
AD-1565611.1	A-2894279.1	299	asgscuc(Uhd)UfuGfCfCfugggacaascsa	A-2894280.1	743	VPusGfsudTg(Tgn)cccaggcAfaAfgagcususc	GAAGCUCUUUGCCUGGGACAACU	1187
AD-1565767.1	A-2894281.1	300	gscscuggGfaCfAfAfcuug(Ahd)acasusa	A-2894543.1	744	VPusAfsugdTu(C2p)aaguugUfcCfcaggcsasa	UUGCCUGGGACAACUUGAACAUG	1188
AD-1565612.1	A-2894281.1	301	gscscuggGfaCfAfAfcuug(Ahd)acasusa	A-2894282.1	745	VPusAfsudGu(Tgn)caaguugUfcCfcaggcsasa	UUGCCUGGGACAACUUGAACAUG	1189
AD-1565613.1	A-2894281.1	302	gscscuggGfaCfAfAfcuug(Ahd)acasusa	A-2894283.1	746	VPusAfsugudTc(Agn)aguugUfcCfcaggcsasa	UUGCCUGGGACAACUUGAACAUG	1190
AD-1565614.1	A-2894284.1	303	csusggg(Ahd)CfaAfCfUfugaacaugsgsa	A-2894285.1	747	VPusCfscadTg(Tgn)ucaaguUfgUfcccagsgsc	GCCUGGGACAACUUGAACAUGGU	1191
AD-1565768.1	A-2894544.1	304	usgsgga(Chd)AfaCfUfUfgaacauggsusa	A-2894545.1	748	VPusAfsccdAu(G2p)uucaagUfuGfucccasgsg	CCUGGGACAACUUGAACAUGGUC	1192
AD-1565615.1	A-2894286.1	305	gsgsgac(Ahd)AfcUfUfGfaacaugguscsa	A-2894287.1	749	VPusGfsacdCa(Tgn)gucaaGfuUfgucccsasg	CUGGGACAACUUGAACAUGGUCA	1193
AD-1565616.1	A-2894288.1	306	gsgsgac(Ahd)acUfUfGfaacaugguscsa	A-2894289.1	750	VPusdGsacdCadTguucdAaGfuugucccsasg	CUGGGACAACUUGAACAUGGUCA	1194
AD-1565617.1	A-2894290.1	307	gsgsaca(Ahd)CfuUfGfAfacauggucsasa	A-2894291.1	751	VPusUfsgadCc(Agn)uguucaAfgUfuguccscsa	UGGGACAACUUGAACAUGGUCAC	1195
AD-1565769.1	A-2894292.1	308	gsascaa(Chd)UfuGfAfAfcauggucascsa	A-2894546.1	752	VPusGfsugdAc(C2p)auguucAfaGfuugucscsc	GGGACAACUUGAACAUGGUCACU	1196

AD-1565618.1	A-2894292.1	309	gsascaa(Chd)UfuGfAfAfcauggucascsa	A-2894293.1	753	VPusGfsugadCc(Agn)uguucAfaGfuugucscsc	GGGACAACUUGAACAUGGUCACU	1197
AD-1565770.1	A-2894294.1	310	ascsaac(Uhd)UfgAfAfCfauggucacsusa	A-2894547.1	754	VPusAfsgudGa(C2p)cauguuCfaAfguuguscsc	GGACAACUUGAACAUGGUCACUU	1198
AD-1565619.1	A-2894294.1	311	ascsaac(Uhd)UfgAfAfCfauggucacsusa	A-2894295.1	755	VPusAfsgdTg(Agn)ccauguuCfaAfguuguscsc	GGACAACUUGAACAUGGUCACUU	1199
AD-1565620.1	A-2894296.1	312	csasacu(Uhd)GfaAfCfAfuggucacususa	A-2894297.1	756	VPusAfsagdTg(Agn)ccauguUfcAfaguugsusc	GACAACUUGAACAUGGUCACUUA	1200
AD-1565771.1	A-2894548.1	313	asascuug(Ahd)aCfAfUfggucacuusasa	A-2894549.1	757	VPusUfsaadGu(G2p)accaugUfuCfaaguusgsu	ACAACUUGAACAUGGUCACUUAU	1201
AD-1565621.1	A-1577540.1	314	ascsuug(Ahd)AfcAfUfGfgucacuuasusa	A-2894298.1	758	VPusAfsuadAg(Tgn)gaccauGfuUfcaagususg	CAACUUGAACAUGGUCACUUAUG	1202
AD-1565772.1	A-1577508.1	315	csusuga(Ahd)CfaUfGfGfucacuuausgsa	A-2894550.1	759	VPusCfsaudAa(G2p)ugaccaUfgUfucaagsusu	AACUUGAACAUGGUCACUUAUGA	1203
AD-1565836.1	A-1577508.1	316	csusuga(Ahd)CfaUfGfGfucacuuausgsa	A-2894300.1	760	VPusdCsaudAadGugacdCaUfguucaagsusu	AACUUGAACAUGGUCACUUAUGA	1204
AD-1565837.1	A-1577508.1	317	csusuga(Ahd)CfaUfGfGfucacuuausgsa	A-2894650.1	761	VPusCfsadTa(Agn)gugaccaUfgUfucaagsusu	AACUUGAACAUGGUCACUUAUGA	1205
AD-1565838.1	A-1577508.1	318	csusuga(Ahd)CfaUfGfGfucacuuausgsa	A-2894651.1	762	VPusdCsaudAa(G2p)ugacdCaUfguucaagsusu	AACUUGAACAUGGUCACUUAUGA	1206
AD-1565840.1	A-2894654.1	319	csusugu(Ahd)CfaUfGfGfucacuuausgsa	A-2894655.1	763	VPusCfsaudAa(G2p)ugaccaUfgUfacaagsusu	AACUUGAACAUGGUCACUUAUGA	1207

AD-1565841.1	A-2894656.1	320	csusuca(Ahd)CfaUfGfGfucacuuausgsa	A-2894657.1	764	VPusCfsaudAa(G2p)ugaccaUfgUfugaagsusu	AACUUGAACAUGGUCACUUAUGA	1208
AD-1565842.1	A-2894658.1	321	csusaga(Ahd)CfaUfGfGfucacuuausgsa	A-2894659.1	765	VPusCfsaudAa(G2p)ugaccaUfgUfucuagsusu	AACUUGAACAUGGUCACUUAUGA	1209
AD-822899.17	A-1577508.1	322	csusuga(Ahd)CfaUfGfGfucacuuausgsa	A-1577509.1	766	VPusCfsauaAfgUfGfaccaUfgUfucaagsusu	AACUUGAACAUGGUCACUUAUGA	1210
AD-1565772.2	A-1577508.1	323	csusuga(Ahd)CfaUfGfGfucacuuausgsa	A-2894550.1	767	VPusCfsaudAa(G2p)ugaccaUfgUfucaagsusu	AACUUGAACAUGGUCACUUAUGA	1211
AD-1565622.1	A-2894299.1	324	csusuga(Ahd)caUfGfGfucacuuausgsa	A-2894300.1	768	VPusdCsaudAadGugacdCaUfguucaagsusu	AACUUGAACAUGGUCACUUAUGA	1212
AD-1565843.1	A-1577562.1	325	ususgaa(Chd)AfuGfGfUfcacuuaugsasa	A-2894660.1	769	VPusUfscadTa(Agn)gugaccAfuGfuucaasgsu	ACUUGAACAUGGUCACUUAUGAC	1213
AD-1565844.1	A-1577562.1	326	ususgaa(Chd)AfuGfGfUfcacuuaugsasa	A-2894661.1	770	VPusUfscadTa(A2p)gugaccAfuGfuucaasgsu	ACUUGAACAUGGUCACUUAUGAC	1214
AD-1565845.1	A-1577562.1	327	ususgaa(Chd)AfuGfGfUfcacuuaugsasa	A-2894662.1	771	VPusdTscadTa(Agn)gugadCcAfuguucaasgsu	ACUUGAACAUGGUCACUUAUGAC	1215
AD-1565846.1	A-2894663.1	328	ususgaa(Chd)audGgUfCfacuuaugsasa	A-2894662.1	772	VPusdTscadTa(Agn)gugadCcAfuguucaasgsu	ACUUGAACAUGGUCACUUAUGAC	1216
AD-1565848.1	A-2894666.1	329	ususgau(Chd)AfuGfGfUfcacuuaugsasa	A-2894667.1	773	VPusUfscadTa(Agn)gugaccAfuGfaucaasgsu	ACUUGAACAUGGUCACUUAUGAC	1217
AD-1565849.1	A-2894668.1	330	ususgua(Chd)AfuGfGfUfcacuuaugsasa	A-2894669.1	774	VPusUfscadTa(Agn)gugaccAfuGfuacaasgsu	ACUUGAACAUGGUCACUUAUGAC	1218

AD-1565850.1	A-2894670.1	331	ususcaa(Chd)AfuGfGfUfcacuuaugsasa	A-2894671.1	775	VPusUfscadTa(Agn)gugaccAfuGfuugaasgsu	ACUUGAACAUGGUCACUUAUGAC	1219
AD-822926.4	A-1577562.1	332	ususgaa(Chd)AfuGfGfUfcacuuaugsasa	A-1577563.1	776	VPusUfscauAfaGfUfgaccAfuGfuucaasgsu	ACUUGAACAUGGUCACUUAUGAC	1220
AD-1565623.1	A-2894301.1	333	ususgaa(Chd)auGfGfUfcacuuaugsasa	A-2894302.1	777	VPusdTscadTadAgugadCcAfuguucaasgsu	ACUUGAACAUGGUCACUUAUGAC	1221
AD-1565839.1	A-2894652.1	334	usgsa(Ahd)CfaUfGfGfucacuuausgsa	A-2894653.1	778	VPusCfsaudAa(G2p)ugaccaUfgUfucasgsg	CUUGAACAUGGUCACUUAUGA	1222
AD-1565624.1	A-2894303.1	335	usgsaac(Ahd)ugGfUfCfacuuaugascsa	A-2894304.1	779	VPusdGsucdAudAagugdAcCfauguucasasg	CUUGAACAUGGUCACUUAUGACA	1223
AD-1565847.1	A-2894664.1	336	gsasaca(Uhd)GfGfUfcacuuaugsasa	A-2894665.1	780	VPusUfscadTa(Agn)gugaccAfuGfuucsgsg	UUGAACAUGGUCACUUAUGAC	1224
AD-1565625.1	A-2894305.1	337	gsasaca(Uhd)GfgUfCfAfcuuaugacsasa	A-2894306.1	781	VPusUfsgudCa(Tgn)aagugaCfcAfuguucsasa	UUGAACAUGGUCACUUAUGACAU	1225
AD-1565626.1	A-2894307.1	338	asasca(Uhd)gGfuCfAfCfuuaugacasusa	A-2894308.1	782	VPusAfsugdTc(Agn)uaagugAfcCfauguuscsa	UGAACAUGGUCACUUAUGACAUC	1226
AD-1565773.1	A-2894309.1	339	ascsaugg(Uhd)cAfCfUfuaugacauscsa	A-2894551.1	783	VPusGfsaudGu(C2p)auaaguGfaCfcaugususc	GAACAUGGUCACUUAUGACAUCA	1227
AD-1565627.1	A-2894309.1	340	ascsaugg(Uhd)cAfCfUfuaugacauscsa	A-2894310.1	784	VPusGfsadTg(Tgn)cauaaguGfaCfcaugususc	GAACAUGGUCACUUAUGACAUCA	1228
AD-1565628.1	A-2894309.1	341	ascsaugg(Uhd)cAfCfUfuaugacauscsa	A-2894311.1	785	VPusGfsaugdTc(Agn)uaaguGfaCfcaugususc	GAACAUGGUCACUUAUGACAUCA	1229

AD-1565629.1	A-2894312.1	342	csasugg(Uhd)CfaCfUfUfaugacaucsasa	A-2894313.1	786	VPusUfsgadTg(Tgn)cauaagUfgAfccaugsusu	AACAUGGUCACUUAUGACAUCAA	1230
AD-1565774.1	A-2894552.1	343	asusggu(Chd)AfcUfUfAfugacaucasasa	A-2894553.1	787	VPusUfsugdAu(G2p)ucauaaGfuGfaccausgsu	ACAUGGUCACUUAUGACAUCAAG	1231
AD-1565630.1	A-2894314.1	344	usgsguc(Ahd)CfuUfAfUfgacaucaasgsa	A-2894315.1	788	VPusCfsuudGa(Tgn)gucauaAfgUfgaccasusg	CAUGGUCACUUAUGACAUCAAGC	1232
AD-1565631.1	A-2894316.1	345	gsgsuca(Chd)UfuAfUfGfacaucaagscsa	A-2894317.1	789	VPusGfscudTg(Agn)ugucauAfaGfugaccsasu	AUGGUCACUUAUGACAUCAAGCU	1233
AD-1565775.1	A-2894554.1	346	gsuscac(Uhd)UfaUfGfAfcaucaagcsusa	A-2894555.1	790	VPusAfsgcdTu(G2p)augucaUfaAfgugacscsa	UGGUCACUUAUGACAUCAAGCUC	1234
AD-1565632.1	A-2894318.1	347	gscsaga(Ahd)GfgAfGfAfugcucaggsgsa	A-2894319.1	791	VPusCfsccdTg(Agn)gcaucuCfcUfucugcscsa	UGGCAGAAGGAGAUGCUCAGGGC	1235
AD-1565776.1	A-2894556.1	348	asasggg(Ahd)GfaGfCfCfagccagccsasa	A-2894557.1	792	VPusUfsggdCu(G2p)gcuggcUfcUfcccuuscsa	UGAAGGGAGAGCCAGCCAGCCAG	1236
AD-1565777.1	A-2894558.1	349	gsasuga(Ahd)CfaUfGfGfucaccaucsusa	A-2894559.1	793	VPusAfsgadTg(G2p)ugaccaUfgUfucaucscsu	AGGAUGAACAUGGUCACCAUCUA	1237
AD-1565634.1	A-2894322.1	350	gscsauu(Uhd)auGfGfGfauguuuaasusa	A-2894323.1	794	VPusdAsuudAadAcaucdCcAfuaaaugcsusg	CAGCAUUUAUGGGAUGUUUAAUG	1238
AD-1565778.1	A-2894324.1	351	usgsuuu(Ahd)AfuGfAfCfauaguucasasa	A-2894560.1	795	VPusUfsugdAa(C2p)uaugucAfuUfaaacasusc	GAUGUUUAAUGACAUAGUUCAAG	1239
AD-1565635.1	A-2894324.1	352	usgsuuu(Ahd)AfuGfAfCfauaguucasasa	A-2894325.1	796	VPusUfsudGa(Agn)cuaugucAfuUfaaacasusc	GAUGUUUAAUGACAUAGUUCAAG	1240

AD-1565636.1	A-2894324.1	353	usgsuuu(Ahd)AfuGfAfCfauaguucasasa	A-2894326.1	797	VPusUfsugadAc(Tgn)augucAfuUfaaacasusc	GAUGUUUAAUGACAUAGUUCAAG	1241
AD-1565637.1	A-2894327.1	354	gsusuua(Ahd)UfgAfCfAfuaguucaasgsa	A-2894328.1	798	VPusCfsuudGa(Agn)cuauguCfaUfuaaacsasu	AUGUUUAAUGACAUAGUUCAAGU	1242
AD-1565638.1	A-2894329.1	355	gsusuua(Ahd)ugAfCfAfuaguucaasgsa	A-2894330.1	799	VPusdCsuudGadAcuaudGuCfauuaaacsasu	AUGUUUAAUGACAUAGUUCAAGU	1243
AD-1565639.1	A-2894331.1	356	ususuaa(Uhd)GfaCfAfUfaguucaagsusa	A-2894332.1	800	VPusAfscudTg(Agn)acuaugUfcAfuuaaascsa	UGUUUAAUGACAUAGUUCAAGUU	1244
AD-1565779.1	A-2894561.1	357	ususaaug(Ahd)cAfUfAfguucaagususa	A-2894562.1	801	VPusAfsacdTu(G2p)aacuauGfuCfauuaasasc	GUUUAAUGACAUAGUUCAAGUUU	1245
AD-1565640.1	A-2894333.1	358	usasaug(Ahd)CfaUfAfGfuucaaguususa	A-2894334.1	802	VPusAfsaadCu(Tgn)gaacuaUfgUfcauuasasa	UUUAAUGACAUAGUUCAAGUUUU	1246
AD-1565641.1	A-2894335.1	359	usasaug(Ahd)caUfAfGfuucaaguususa	A-2894336.1	803	VPusdAsaadCudTgaacdTaUfgucauuasasa	UUUAAUGACAUAGUUCAAGUUUU	1247
AD-1565642.1	A-2894337.1	360	asasuga(Chd)AfuAfGfUfucaaguuususa	A-2894338.1	804	VPusAfsaadAc(Tgn)ugaacuAfuGfucauusasa	UUAAUGACAUAGUUCAAGUUUUC	1248
AD-1565643.1	A-2894339.1	361	asasuga(Chd)auAfGfUfucaaguuususa	A-2894340.1	805	VPusdAsaadAcdTugaadCuAfugucauusasa	UUAAUGACAUAGUUCAAGUUUUC	1249
AD-1565644.1	A-2894341.1	362	asusgac(Ahd)uaGfUfUfcaaguuuuscsa	A-2894342.1	806	VPusdGsaadAadCuugadAcUfaugucaususa	UAAUGACAUAGUUCAAGUUUUCU	1250
AD-1565645.1	A-2894343.1	363	usgsaca(Uhd)agUfUfCfaaguuuucsusa	A-2894344.1	807	VPusdAsgadAadAcuugdAaCfuaugucasusu	AAUGACAUAGUUCAAGUUUUCUU	1251

AD-1565646.1	A-2894345.1	364	gsascau(Ahd)guUfCfAfaguuuucususa	A-2894346.1	808	VPusdAsagdAadAacuudGaAfcuaugucsasu	AUGACAUAGUUCAAGUUUUCUUG	1252
AD-1565851.1	A-2894672.1	365	ascsaua(Ghd)UfuCfAfAfguuuucuusgsa	A-1806645.1	809	VPusCfsaaga(Agn)aacuugAfaCfuauguscsa	UGACAUAGUUCAAGUUUUCUUGU	1253
AD-1565852.1	A-2894347.1	366	ascsauag(Uhd)uCfAfAfguuuucuusgsa	A-2894673.1	810	VPusdCsaadGadAaacudTgAfacuauguscsg	UGACAUAGUUCAAGUUUUCUUGU	1254
AD-1565853.1	A-2894347.1	367	ascsauag(Uhd)uCfAfAfguuuucuusgsa	A-2894674.1	811	VPusCfsaadGa(Agn)aacuugAfaCfuauguscsg	UGACAUAGUUCAAGUUUUCUUGU	1255
AD-1565854.1	A-2894347.1	368	ascsauag(Uhd)uCfAfAfguuuucuusgsa	A-2894675.1	812	VPusdCsaadGa(Agn)aacudTgAfaCfuauguscsg	UGACAUAGUUCAAGUUUUCUUGU	1256
AD-1565857.1	A-2894679.1	369	ascsauug(Uhd)uCfAfAfguuuucuusgsa	A-2894680.1	813	VPusCfsaadGa(Agn)aacuugAfaCfaauguscsg	UGACAUAGUUCAAGUUUUCUUGU	1257
AD-1565858.1	A-2894681.1	370	ascsaaag(Uhd)uCfAfAfguuuucuusgsa	A-2894682.1	814	VPusCfsaadGa(Agn)aacuugAfaCfuuuguscsg	UGACAUAGUUCAAGUUUUCUUGU	1258
AD-1565859.1	A-2894683.1	371	ascsuuag(Uhd)uCfAfAfguuuucuusgsa	A-2894684.1	815	VPusCfsaadGa(Agn)aacuugAfaCfuaaguscsg	UGACAUAGUUCAAGUUUUCUUGU	1259
AD-1565647.1	A-2894347.1	372	ascsauag(Uhd)uCfAfAfguuuucuusgsa	A-2894348.1	816	VPusdCsaadGadAaacudTgAfacuauguscsa	UGACAUAGUUCAAGUUUUCUUGU	1260
AD-1565648.1	A-2894349.1	373	csasuag(Uhd)ucAfAfGfuuuucuugsusa	A-2894350.1	817	VPusdAscadAgdAaaacdTuGfaacuaugsusc	GACAUAGUUCAAGUUUUCUUGUG	1261
AD-1565855.1	A-2894676.1	374	asusagu(Uhd)CfAfAfguuuucuusgsa	A-2894677.1	818	VPusCfsaadGa(Agn)aacuugAfaCfuausgsu	ACAUAGUUCAAGUUUUCUUGU	1262

AD-1565856.1	A-2894676.1	375	asusagu(Uhd)CfAfAfguuuucuusgsa	A-2894678.1	819	VPusdCsaadGa(Agn)aacudTgAfaCfuausgsu	ACAUAGUUCAAGUUUUCUUGU	1263
AD-1565649.1	A-2894351.1	376	asusagu(Uhd)caAfGfUfuuucuugusgsa	A-2894352.1	820	VPusdCsacdAadGaaaadCuUfgaacuausgsu	ACAUAGUUCAAGUUUUCUUGUGA	1264
AD-1565650.1	A-2894353.1	377	usasguu(Chd)AfaGfUfUfuucuugugsasa	A-2894354.1	821	VPusUfscadCa(Agn)gaaaacUfuGfaacuasusg	CAUAGUUCAAGUUUUCUUGUGAU	1265
AD-1565651.1	A-2894355.1	378	usasguu(Chd)aaGfUfUfuucuugugsasa	A-2894356.1	822	VPusdTscadCadAgaaadAcUfugaacuasusg	CAUAGUUCAAGUUUUCUUGUGAU	1266
AD-1565652.1	A-2894357.1	379	asgsuuc(Ahd)AfgUfUfUfucuugugasusa	A-2894358.1	823	VPusAfsucdAc(Agn)agaaaaCfuUfgaacusasu	AUAGUUCAAGUUUUCUUGUGAUU	1267
AD-1565653.1	A-2894359.1	380	asgsuuc(Ahd)agUfUfUfucuugugasusa	A-2894360.1	824	VPusdAsucdAcdAagaadAaCfuugaacusasu	AUAGUUCAAGUUUUCUUGUGAUU	1268
AD-1565780.1	A-2894361.1	381	gsusuca(Ahd)GfuUfUfUfcuugugaususa	A-2894563.1	825	VPusAfsaudCa(C2p)aagaaaAfcUfugaacsusa	UAGUUCAAGUUUUCUUGUGAUUU	1269
AD-1565654.1	A-2894361.1	382	gsusuca(Ahd)GfuUfUfUfcuugugaususa	A-2894362.1	826	VPusAfsadTc(Agn)caagaaaAfcUfugaacsusa	UAGUUCAAGUUUUCUUGUGAUUU	1270
AD-1565655.1	A-2894363.1	383	ususcaag(Uhd)uUfUfCfuugugauususa	A-2894364.1	827	VPusAfsaadTc(Agn)caagaaAfaCfuugaascsu	AGUUCAAGUUUUCUUGUGAUUUG	1271
AD-1565781.1	A-2894365.1	384	uscsaag(Uhd)UfuUfCfUfugugauuusgsa	A-2894564.1	828	VPusCfsaadAu(C2p)acaagaAfaAfcuugasasc	GUUCAAGUUUUCUUGUGAUUUGG	1272
AD-1565656.1	A-2894365.1	385	uscsaag(Uhd)UfuUfCfUfugugauuusgsa	A-2894366.1	829	VPusCfsadAa(Tgn)cacaagaAfaAfcuugasasc	GUUCAAGUUUUCUUGUGAUUUGG	1273

AD-1565657.1	A-2894365.1	386	uscsaag(Uhd)UfuUfCfUfugugauuusgsa	A-2894367.1	830	VPusCfsaaadTc(Agn)caagaAfaAfcuugasasc	GUUCAAGUUUUCUUGUGAUUUGG	1274
AD-1565658.1	A-2894368.1	387	uscsaag(Uhd)uuUfCfUfugugauuusgsa	A-2894369.1	831	VPusdCsaadAudCacaadGaAfaacuugasasc	GUUCAAGUUUUCUUGUGAUUUGG	1275
AD-1565659.1	A-2894370.1	388	csasagu(Uhd)uuCfUfUfgugauuugsgsa	A-2894371.1	832	VPusdCscadAadTcacadAgAfaaacuugsasa	UUCAAGUUUUCUUGUGAUUUGGG	1276
AD-1565660.1	A-2894372.1	389	asasguu(Uhd)ucUfUfGfugauuuggsgsa	A-2894373.1	833	VPusdCsccdAadAucacdAaGfaaaacuusgsa	UCAAGUUUUCUUGUGAUUUGGGG	1277
AD-1565782.1	A-2894565.1	390	usgsaaa(Ahd)CfcAfUfUfgcucuugcsasa	A-2894566.1	834	VPusUfsgcdAa(G2p)agcaauGfgUfuuucasgsg	CCUGAAAACCAUUGCUCUUGCAU	1278
AD-1565661.1	A-2894374.1	391	gsasaaa(Chd)CfaUfUfGfcucuugcasusa	A-2894375.1	835	VPusAfsugdCa(Agn)gagcaaUfgGfuuuucsasg	CUGAAAACCAUUGCUCUUGCAUG	1279
AD-1565662.1	A-2894376.1	392	gsasaaa(Chd)caUfUfGfcucuugcasusa	A-2894377.1	836	VPusdAsugdCadAgagcdAaUfgguuuucsasg	CUGAAAACCAUUGCUCUUGCAUG	1280
AD-1565663.1	A-2894378.1	393	asasaac(Chd)AfuUfGfCfucuugcausgsa	A-2894379.1	837	VPusCfsaudGc(Agn)agagcaAfuGfguuuuscsa	UGAAAACCAUUGCUCUUGCAUGU	1281
AD-1565664.1	A-2894380.1	394	asasaac(Chd)auUfGfCfucuugcausgsa	A-2894381.1	838	VPusdCsaudGcdAagagdCaAfugguuuuscsa	UGAAAACCAUUGCUCUUGCAUGU	1282
AD-1565783.1	A-2894382.1	395	asasacc(Ahd)UfuGfCfUfcuugcaugsusa	A-2894567.1	839	VPusAfscadTg(C2p)aagagcAfaUfgguuususc	GAAAACCAUUGCUCUUGCAUGUU	1283
AD-1565665.1	A-2894382.1	396	asasacc(Ahd)UfuGfCfUfcuugcaugsusa	A-2894383.1	840	VPusAfscaudGc(Agn)agagcAfaUfgguuususc	GAAAACCAUUGCUCUUGCAUGUU	1284

AD-1565784.1	A-2894568.1	397	asascca(Uhd)UfgCfUfCfuugcaugususa	A-2894569.1	841	VPusAfsacdAu(G2p)caagagCfaAfugguususu	AAAACCAUUGCUCUUGCAUGUUA	1285
AD-1565666.1	A-2894384.1	398	ascscau(Uhd)GfcUfCfUfugcauguusasa	A-2894385.1	842	VPusUfsaadCa(Tgn)gcaagaGfcAfauggususu	AAACCAUUGCUCUUGCAUGUUAC	1286
AD-1565667.1	A-2894386.1	399	cscsauug(Chd)uCfUfUfgcauguuascsa	A-2894387.1	843	VPusGfsuadAc(Agn)ugcaagAfgCfaauggsusu	AACCAUUGCUCUUGCAUGUUACA	1287
AD-1565785.1	A-2894388.1	400	csasuug(Chd)UfcUfUfGfcauguuacsasa	A-2894570.1	844	VPusUfsgudAa(C2p)augcaaGfaGfcaaugsgsu	ACCAUUGCUCUUGCAUGUUACAU	1288
AD-1565668.1	A-2894388.1	401	csasuug(Chd)UfcUfUfGfcauguuacsasa	A-2894389.1	845	VPusUfsgdTa(Agn)caugcaaGfaGfcaaugsgsu	ACCAUUGCUCUUGCAUGUUACAU	1289
AD-1565669.1	A-2894390.1	402	asusugc(Uhd)cuUfGfCfauguuacasusa	A-2894391.1	846	VPusdAsugdTadAcaugdCaAfgagcaausgsg	CCAUUGCUCUUGCAUGUUACAUG	1290
AD-1565670.1	A-2894392.1	403	ususgcu(Chd)uuGfCfAfuguuacausgsa	A-2894393.1	847	VPusdCsaudGudAacaudGcAfagagcaasusg	CAUUGCUCUUGCAUGUUACAUGG	1291
AD-1565860.1	A-2894685.1	404	ususgcu(Chd)UfuGfCfAfuguuacausgsa	A-1804746.1	848	VPusCfsaugUfaAfCfaugcAfaGfagcaasusg	CAUUGCUCUUGCAUGUUACAUGG	1292
AD-1565861.1	A-2894686.1	405	ususgca(Chd)uuGfCfAfuguuacausgsa	A-2894687.1	849	VPusdCsaudGudAacaudGcAfagugcaasusg	CAUUGCUCUUGCAUGUUACAUGG	1293
AD-1565862.1	A-2894688.1	406	ususggu(Chd)uuGfCfAfuguuacausgsa	A-2894689.1	850	VPusdCsaudGudAacaudGcAfagaccaasusg	CAUUGCUCUUGCAUGUUACAUGG	1294
AD-1565863.1	A-2894690.1	407	ususccu(Chd)uuGfCfAfuguuacausgsa	A-2894691.1	851	VPusdCsaudGudAacaudGcAfagaggaasusg	CAUUGCUCUUGCAUGUUACAUGG	1295

AD-1565864.1	A-2894692.1	408	ususgcu(Chd)UfudGcdAUfguuacausgsa	A-2894393.1	852	VPusdCsaudGudAacaudGcAfagagcaasusg	CAUUGCUCUUGCAUGUUACAUGG	1296
AD-1565866.1	A-2894685.1	409	ususgcu(Chd)UfuGfCfAfuguuacausgsa	A-2894695.1	853	VPusCfsaudGu(Agn)acaugcAfaGfagcaasusg	CAUUGCUCUUGCAUGUUACAUGG	1297
AD-1565670.2	A-2894392.1	410	ususgcu(Chd)uuGfCfAfuguuacausgsa	A-2894393.1	854	VPusdCsaudGudAacaudGcAfagagcaasusg	CAUUGCUCUUGCAUGUUACAUGG	1298
AD-1565671.1	A-2894394.1	411	usgscuc(Uhd)UfgCfAfUfguuacaugsgsa	A-2894395.1	855	VPusCfscadTg(Tgn)aacaugCfaAfgagcasasu	AUUGCUCUUGCAUGUUACAUGGU	1299
AD-1565865.1	A-2894693.1	412	gscsucu(Uhd)GfCfAfuguuacausgsa	A-2894694.1	856	VPusdCsaudGudAacaudGcAfagagcsgsg	UUGCUCUUGCAUGUUACAUGG	1300
AD-1565786.1	A-2894571.1	413	gscsucu(Uhd)GfcAfUfGfuuacauggsusa	A-2894572.1	857	VPusAfsccdAu(G2p)uaacauGfcAfagagcsasa	UUGCUCUUGCAUGUUACAUGGUU	1301
AD-1565672.1	A-2894396.1	414	csuscuug(Chd)aUfGfUfuacauggususa	A-2894397.1	858	VPusAfsacdCa(Tgn)guaacaUfgCfaagagscsa	UGCUCUUGCAUGUUACAUGGUUA	1302
AD-1565673.1	A-2894396.1	415	csuscuug(Chd)aUfGfUfuacauggususa	A-2894398.1	859	VPusdAsacdCadTguaadCaUfgcaagagscsa	UGCUCUUGCAUGUUACAUGGUUA	1303
AD-1565674.1	A-2894399.1	416	uscsuug(Chd)AfuGfUfUfacaugguusasa	A-2894400.1	860	VPusUfsaadCc(Agn)uguaacAfuGfcaagasgsc	GCUCUUGCAUGUUACAUGGUUAC	1304
AD-1565787.1	A-1577564.1	417	csusugc(Ahd)UfgUfUfAfcaugguuascsa	A-2894573.1	861	VPusGfsuadAc(C2p)auguaaCfaUfgcaagsasg	CUCUUGCAUGUUACAUGGUUACC	1305
AD-1565675.1	A-1577564.1	418	csusugc(Ahd)UfgUfUfAfcaugguuascsa	A-2894401.1	862	VPusGfsuaadCc(Agn)uguaaCfaUfgcaagsasg	CUCUUGCAUGUUACAUGGUUACC	1306

AD-1565788.1	A-1577566.1	419	ususgca(Uhd)GfuUfAfCfaugguuacscsa	A-2894574.1	863	VPusGfsgudAa(C2p)caguguaAfcAfugcaasgsa	UCUUGCAUGUUACAUGGUUACCA	1307
AD-1565676.1	A-1577566.1	420	ususgca(Uhd)GfuUfAfCfaugguuacscsa	A-2894402.1	864	VPusGfsgdTa(Agn)ccaguguaAfcAfugcaasgsa	UCUUGCAUGUUACAUGGUUACCA	1308
AD-1565677.1	A-2894403.1	421	ususgca(Uhd)guUfAfCfaugguuacscsa	A-2894404.1	865	VPusdGsgudAadCcaugdTaAfcaugcaasgsa	UCUUGCAUGUUACAUGGUUACCA	1309
AD-1565678.1	A-2894405.1	422	usgscaug(Uhd)uAfCfAfugguuaccsasa	A-2894406.1	866	VPusUfsggdTa(Agn)ccauguAfaCfaugcasasg	CUUGCAUGUUACAUGGUUACCAC	1310
AD-1565679.1	A-1577580.1	423	gscsaug(Uhd)UfaCfAfUfgguuaccascsa	A-2894407.1	867	VPusGfsugdGu(Agn)accaugUfaAfcaugcsasa	UUGCAUGUUACAUGGUUACCACA	1311
AD-1565680.1	A-2894408.1	424	gscsaug(Uhd)uaCfAfUfgguuaccascsa	A-2894409.1	868	VPusdGsugdGudAaccadTgUfaacaugcsasa	UUGCAUGUUACAUGGUUACCACA	1312
AD-1565681.1	A-2894410.1	425	csasugu(Uhd)AfcAfUfGfguuaccacsasa	A-2894411.1	869	VPusUfsgudGg(Tgn)aaccauGfuAfacaugscsa	UGCAUGUUACAUGGUUACCACAA	1313
AD-1565789.1	A-2894575.1	426	asusguu(Ahd)CfaUfGfGfuuaccacasasa	A-2894576.1	870	VPusUfsugdTg(G2p)uaaccaUfgUfaacausgsc	GCAUGUUACAUGGUUACCACAAG	1314
AD-1565790.1	A-2894577.1	427	usgsuua(Chd)AfuGfGfUfuaccacaasgsa	A-2894578.1	871	VPusCfsuudGu(G2p)guaaccAfuGfuaacasusg	CAUGUUACAUGGUUACCACAAGC	1315
AD-1565682.1	A-2894412.1	428	csusccu(Chd)ugGfCfCfagcaucgasasa	A-2894413.1	872	VPusdTsucdGadTgcugdGcCfagaggagscsu	AGCUCCUCUGGCCAGCAUCGAAU	1316
AD-1565791.1	A-2894579.1	429	asusaua(Ahd)GfuAfAfGfaugcauuusasa	A-2894580.1	873	VPusUfsaadAu(G2p)caucuuAfcUfuauaususc	GAAUAUAAGUAAGAUGCAUUUAC	1317

AD-1565683.1	A-2894414.1	430	usasuaag(Uhd)aAfGfAfugcauuuascsa	A-2894415.1	874	VPusdGsuadAadTgcaudCuUfacuuauasusu	AAUAUAAGUAAGAUGCAUUUACU	1318
AD-1565684.1	A-2894416.1	431	asusaag(Uhd)aaGfAfUfgcauuuacsusa	A-2894417.1	875	VPusdAsgudAadAugcadTcUfuacuuausasu	AUAUAAGUAAGAUGCAUUUACUA	1319
AD-1565685.1	A-2894418.1	432	usasagu(Ahd)agAfUfGfcauuuacusasa	A-2894419.1	876	VPusdTsagdTadAaugcdAuCfuuacuuasusa	UAUAAGUAAGAUGCAUUUACUAC	1320
AD-1565867.1	A-2894420.1	433	asasgua(Ahd)GfaUfGfCfauuuacuascsa	A-1804926.1	877	VPusGfsuagUfaAfAfugcaUfcUfuacuusasu	AUAAGUAAGAUGCAUUUACUACA	1321
AD-1565868.1	A-2894420.1	434	asasgua(Ahd)GfaUfGfCfauuuacuascsa	A-2894696.1	878	VPusdGsuadGudAaaugdCaUfcuuacuusgsu	AUAAGUAAGAUGCAUUUACUACA	1322
AD-1565869.1	A-2894420.1	435	asasgua(Ahd)GfaUfGfCfauuuacuascsa	A-2894697.1	879	VPusGfsuadGu(Agn)aaugcaUfcUfuacuusgsu	AUAAGUAAGAUGCAUUUACUACA	1323
AD-1565870.1	A-2894420.1	436	asasgua(Ahd)GfaUfGfCfauuuacuascsa	A-2894698.1	880	VPusGfsuadGu(A2p)aaugcaUfcUfuacuusgsu	AUAAGUAAGAUGCAUUUACUACA	1324
AD-1565871.1	A-2894422.1	437	asasgua(Ahd)gaUfGfCfauuuacuascsa	A-2894697.1	881	VPusGfsuadGu(Agn)aaugcaUfcUfuacuusgsu	AUAAGUAAGAUGCAUUUACUACA	1325
AD-1565872.1	A-2894699.1	438	asasgua(Ahd)gadTgCfdAuuuacuascsa	A-2894700.1	882	VPusdGsuadGu(Agn)aaugdCaUfcUfuacuusgsu	AUAAGUAAGAUGCAUUUACUACA	1326
AD-1565873.1	A-2894422.1	439	asasgua(Ahd)gaUfGfCfauuuacuascsa	A-2894700.1	883	VPusdGsuadGu(Agn)aaugdCaUfcUfuacuusgsu	AUAAGUAAGAUGCAUUUACUACA	1327
AD-1565874.1	A-2894701.1	440	asasguu(Ahd)gaUfGfCfauuuacuascsa	A-2894702.1	884	VPusGfsuadGu(Agn)aaugcaUfcUfaacuusgsu	AUAAGUAAGAUGCAUUUACUACA	1328

AD-1565875.1	A-2894703.1	441	asasgaa(Ahd)gaUfGfCfauuuacuascsa	A-2894704.1	885	VPusGfsuadGu(Agn)aaugcaUfcUfuucuusgsu	AUAAGUAAGAUGCAUUUACUACA	1329
AD-1565876.1	A-2894705.1	442	asascua(Ahd)gaUfGfCfauuuacuascsa	A-2894706.1	886	VPusGfsuadGu(Agn)aaugcaUfcUfuaguusgsu	AUAAGUAAGAUGCAUUUACUACA	1330
AD-1565686.1	A-2894420.1	443	asasgua(Ahd)GfaUfGfCfauuuacuascsa	A-2894421.1	887	VPusGfsuadGu(Agn)aaugcaUfcUfuacuusasu	AUAAGUAAGAUGCAUUUACUACA	1331
AD-1565687.1	A-2894422.1	444	asasgua(Ahd)gaUfGfCfauuuacuascsa	A-2894423.1	888	VPusdGsuadGudAaaugdCaUfcuuacuusasu	AUAAGUAAGAUGCAUUUACUACA	1332
AD-1565688.1	A-2894424.1	445	asgsuaag(Ahd)uGfCfAfauuuacuacsasa	A-2894425.1	889	VPusUfsgudAg(Tgn)aaaugcAfuCfuuacususa	UAAGUAAGAUGCAUUUACUACAG	1333
AD-1565877.1	A-2894707.1	446	gsusaag(Ahd)UfGfCfauuuacuascsa	A-2894708.1	890	VPusGfsuadGu(Agn)aaugcaUfcUfuacsusu	AAGUAAGAUGCAUUUACUACA	1334
AD-1565689.1	A-2894426.1	447	ususggc(Uhd)UfcUfAfAfugcuucagsasa	A-2894427.1	891	VPusUfscudGa(Agn)gcauuaGfaAfgccaascsu	AGUUGGCUUCUAAUGCUUCAGAU	1335
AD-1565792.1	A-2894428.1	448	csususcu(Ahd)AfuGfCfUfucagauagsasa	A-2894581.1	892	VPusUfscudAu(C2p)ugaagcAfuUfagaagscsc	GGCUUCUAAUGCUUCAGAUAGAA	1336
AD-1565690.1	A-2894428.1	449	csususcu(Ahd)AfuGfCfUfucagauagsasa	A-2894429.1	893	VPusUfscdTa(Tgn)cugaagcAfuUfagaagscsc	GGCUUCUAAUGCUUCAGAUAGAA	1337
AD-1565691.1	A-2894428.1	450	csususcu(Ahd)AfuGfCfUfucagauagsasa	A-2894430.1	894	VPusUfscuadTc(Tgn)gaagcAfuUfagaagscsc	GGCUUCUAAUGCUUCAGAUAGAA	1338

Table 2B. Exemplary Human MYOC siRNA Unmodified Single Strands and Duplex Sequences

Column 1 indicates duplex name; the number following the decimal point in a duplex name merely refers to a batch production number. Column 2 indicates the sense sequence name. Column 3 indicates the sequence ID for the sequence of column 4. Column 4 provides the unmodified sequence of a sense strand suitable for use in a duplex described herein. Column 5 provides the position in the target mRNA (NM_000261.2) of the sense strand of Column 4. Column 6 indicates the antisense sequence name. Column 7 indicates the sequence ID for the sequence of column 8. Column 8 provides the sequence of an antisense strand suitable for use in a duplex described herein, without specifying chemical modifications. Column 9 indicates the position in the target mRNA (NM_000261.2) that is complementary to the antisense strand of Column 8.

Duplex Name	Sense sequence name	Seq ID NO: (sense)	Sense sequence (5'-3')	mRNA target range in NM_000261.2	Antisense sequence name	Seq ID NO: (antisense)	antisense sequence (5'-3')	mRNA target range in NM_000261.2
AD-1565444.1	A-2893965.1	1339	CUCUCAGCACAGCAGAGCUUA	29-49	A-2893966.1	1783	UAAGCTCUGCUGUGCUGAGAGGU	27-49
AD-1565445.1	A-2893965.1	1340	CUCUCAGCACAGCAGAGCUUA	29-49	A-2893967.1	1784	UAAGCTCTGCUGUGCUGAGAGGU	27-49
AD-1565692.1	A-2893965.1	1341	CUCUCAGCACAGCAGAGCUUA	29-49	A-2894431.1	1785	UAAGCUCUGCUGUGCUGAGAGGU	27-49
AD-1565446.1	A-2893968.1	1342	UCUCAGCACAGCAGAGCUUUA	30-50	A-2893969.1	1786	UAAAGCTCUGCUGUGCUGAGAGG	28-50
AD-1565447.1	A-2893970.1	1343	CAGCAGAGCUUUCCAGAGGAA	38-58	A-2893971.1	1787	UUCCTCTGGAAAGCUCUGCUGUG	36-58
AD-1565448.1	A-2893972.1	1344	AAUGAGGUUCUUCUGUGCACA	77-97	A-2893973.1	1788	UGUGCACAGAAGAACCUCAUUGC	75-97
AD-1565693.1	A-2893972.1	1345	AAUGAGGUUCUUCUGUGCACA	77-97	A-2894432.1	1789	UGUGCACAGAAGAACCUCAUUGC	75-97
AD-1565449.1	A-2893974.1	1346	AUGAGGUUCUUCUGUGCACGA	78-98	A-2893975.1	1790	UCGUGCACAGAAGAACCUCAUUG	76-98
AD-1565450.1	A-2893976.1	1347	UGAGGUUCUUCUGUGCACGUA	79-99	A-2893977.1	1791	UACGUGCACAGAAGAACCUCAUU	77-99

AD-1565694.1	A-2893976.1	1348	UGAGGUUCUUCUGUGCACGUA	79-99	A-2894433.1	1792	UACGTGCACAGAAGAACCUCAUU	77-99
AD-1565695.1	A-2894434.1	1349	GAGGUUCUUCUGUGCACGUUA	80-100	A-2894435.1	1793	UAACGUGCACAGAAGAACCUCAU	78-100
AD-1193175.5	A-2058874.1	1350	AGGUUCUUCUGUGCACGUUGA	81-101	A-1801665.1	1794	UCAACGUGCACAGAAGAACCUCA	79-101
AD-1565793.1	A-2893978.1	1351	AGGUUCUUCUGUGCACGUUGA	81-101	A-2894582.1	1795	UCAACGTGCACAGAAGAACCUCG	79-101
AD-1565795.1	A-2058874.1	1352	AGGUUCUUCUGUGCACGUUGA	81-101	A-2894585.1	1796	UCAACGTGCACAGAAGAACCUCG	79-101
AD-1565796.1	A-2058874.1	1353	AGGUUCUUCUGUGCACGUUGA	81-101	A-2894586.1	1797	UCAACGTGCACAGAAGAACCUCG	79-101
AD-1565797.1	A-2894587.1	1354	AGGUACUUCUGUGCACGUUGA	81-101	A-2894588.1	1798	UCAACGTGCACAGAAGUACCUCG	79-101
AD-1565798.1	A-2894589.1	1355	AGGAUCUUCUGUGCACGUUGA	81-101	A-2894590.1	1799	UCAACGTGCACAGAAGAUCCUCG	79-101
AD-1565799.1	A-2894591.1	1356	AGCUUCUUCUGUGCACGUUGA	81-101	A-2894592.1	1800	UCAACGTGCACAGAAGAAGCUCG	79-101
AD-1565451.1	A-2893978.1	1357	AGGUUCUUCUGUGCACGUUGA	81-101	A-2893979.1	1801	UCAACGTGCACAGAAGAACCUCA	79-101
AD-1565452.1	A-2893980.1	1358	GGUUCUUCUGUGCACGUUGCA	82-102	A-2893981.1	1802	UGCAACGUGCACAGAAGAACCUC	80-102
AD-1565696.1	A-2894436.1	1359	GGUUCUUCUGUGCACGUUGCA	82-102	A-2894437.1	1803	UGCAACGUGCACAGAAGAACCUC	80-102
AD-1565794.1	A-2894583.1	1360	GUUCUUCUGUGCACGUUGA	83-101	A-2894584.1	1804	UCAACGTGCACAGAAGAACCU	81-101
AD-1565453.1	A-2893982.1	1361	GUUCUUCUGUGCACGUUGCUA	83-103	A-2893983.1	1805	UAGCAACGUGCACAGAAGAACCU	81-103
AD-1565454.1	A-2893984.1	1362	UUCUUCUGUGCACGUUGCUGA	84-104	A-2893985.1	1806	UCAGCAACGUGCACAGAAGAACC	82-104
AD-1565455.1	A-2893986.1	1363	UCUUCUGUGCACGUUGCUGCA	85-105	A-2893987.1	1807	UGCAGCAACGUGCACAGAAGAAC	83-105
AD-1565456.1	A-2893988.1	1364	UCUUCUGUGCACGUUGCUGCA	85-105	A-2893989.1	1808	UGCAGCAACGUGCACAGAAGAAC	83-105

AD-1565457.1	A-2893990.1	1365	CUUCUGUGCACGUUGCUGCAA	86-106	A-2893991.1	1809	UUGCAGCAACGUGCACAGAAGAA	84-106
AD-1565697.1	A-2893990.1	1366	CUUCUGUGCACGUUGCUGCAA	86-106	A-2894438.1	1810	UUGCAGCAACGUGCACAGAAGAA	84-106
AD-1565698.1	A-2894439.1	1367	UUCUGUGCACGUUGCUGCAGA	87-107	A-2894440.1	1811	UCUGCAGCAACGUGCACAGAAGA	85-107
AD-1565458.1	A-2893992.1	1368	UCUGUGCACGUUGCUGCAGCA	88-108	A-2893993.1	1812	UGCUGCAGCAACGUGCACAGAAG	86-108
AD-1565459.1	A-2893994.1	1369	CUGUGCACGUUGCUGCAGCUA	89-109	A-2893995.1	1813	UAGCUGCAGCAACGUGCACAGAA	87-109
AD-1565699.1	A-2893994.1	1370	CUGUGCACGUUGCUGCAGCUA	89-109	A-2894441.1	1814	UAGCTGCAGCAACGUGCACAGAA	87-109
AD-1565700.1	A-2894442.1	1371	UGUGCACGUUGCUGCAGCUUA	90-110	A-2894443.1	1815	UAAGCUGCAGCAACGUGCACAGA	88-110
AD-1565460.1	A-2893996.1	1372	GUGCACGUUGCUGCAGCUUUA	91-111	A-2893997.1	1816	UAAAGCTGCAGCAACGUGCACAG	89-111
AD-1565461.1	A-2893998.1	1373	GCACGUUGCUGCAGCUUUGGA	93-113	A-2893999.1	1817	UCCAAAGCUGCAGCAACGUGCAC	91-113
AD-1565462.1	A-2894000.1	1374	CACGUUGCUGCAGCUUUGGGA	94-114	A-2894001.1	1818	UCCCAAAGCUGCAGCAACGUGCA	92-114
AD-1565701.1	A-2894444.1	1375	GAGAUGCCAGCUGUCCAGCUA	117-137	A-2894445.1	1819	UAGCTGGACAGCUGGCAUCUCAG	115-137
AD-1565463.1	A-2894002.1	1376	GAUGCCAGCUGUCCAGCUGCA	119-139	A-2894003.1	1820	UGCAGCTGGACAGCUGGCAUCUC	117-139
AD-1565464.1	A-2894004.1	1377	CUGUCCAGCUGCUGCUUCUGA	127-147	A-2894005.1	1821	UCAGAAGCAGCAGCUGGACAGCU	125-147
AD-1565465.1	A-2894006.1	1378	CCAGCUGCUGCUUCUGGCCUA	131-151	A-2894007.1	1822	UAGGCCAGAAGCAGCAGCUGGAC	129-151
AD-1565466.1	A-2894008.1	1379	CUGGCCUGCCUGGUGUGGGAA	144-164	A-2894009.1	1823	UUCCCACACCAGGCAGGCCAGAA	142-164
AD-1565467.1	A-2894008.1	1380	CUGGCCUGCCUGGUGUGGGAA	144-164	A-2894010.1	1824	UUCCCACACCAGGCAGGCCAGAA	142-164
AD-1565702.1	A-2894008.1	1381	CUGGCCUGCCUGGUGUGGGAA	144-164	A-2894446.1	1825	UUCCCACACCAGGCAGGCCAGAA	142-164

AD-1565468.1	A-2894011.1	1382	UGGCCUGCCUGGUGUGGGAUA	145-165	A-2894012.1	1826	UAUCCCACACCAGGCAGGCCAGA	143-165
AD-1565469.1	A-2894013.1	1383	GCCUGCCUGGUGUGUGAUGUA	147-167	A-2894014.1	1827	UACATCACACACCAGGCAGGCCA	145-167
AD-1565703.1	A-2894447.1	1384	GCCUGCCUGGUGUGGGAUGUA	147-167	A-2894448.1	1828	UACATCCCACACCAGGCAGGCCA	145-167
AD-1565704.1	A-2894449.1	1385	AGAGUGGCCGAUGCCAGUAUA	205-225	A-2894450.1	1829	UAUACUGGCAUCGGCCACUCUGG	203-225
AD-1565705.1	A-2894451.1	1386	AGUGUGGCCAGUCCCAAUGAA	231-251	A-2894452.1	1830	UUCATUGGGACUGGCCACACUGA	229-251
AD-1565470.1	A-2894015.1	1387	GUGUGGCCAGUCCCAAUGAAA	232-252	A-2894016.1	1831	UUUCAUTGGGACUGGCCACACUG	230-252
AD-1565471.1	A-2894015.1	1388	GUGUGGCCAGUCCCAAUGAAA	232-252	A-2894017.1	1832	UTUCAUTGGGACUGGCCACACUG	230-252
AD-1565472.1	A-2894018.1	1389	GCCAGGCCAUGUCAGUCAUCA	271-291	A-2894019.1	1833	UGATGACUGACAUGGCCUGGCUC	269-291
AD-1565473.1	A-2894018.1	1390	GCCAGGCCAUGUCAGUCAUCA	271-291	A-2894020.1	1834	UGAUGACTGACAUGGCCUGGCUC	269-291
AD-1565706.1	A-2894018.1	1391	GCCAGGCCAUGUCAGUCAUCA	271-291	A-2894453.1	1835	UGAUGACUGACAUGGCCUGGCUC	269-291
AD-1565474.1	A-2894021.1	1392	GGCCAUGUCAGUCAUCCAUAA	275-295	A-2894022.1	1836	UUAUGGAUGACUGACAUGGCCUG	273-295
AD-1565707.1	A-2894454.1	1393	GACCUGGAGGCCACCAAAGCA	327-347	A-2894455.1	1837	UGCUTUGGUGGCCUCCAGGUCUA	325-347
AD-1565475.1	A-2894023.1	1394	CUGGAGGCCACCAAAGCUCGA	330-350	A-2894024.1	1838	UCGAGCTUUGGUGGCCUCCAGGU	328-350
AD-1565476.1	A-2894025.1	1395	GAAACCCAAACCAGAGAGUUA	471-491	A-2894026.1	1839	UAACTCTCUGGUUUGGGUUUCCA	469-491
AD-1565477.1	A-2894027.1	1396	CAGCAACCUCCUCCGAGACAA	503-523	A-2894028.1	1840	UUGTCTCGGAGGAGGUUGCUGUA	501-523
AD-1565708.1	A-2894027.1	1397	CAGCAACCUCCUCCGAGACAA	503-523	A-2894456.1	1841	UUGUCUCGGAGGAGGUUGCUGUA	501-523
AD-1565478.1	A-2894029.1	1398	GCAACCUCCUCCGAGACAAGA	505-525	A-2894030.1	1842	UCUTGTCUCGGAGGAGGUUGCUG	503-525

AD-1565479.1	A-2894029.1	1399	GCAACCUCCUCCGAGACAAGA	505-525	A-2894031.1	1843	UCUUGTCTCGGAGGAGGUUGCUG	503-525
AD-1565709.1	A-2894029.1	1400	GCAACCUCCUCCGAGACAAGA	505-525	A-2894457.1	1844	UCUUGUCUCGGAGGAGGUUGCUG	503-525
AD-1565480.1	A-2894032.1	1401	AGACAAGUCAGUUCUGGAGGA	518-538	A-2894033.1	1845	UCCUCCAGAACTGACUUGUCUCG	516-538
AD-1565481.1	A-2894034.1	1402	CAAGUCAGUUCUGGAGGAAGA	521-541	A-2894035.1	1846	UCUUCCTCCAGAACUGACUUGUC	519-541
AD-1565482.1	A-2894036.1	1403	GUCAGUUCUGGAGGAAGAGAA	524-544	A-2894037.1	1847	UUCUCUTCCUCCAGAACUGACUU	522-544
AD-1565483.1	A-2894038.1	1404	AGAAUCUGGCCAGGAGGUUGA	568-588	A-2894039.1	1848	UCAACCTCCUGGCCAGAUUCUCA	566-588
AD-1565484.1	A-2894040.1	1405	UGGCCAGGAGGUUGGAAAGCA	574-594	A-2894041.1	1849	UGCTUTCCAACCUCCUGGCCAGA	572-594
AD-1565710.1	A-2894040.1	1406	UGGCCAGGAGGUUGGAAAGCA	574-594	A-2894458.1	1850	UGCUTUCCAACCUCCUGGCCAGA	572-594
AD-1565711.1	A-2894459.1	1407	CAGCCAGGAGGUAGCAAGGCA	596-616	A-2894460.1	1851	UGCCTUGCUACCUCCUGGCUGCU	594-616
AD-1565485.1	A-2894042.1	1408	UGCCACCAGGCUCCAGAGAAA	661-681	A-2894043.1	1852	UUUCTCTGGAGCCUGGUGGCACA	659-681
AD-1565486.1	A-2894044.1	1409	AAUUUGGACACUUUGGCCUUA	693-713	A-2894045.1	1853	UAAGGCCAAAGUGUCCAAAUUCC	691-713
AD-1565712.1	A-2894044.1	1410	AAUUUGGACACUUUGGCCUUA	693-713	A-2894461.1	1854	UAAGGCCAAAGUGUCCAAAUUCC	691-713
AD-1565713.1	A-2894462.1	1411	ACACUUUGGCCUUCCAGGAAA	700-720	A-2894463.1	1855	UUUCCUGGAAGGCCAAAGUGUCC	698-720
AD-1565487.1	A-2894046.1	1412	CUUUGGCCUUCCAGGAACUGA	703-723	A-2894047.1	1856	UCAGTUCCUGGAAGGCCAAAGUG	701-723
AD-1565714.1	A-2894048.1	1413	AGGAACUGAAGUCCGAGCUAA	715-735	A-2894464.1	1857	UUAGCUCGGACUUCAGUUCCUGG	713-735
AD-1565488.1	A-2894048.1	1414	AGGAACUGAAGUCCGAGCUAA	715-735	A-2894049.1	1858	UUAGCTCGGACUUCAGUUCCUGG	713-735
AD-1565715.1	A-2894465.1	1415	GAAGUCCGAGCUAACUGAAGA	722-742	A-2894466.1	1859	UCUUCAGUUAGCUCGGACUUCAG	720-742

AD-1565716.1	A-2894467.1	1416	GCUAACUGAAGUUCCUGCUUA	731-751	A-2894468.1	1860	UAAGCAGGAACUUCAGUUAGCUC	729-751
AD-1565489.1	A-2894050.1	1417	CUAACUGAAGUUCCUGCUUCA	732-752	A-2894051.1	1861	UGAAGCAGGAACUUCAGUUAGCU	730-752
AD-1565717.1	A-2894469.1	1418	GAAGUUCCUGCUUCCCGAAUA	738-758	A-2894470.1	1862	UAUUCGGGAAGCAGGAACUUCAG	736-758
AD-1565718.1	A-2894052.1	1419	GAGAACUAGUUUGGGUAGGAA	814-834	A-2894471.1	1863	UUCCTACCCAAACUAGUUCUCCA	812-834
AD-1565490.1	A-2894052.1	1420	GAGAACUAGUUUGGGUAGGAA	814-834	A-2894053.1	1864	UUCCUACCCAAACUAGUUCUCCA	812-834
AD-1565719.1	A-2894054.1	1421	ACUAGUUUGGGUAGGAGAGCA	818-838	A-2894472.1	1865	UGCUCUCCUACCCAAACUAGUUC	816-838
AD-1565491.1	A-2894054.1	1422	ACUAGUUUGGGUAGGAGAGCA	818-838	A-2894055.1	1866	UGCTCTCUACCCAAACUAGUUC	816-837
AD-1565720.1	A-2894473.1	1423	GGGUAGGAGAGCCUCUCACGA	826-846	A-2894474.1	1867	UCGUGAGAGGCUCUCCUACCCAA	824-846
AD-1565721.1	A-2894475.1	1424	GUAGGAGAGCCUCUCACGCUA	828-848	A-2894476.1	1868	UAGCGUGAGAGGCUCUCCUACCC	826-848
AD-1565492.1	A-2894056.1	1425	GUAGGAGAGCCUCUCACGCUA	828-848	A-2894057.1	1869	UAGCGUGAGAGGCUCUCCUACCC	826-848
AD-1565493.1	A-2894058.1	1426	UAGGAGAGCCUCUCACGCUGA	829-849	A-2894059.1	1870	UCAGCGTGAGAGGCUCUCCUACC	827-849
AD-1565722.1	A-2894477.1	1427	AGGAGAGCCUCUCACGCUGAA	830-850	A-2894478.1	1871	UUCAGCGUGAGAGGCUCUCCUAC	828-850
AD-1565723.1	A-2894060.1	1428	GCCUCUCACGCUGAGAACAGA	836-856	A-2894479.1	1872	UCUGTUCUCAGCGUGAGAGGCUC	834-856
AD-1565494.1	A-2894060.1	1429	GCCUCUCACGCUGAGAACAGA	836-856	A-2894061.1	1873	UCUGUTCUCAGCGUGAGAGGCUC	834-856
AD-1565495.1	A-2894060.1	1430	GCCUCUCACGCUGAGAACAGA	836-856	A-2894062.1	1874	UCUGUTCTCAGCGUGAGAGGCUC	834-856
AD-1565724.1	A-2894480.1	1431	CACGCUGAGAACAGCAGAAAA	842-862	A-2894481.1	1875	UUUUCUGCUGUUCUCAGCGUGAG	840-862
AD-1565496.1	A-2894063.1	1432	ACGCUGAGAACAGCAGAAACA	843-863	A-2894064.1	1876	UGUUTCTGCUGUUCUCAGCGUGA	841-863

AD-1565725.1	A-2894065.1	1433	CGCUGAGAACAGCAGAAACAA	844-864	A-2894482.1	1877	UUGUTUCUGCUGUUCUCAGCGUG	842-864
AD-1565497.1	A-2894065.1	1434	CGCUGAGAACAGCAGAAACAA	844-864	A-2894066.1	1878	UUGTUTCUGCUGUUCUCAGCGUG	842-864
AD-1565498.1	A-2894067.1	1435	GCUGAGAACAGCAGAAACAAA	845-865	A-2894068.1	1879	UUUGTUTCUGCUGUUCUCAGCGU	843-865
AD-1565499.1	A-2894069.1	1436	CUGAGAACAGCAGAAACAAUA	846-866	A-2894070.1	1880	UAUUGUTUCUGCUGUUCUCAGCG	844-866
AD-1565500.1	A-2894071.1	1437	UGAGAACAGCAGAAACAAUUA	847-867	A-2894072.1	1881	UAAUTGTUUCUGCUGUUCUCAGC	845-867
AD-1565726.1	A-2894483.1	1438	GAGAACAGCAGAAACAAUUAA	848-868	A-2894484.1	1882	UUAATUGUUUCUGCUGUUCUCAG	846-868
AD-1565501.1	A-2894073.1	1439	AGAACAGCAGAAACAAUUACA	849-869	A-2894074.1	1883	UGUAAUTGUUUCUGCUGUUCUCA	847-869
AD-1565502.1	A-2894075.1	1440	AGAACAGCAGAAACAAUUACA	849-869	A-2894076.1	1884	UGUAAUTGUUUCUGCUGUUCUCA	847-869
AD-1565503.1	A-2894077.1	1441	GAACAGCAGAAACAAUUACUA	850-870	A-2894078.1	1885	UAGUAATUGUUTCUGCUGUUCUC	848-870
AD-1565801.1	A-1991726.1	1442	AACAGCAGAAACAAUUACUGA	851-871	A-2894595.1	1886	UCAGTAAUUGUUUCUGCUGUUCU	849-871
AD-1565802.1	A-1991726.1	1443	AACAGCAGAAACAAUUACUGA	851-871	A-2894596.1	1887	UCAGTAAUUGUUUCUGCUGUUCU	849-871
AD-1565803.1	A-2894597.1	1444	AACACCAGAAACAAUUACUGA	851-871	A-2894598.1	1888	UCAGTAAUUGUTUCUGGUGUUCU	849-871
AD-1565804.1	A-2894599.1	1445	AACUGCAGAAACAAUUACUGA	851-871	A-2894600.1	1889	UCAGTAAUUGUTUCUGCAGUUCU	849-871
AD-1565805.1	A-2894601.1	1446	AAGAGCAGAAACAAUUACUGA	851-871	A-2894602.1	1890	UCAGTAAUUGUTUCUGCUCUUCU	849-871
AD-1073418.5	A-1991726.1	1447	AACAGCAGAAACAAUUACUGA	851-871	A-1802980.1	1891	UCAGUAAUUGUUUCUGCUGUUCU	849-871
AD-1565504.1	A-2894079.1	1448	AACAGCAGAAACAAUUACUGA	851-871	A-2894080.1	1892	UCAGTAAUUGUTUCUGCUGUUCU	849-871
AD-1565504.2	A-2894079.1	1449	AACAGCAGAAACAAUUACUGA	851-871	A-2894080.1	1893	UCAGTAAUUGUTUCUGCUGUUCU	849-871

AD-1565505.1	A-2894081.1	1450	ACAGCAGAAACAAUUACUGGA	852-872	A-2894082.1	1894	UCCAGUAAUUGTUUCUGCUGUUC	850-872
AD-1565800.1	A-2894593.1	1451	CAGCAGAAACAAUUACUGA	853-871	A-2894594.1	1895	UCAGTAAUUGUTUCUGCUGUU	851-871
AD-1565506.1	A-2894083.1	1452	CAGCAGAAACAAUUACUGGCA	853-873	A-2894084.1	1896	UGCCAGTAAUUGUUUCUGCUGUU	851-873
AD-1565727.1	A-2894485.1	1453	AGCAGAAACAAUUACUGGCAA	854-874	A-2894486.1	1897	UUGCCAGUAAUUGUUUCUGCUGU	852-874
AD-1565507.1	A-2894085.1	1454	GCAGAAACAAUUACUGGCAAA	855-875	A-2894086.1	1898	UUUGCCAGUAAUUGUUUCUGCUG	853-875
AD-1565728.1	A-1577510.1	1455	CAGAAACAAUUACUGGCAAGA	856-876	A-2894487.1	1899	UCUUGCCAGUAAUUGUUUCUGCU	854-876
AD-1565508.1	A-1577510.1	1456	CAGAAACAAUUACUGGCAAGA	856-876	A-2894087.1	1900	UCUUGCCAGUAAUUGUUUCUGCU	854-876
AD-1565509.1	A-2894088.1	1457	AGAAACAAUUACUGUCAAGUA	857-877	A-2894089.1	1901	UACUTGACAGUAAUUGUUUCUGC	855-877
AD-1565730.1	A-2894490.1	1458	GAAACAAUUACUGGCAAGUAA	858-878	A-2894491.1	1902	UUACTUGCCAGUAAUUGUUUCUG	856-878
AD-1565510.1	A-2894090.1	1459	AAACAAUUACUGGCAAGUAUA	859-879	A-2894091.1	1903	UAUACUTGCCAGUAAUUGUUUCU	857-879
AD-1565511.1	A-2894092.1	1460	AACAAUUACUGGCAAGUAUGA	860-880	A-2894093.1	1904	UCAUACTUGCCAGUAAUUGUUUC	858-880
AD-1565512.1	A-2894094.1	1461	AACAAUUACUGGCAAGUAUGA	860-880	A-2894095.1	1905	UCAUACTUGCCAGUAAUUGUUUC	858-880
AD-1565731.1	A-2894096.1	1462	GGCAAGUAUGGUGUGUGGAUA	870-890	A-2894492.1	1906	UAUCCACACACCAUACUUGCCAG	868-890
AD-1565513.1	A-2894096.1	1463	GGCAAGUAUGGUGUGUGGAUA	870-890	A-2894097.1	1907	UAUCCACACACCAUACUUGCCAG	868-890
AD-1565514.1	A-2894096.1	1464	GGCAAGUAUGGUGUGUGGAUA	870-890	A-2894098.1	1908	UAUCCACACACCAUACUUGCCAG	868-890
AD-1565732.1	A-2894099.1	1465	CAAGUAUGGUGUGUGGAUGCA	872-892	A-2894493.1	1909	UGCATCCACACACCAUACUUGCC	870-892
AD-1565515.1	A-2894099.1	1466	CAAGUAUGGUGUGUGGAUGCA	872-892	A-2894100.1	1910	UGCAUCCACACACCAUACUUGCC	870-892

AD-1565516.1	A-2894101.1	1467	UGAGUAUGACCUCAUCAGCCA	974-994	A-2894102.1	1911	UGGCTGAUGAGGUCAUACUCAAA	972-994
AD-1565733.1	A-2894494.1	1468	GAGUAUGACCUCAUCAGCCAA	975-995	A-2894495.1	1912	UUGGCUGAUGAGGUCAUACUCAA	973-995
AD-1565517.1	A-2894103.1	1469	AGUAUGACCUCAUCAGCCAGA	976-996	A-2894104.1	1913	UCUGGCTGAUGAGGUCAUACUCA	974-996
AD-1565734.1	A-2894105.1	1470	GUAUGACCUCAUCAGCCAGUA	977-997	A-2894496.1	1914	UACUGGCUGAUGAGGUCAUACUC	975-997
AD-1565518.1	A-2894105.1	1471	GUAUGACCUCAUCAGCCAGUA	977-997	A-2894106.1	1915	UACUGGCTGAUGAGGUCAUACUC	975-997
AD-1565735.1	A-2894497.1	1472	UAUGACCUCAUCAGCCAGUUA	978-998	A-2894498.1	1916	UAACTGGCUGAUGAGGUCAUACU	976-998
AD-1565736.1	A-2894499.1	1473	AUGACCUCAUCAGCCAGUUUA	979-999	A-2894500.1	1917	UAAACUGGCUGAUGAGGUCAUAC	977-999
AD-1565519.1	A-2894107.1	1474	UGACCUCAUCAGCCAGUUUAA	980-1000	A-2894108.1	1918	UUAAACTGGCUGAUGAGGUCAUA	978-1000
AD-1565520.1	A-2894109.1	1475	GACCUCAUCAGCCAGUUUUAUA	981-1001	A-2894110.1	1919	UAUAAACUGGCTGAUGAGGUCAU	979-1001
AD-1565521.1	A-2894111.1	1476	ACCUCAUCAGCCAGUUUAUGA	982-1002	A-2894112.1	1920	UCAUAAACUGGCUGAUGAGGUCA	980-1002
AD-1244360.3	A-2298063.1	1477	CCUCAUCAGCCAGUUUAUGCA	983-1003	A-1803173.1	1921	UGCAUAAACUGGCUGAUGAGGUC	981-1003
AD-1565522.1	A-2894113.1	1478	CCUCAUCAGCCAGUUUAUGCA	983-1003	A-2894114.1	1922	UGCATAAACUGGCUGAUGAGGUC	981-1003
AD-1565806.1	A-2298063.1	1479	CCUCAUCAGCCAGUUUAUGCA	983-1003	A-2894603.1	1923	UGCATAAACUGGCUGAUGAGGUC	981-1003
AD-1565807.1	A-2298063.1	1480	CCUCAUCAGCCAGUUUAUGCA	983-1003	A-2894604.1	1924	UGCATAAACUGGCUGAUGAGGUC	981-1003
AD-1565808.1	A-2894113.1	1481	CCUCAUCAGCCAGUUUAUGCA	983-1003	A-2894605.1	1925	UGCATAAACUGGCUGAUGAGGUC	981-1003
AD-1565809.1	A-2894606.1	1482	CCUCAUCAGCCAGUUUAUGCA	983-1003	A-2894603.1	1926	UGCATAAACUGGCUGAUGAGGUC	981-1003
AD-1565810.1	A-2894607.1	1483	CCUCAUCAGCCAGUUUAUGCA	983-1003	A-2894608.1	1927	UGCATAAACUGGCUGAUGAGGUC	981-1003

AD-1565812.1	A-2894611.1	1484	CCUCUUCAGCCAGUUUAUGCA	983-1003	A-2894612.1	1928	UGCATAAACUGGCUGAAGAGGUC	981-1003
AD-1565813.1	A-2894613.1	1485	CCUGAUCAGCCAGUUUAUGCA	983-1003	A-2894614.1	1929	UGCATAAACUGGCUGAUCAGGUC	981-1003
AD-1565814.1	A-2894615.1	1486	CCACAUCAGCCAGUUUAUGCA	983-1003	A-2894616.1	1930	UGCATAAACUGGCUGAUGUGGUC	981-1003
AD-1565522.2	A-2894113.1	1487	CCUCAUCAGCCAGUUUAUGCA	983-1003	A-2894114.1	1931	UGCATAAACUGGCUGAUGAGGUC	981-1003
AD-1565523.1	A-2894115.1	1488	CUCAUCAGCCAGUUUAUGCAA	984-1004	A-2894116.1	1932	UTGCAUAAACUGGCUGAUGAGGU	982-1004
AD-1565811.1	A-2894609.1	1489	UCAUCAGCCAGUUUAUGCA	985-1003	A-2894610.1	1933	UGCATAAACUGGCUGAUGAGG	983-1003
AD-1565524.1	A-2894117.1	1490	UCAUCAGCCAGUUUAUGCAGA	985-1005	A-2894118.1	1934	UCUGCATAAACUGGCUGAUGAGG	983-1005
AD-1565525.1	A-2894119.1	1491	UCAUCAGCCAGUUUAUGCAGA	985-1005	A-2894120.1	1935	UCUGCATAAACTGGCUGAUGAGG	983-1005
AD-1565526.1	A-2894121.1	1492	CAUCAGCCAGUUUAUGCAGGA	986-1006	A-2894122.1	1936	UCCUGCAUAAACUGGCUGAUGAG	984-1006
AD-1565737.1	A-2894123.1	1493	AUCAGCCAGUUUAUGCAGGGA	987-1007	A-2894501.1	1937	UCCCTGCAUAAACUGGCUGAUGA	985-1007
AD-1565527.1	A-2894123.1	1494	AUCAGCCAGUUUAUGCAGGGA	987-1007	A-2894124.1	1938	UCCCUGCAUAAACUGGCUGAUGA	985-1007
AD-1565738.1	A-2894502.1	1495	UCAGCCAGUUUAUGCAGGGCA	988-1008	A-2894503.1	1939	UGCCCUGCAUAAACUGGCUGAUG	986-1008
AD-1565528.1	A-2894125.1	1496	CAGCCAGUUUAUGCAGGGCUA	989-1009	A-2894126.1	1940	UAGCCCTGCAUAAACUGGCUGAU	987-1009
AD-1565739.1	A-2894127.1	1497	AGCCAGUUUAUGCAGGGCUAA	990-1010	A-2894504.1	1941	UUAGCCCUGCAUAAACUGGCUGA	988-1010
AD-1565529.1	A-2894127.1	1498	AGCCAGUUUAUGCAGGGCUAA	990-1010	A-2894128.1	1942	UUAGCCCTGCAUAAACUGGCUGA	988-1010
AD-1565740.1	A-2894505.1	1499	GCCAGUUUAUGCAGGGCUACA	991-1011	A-2894506.1	1943	UGUAGCCCUGCAUAAACUGGCUG	989-1011
AD-1565530.1	A-2894129.1	1500	GCCAGUUUAUGCAGUGCUACA	991-1011	A-2894130.1	1944	UGUAGCACUGCAUAAACUGGCUG	989-1011

AD-1565741.1	A-2894507.1	1501	CCAGUUUAUGCAGGGCUACCA	992-1012	A-2894508.1	1945	UGGUAGCCCUGCAUAAACUGGCU	990-1012
AD-1565531.1	A-2894131.1	1502	CCAGUUUAUGCAGGUCUACCA	992-1012	A-2894132.1	1946	UGGUAGACCUGCAUAAACUGGCU	990-1012
AD-1565532.1	A-2894133.1	1503	UGCAGGGCUACCCUUCUAAGA	1000-1020	A-2894134.1	1947	UCUUAGAAGGGTAGCCCUGCAUA	998-1020
AD-1565533.1	A-2894135.1	1504	GGGUGCUGUGGUGUACUCGGA	1052-1072	A-2894136.1	1948	UCCGAGTACACCACAGCACCCGU	1050-1072
AD-1565534.1	A-2894137.1	1505	GGGUGCUGUGGUGUACUCGGA	1052-1072	A-2894138.1	1949	UCCGAGTACACCACAGCACCCGU	1050-1072
AD-1565535.1	A-2894139.1	1506	GAGCCUCUAUUUCCAGGGCGA	1073-1093	A-2894140.1	1950	UCGCCCTGGAAAUAGAGGCUCCC	1071-1093
AD-1565536.1	A-2894141.1	1507	GAGCCUCUAUUUCCAGGGCGA	1073-1093	A-2894142.1	1951	UCGCCCTGGAAAUAGAGGCUCCC	1071-1093
AD-1565537.1	A-2894143.1	1508	CCUCUAUUUCCAGGGCGCUGA	1076-1096	A-2894144.1	1952	UCAGCGCCCUGGAAAUAGAGGCU	1074-1096
AD-1565742.1	A-2894145.1	1509	UUCCAGGGCGCUGAGUCCAGA	1083-1103	A-2894509.1	1953	UCUGGACUCAGCGCCCUGGAAAU	1081-1103
AD-1565538.1	A-2894145.1	1510	UUCCAGGGCGCUGAGUCCAGA	1083-1103	A-2894146.1	1954	UCUGGACUCAGCGCCCUGGAAAU	1081-1103
AD-1565539.1	A-2894145.1	1511	UUCCAGGGCGCUGAGUCCAGA	1083-1103	A-2894147.1	1955	UCUGGACTCAGCGCCCUGGAAAU	1081-1103
AD-1565540.1	A-2894148.1	1512	AGGGCGCUGAGUCCAGAACUA	1087-1107	A-2894149.1	1956	UAGUTCTGGACUCAGCGCCCUGG	1085-1107
AD-1565743.1	A-2894150.1	1513	GGGCGCUGAGUCCAGAACUGA	1088-1108	A-2894510.1	1957	UCAGTUCUGGACUCAGCGCCCUG	1086-1108
AD-1565541.1	A-2894150.1	1514	GGGCGCUGAGUCCAGAACUGA	1088-1108	A-2894151.1	1958	UCAGUTCUGGACUCAGCGCCCUG	1086-1108
AD-1565542.1	A-2894152.1	1515	GGGCGCUGAGUCCAGAACUGA	1088-1108	A-2894153.1	1959	UCAGTUCUGGACUCAGCGCCCUG	1086-1108
AD-1565543.1	A-2894154.1	1516	GGCGCUGAGUCCAGAACUGUA	1089-1109	A-2894155.1	1960	UACAGUTCUGGACUCAGCGCCCU	1087-1109
AD-1565544.1	A-2894156.1	1517	GCGCUGAGUCCAGAACUGUCA	1090-1110	A-2894157.1	1961	UGACAGTUCUGGACUCAGCGCCC	1088-1110

AD-1565744.1	A-2894511.1	1518	CGCUGAGUCCAGAACUGUCAA	1091-1111	A-2894512.1	1962	UUGACAGUUCUGGACUCAGCGCC	1089-1111
AD-1565545.1	A-2894158.1	1519	GCUGAGUCCAGAACUGUCAUA	1092-1112	A-2894159.1	1963	UAUGACAGUUCUGGACUCAGCGC	1090-1112
AD-1565745.1	A-1577552.1	1520	CUGAGUCCAGAACUGUCAUAA	1093-1113	A-2894513.1	1964	UUAUGACAGUUCUGGACUCAGCG	1091-1113
AD-1565546.1	A-1577552.1	1521	CUGAGUCCAGAACUGUCAUAA	1093-1113	A-2894160.1	1965	UUATGACAGUUCUGGACUCAGCG	1091-1113
AD-1565547.1	A-1577552.1	1522	CUGAGUCCAGAACUGUCAUAA	1093-1113	A-2894161.1	1966	UUAUGACAGUUCUGGACUCAGCG	1091-1113
AD-1565548.1	A-2894162.1	1523	UGAGUCCAGAACUGUCAUAAA	1094-1114	A-2894163.1	1967	UUUATGACAGUUCUGGACUCAGC	1092-1114
AD-1565746.1	A-1577518.1	1524	GAGUCCAGAACUGUCAUAAGA	1095-1115	A-2894514.1	1968	UCUUAUGACAGUUCUGGACUCAG	1093-1115
AD-1565549.1	A-2894164.1	1525	GAGUCCAGAACUGUCAUAAGA	1095-1115	A-2894165.1	1969	UCUUAUGACAGTUCUGGACUCAG	1093-1115
AD-1565550.1	A-2894166.1	1526	AGUCCAGAACUGUCAUAAGAA	1096-1116	A-2894167.1	1970	UUCUTATGACAGUUCUGGACUCA	1094-1116
AD-1565551.1	A-2894168.1	1527	AGUCCAGAACUGUCAUAAGAA	1096-1116	A-2894169.1	1971	UTCUTATGACAGUUCUGGACUCA	1094-1116
AD-1244365.3	A-2324726.1	1528	GUCCAGAACUGUCAUAAGAUA	1097-1117	A-1803353.1	1972	UAUCUUAUGACAGUUCUGGACUC	1095-1117
AD-1565552.1	A-2894170.1	1529	GUCCAGAACUGUCAUAAGAUA	1097-1117	A-2894171.1	1973	UAUCTUAUGACAGUUCUGGACUC	1095-1117
AD-1565553.1	A-2894170.1	1530	GUCCAGAACUGUCAUAAGAUA	1097-1117	A-2894172.1	1974	UAUCTUAUGACAGUUCUGGACUC	1095-1117
AD-1565815.1	A-2894170.1	1531	GUCCAGAACUGUCAUAAGAUA	1097-1117	A-2894617.1	1975	UAUCTUAUGACAGUUCUGGACUC	1095-1117
AD-1565816.1	A-2894170.1	1532	GUCCAGAACUGUCAUAAGAUA	1097-1117	A-2894618.1	1976	UAUCTUAUGACAGUUCUGGACUC	1095-1117
AD-1565818.1	A-2894621.1	1533	GUCCUGAACUGUCAUAAGAUA	1097-1117	A-2894622.1	1977	UAUCTUAUGACAGUUCAGGACUC	1095-1117
AD-1565819.1	A-2894623.1	1534	GUCGAGAACUGUCAUAAGAUA	1097-1117	A-2894624.1	1978	UAUCTUAUGACAGUUCUCGACUC	1095-1117

AD-1565820.1	A-2894625.1	1535	GUGCAGAACUGUCAUAAGAUA	1097-1117	A-2894626.1	1979	UAUCTUAUGACAGUUCUGGACUC	1095-1117
AD-1565552.2	A-2894170.1	1536	GUCCAGAACUGUCAUAAGAUA	1097-1117	A-2894171.1	1980	UAUCTUAUGACAGUUCUGGACUC	1095-1117
AD-1565553.2	A-2894170.1	1537	GUCCAGAACUGUCAUAAGAUA	1097-1117	A-2894172.1	1981	UAUCTUAUGACAGUUCUGGACUC	1095-1117
AD-1565554.1	A-2894173.1	1538	UCCAGAACUGUCAUAAGAUAA	1098-1118	A-2894174.1	1982	UUAUCUTAUGACAGUUCUGGACU	1096-1118
AD-1565817.1	A-2894619.1	1539	CCAGAACUGUCAUAAGAUA	1099-1117	A-2894620.1	1983	UAUCTUAUGACAGUUCUGGGC	1097-1117
AD-1565555.1	A-2894175.1	1540	CCAGAACUGUCAUAAGAUAUA	1099-1119	A-2894176.1	1984	UAUATCTUAUGACAGUUCUGGAC	1097-1119
AD-1565747.1	A-2894177.1	1541	CAGAACUGUCAUAAGAUAUGA	1100-1120	A-2894515.1	1985	UCAUAUCUUAUGACAGUUCUGGA	1098-1120
AD-1565556.1	A-2894177.1	1542	CAGAACUGUCAUAAGAUAUGA	1100-1120	A-2894178.1	1986	UCATATCUUAUGACAGUUCUGGA	1098-1120
AD-1565557.1	A-2894179.1	1543	CAGAACUGUCAUAAGAUAUGA	1100-1120	A-2894180.1	1987	UCAUAUCUUAUGACAGUUCUGGA	1098-1120
AD-1565558.1	A-2894181.1	1544	AGAACUGUCAUAAGAUAUGAA	1101-1121	A-2894182.1	1988	UUCATATCUUAUGACAGUUCUGG	1099-1121
AD-1565559.1	A-2894183.1	1545	AGAACUGUCAUAAGAUAUGAA	1101-1121	A-2894184.1	1989	UTCATATCUUATGACAGUUCUGG	1099-1121
AD-1565560.1	A-2894185.1	1546	GAACUGUCAUAAGAUAUGAGA	1102-1122	A-2894186.1	1990	UCUCAUAUCUUAUGACAGUUCUG	1100-1122
AD-1565561.1	A-2894187.1	1547	AACUGUCAUAAGAUAUGAGCA	1103-1123	A-2894188.1	1991	UGCUCATAUCUUAUGACAGUUCU	1101-1123
AD-1565562.1	A-2894189.1	1548	ACUGUCAUAAGAUAUGAGCUA	1104-1124	A-2894190.1	1992	UAGCTCAUAUCUUAUGACAGUUC	1102-1124
AD-1565748.1	A-2894191.1	1549	CUGUCAUAAGAUAUGAGCUGA	1105-1125	A-2894516.1	1993	UCAGCUCAUAUCUUAUGACAGUU	1103-1125
AD-1565563.1	A-2894191.1	1550	CUGUCAUAAGAUAUGAGCUGA	1105-1125	A-2894192.1	1994	UCAGCTCAUAUCUUAUGACAGUU	1103-1125
AD-1565564.1	A-2894191.1	1551	CUGUCAUAAGAUAUGAGCUGA	1105-1125	A-2894193.1	1995	UCAGCTCAUAUCUUAUGACAGUU	1103-1125

AD-1565565.1	A-2894194.1	1552	UGUCAUAAGAUAUGAGCUGAA	1106-1126	A-2894195.1	1996	UUCAGCTCAUAUCUUAUGACAGU	1104-1126
AD-1565566.1	A-2894196.1	1553	CAUAAGAUAUGAGCUGAAUAA	1109-1129	A-2894197.1	1997	UUAUTCAGCUCAUAUCUUAUGAC	1107-1129
AD-1565567.1	A-2894198.1	1554	CUGAAUACCGAGACAGUGAAA	1122-1142	A-2894199.1	1998	UUUCACTGUCUCGGUAUUCAGCU	1120-1142
AD-1565568.1	A-2894200.1	1555	UACCGAGACAGUGAAGGCUGA	1127-1147	A-2894201.1	1999	UCAGCCTUCACUGUCUCGGUAUU	1125-1147
AD-1565569.1	A-2894202.1	1556	UACCGAGACAGUGAAGGCUGA	1127-1147	A-2894203.1	2000	UCAGCCTUCACTGUCUCGGUAUU	1125-1147
AD-1565749.1	A-2894204.1	1557	ACAGUGAAGGCUGAGAAGGAA	1134-1154	A-2894517.1	2001	UUCCTUCUCAGCCUUCACUGUCU	1132-1154
AD-1565570.1	A-2894204.1	1558	ACAGUGAAGGCUGAGAAGGAA	1134-1154	A-2894205.1	2002	UUCCUTCUCAGCCUUCACUGUCU	1132-1154
AD-1565571.1	A-2894204.1	1559	ACAGUGAAGGCUGAGAAGGAA	1134-1154	A-2894206.1	2003	UUCCUTCTCAGCCUUCACUGUCU	1132-1154
AD-1565750.1	A-2894518.1	1560	UGAGAAGGAAAUCCCUGGAGA	1145-1165	A-2894519.1	2004	UCUCCAGGGAUUUCCUUCUCAGC	1143-1165
AD-1565573.1	A-2894209.1	1561	GCCAAAGGUGCCAUUGUCCUA	1266-1286	A-2894210.1	2005	UAGGACAAUGGCACCUUUGGCCU	1264-1286
AD-1565751.1	A-2894211.1	1562	CCAAACUGAACCCAGAGAAUA	1288-1308	A-2894520.1	2006	UAUUCUCUGGGUUCAGUUUGGAG	1286-1308
AD-1565574.1	A-2894211.1	1563	CCAAACUGAACCCAGAGAAUA	1288-1308	A-2894212.1	2007	UAUTCTCUGGGUUCAGUUUGGAG	1286-1308
AD-1565575.1	A-2894211.1	1564	CCAAACUGAACCCAGAGAAUA	1288-1308	A-2894213.1	2008	UAUUCTCTGGGUUCAGUUUGGAG	1286-1308
AD-1565576.1	A-2894214.1	1565	CAAACUGAACCCAGAGAAUCA	1289-1309	A-2894215.1	2009	UGAUTCTCUGGGUUCAGUUUGGA	1287-1309
AD-1565752.1	A-2894216.1	1566	UCCGUAAGCAGUCAGUCGCCA	1339-1359	A-2894521.1	2010	UGGCGACUGACUGCUUACGGAUG	1337-1359
AD-1565577.1	A-2894216.1	1567	UCCGUAAGCAGUCAGUCGCCA	1339-1359	A-2894217.1	2011	UGGCGACUGACUGCUUACGGAUG	1337-1359
AD-1565578.1	A-2894216.1	1568	UCCGUAAGCAGUCAGUCGCCA	1339-1359	A-2894218.1	2012	UGGCGACTGACUGCUUACGGAUG	1337-1359

AD-1565579.1	A-2894219.1	1569	UCCGUAAGCAGUCAGUCGCCA	1339-1359	A-2894220.1	2013	UGGCGACUGACTGCUUACGGAUG	1337-1359
AD-1565753.1	A-2894522.1	1570	UAAGCAGUCAGUCGCCAAUGA	1343-1363	A-2894523.1	2014	UCAUTGGCGACUGACUGCUUACG	1341-1363
AD-1565580.1	A-2894221.1	1571	CAGUCAGUCGCCAAUGCCUUA	1347-1367	A-2894222.1	2015	UAAGGCAUUGGCGACUGACUGCU	1345-1367
AD-1565754.1	A-2894524.1	1572	UCAGUCGCCAAUGCCUUCAUA	1350-1370	A-2894525.1	2016	UAUGAAGGCAUUGGCGACUGACU	1348-1370
AD-1565581.1	A-2894223.1	1573	AGUCGCCAAUGCCUUCAUCAA	1352-1372	A-2894224.1	2017	UUGATGAAGGCAUUGGCGACUGA	1350-1372
AD-1565582.1	A-2894225.1	1574	GCCAAUGCCUUCAUCAUCUGA	1356-1376	A-2894226.1	2018	UCAGAUGAUGAAGGCAUUGGCGA	1354-1376
AD-1565755.1	A-2894227.1	1575	CCUUCAUCAUCUGUGGCACCA	1363-1383	A-2894526.1	2019	UGGUGCCACAGAUGAUGAAGGCA	1361-1383
AD-1565583.1	A-2894227.1	1576	CCUUCAUCAUCUGUGGCACCA	1363-1383	A-2894228.1	2020	UGGUGCCACAGAUGAUGAAGGCA	1361-1383
AD-1565584.1	A-2894229.1	1577	CUGUGGCACCUUGUACACCGA	1373-1393	A-2894230.1	2021	UCGGTGTACAAGGUGCCACAGAU	1371-1393
AD-1565756.1	A-2894231.1	1578	CUACCGUCAACUUUGCUUAUA	1417-1437	A-2894527.1	2022	UAUAAGCAAAGUUGACGGUAGCA	1415-1437
AD-1565585.1	A-2894231.1	1579	CUACCGUCAACUUUGCUUAUA	1417-1437	A-2894232.1	2023	UAUAAGCAAAGUUGACGGUAGCA	1415-1437
AD-1073420.3	A-1991728.1	1580	UACCGUCAACUUUGCUUAUGA	1418-1438	A-1806295.1	2024	UCAUAAGCAAAGUUGACGGUAGC	1416-1438
AD-1244366.3	A-1991728.1	1581	UACCGUCAACUUUGCUUAUGA	1418-1438	A-1803954.1	2025	UCAUAAGCAAAGUUGACGGUAGC	1416-1438
AD-1565757.1	A-1991728.1	1582	UACCGUCAACUUUGCUUAUGA	1418-1438	A-2894528.1	2026	UCAUAAGCAAAGUUGACGGUAGC	1416-1438
AD-1565821.1	A-1991728.1	1583	UACCGUCAACUUUGCUUAUGA	1418-1438	A-2894627.1	2027	UCATAAGCAAAGUUGACGGUAGC	1416-1438
AD-1565822.1	A-1991728.1	1584	UACCGUCAACUUUGCUUAUGA	1418-1438	A-2894628.1	2028	UCAUAAGCAAAGUUGACGGUAGC	1416-1438
AD-1565824.1	A-2894631.1	1585	UACCCUCAACUUUGCUUAUGA	1418-1438	A-2894632.1	2029	UCAUAAGCAAAGUUGAGGGUAGC	1416-1438

AD-1565825.1	A-2894633.1	1586	UACGGUCAACUUUGCUUAUGA	1418-1438	A-2894634.1	2030	UCAUAAGCAAAGUUGACCGUAGC	1416-1438
AD-1565826.1	A-2894635.1	1587	UAGCGUCAACUUUGCUUAUGA	1418-1438	A-2894636.1	2031	UCAUAAGCAAAGUUGACGCUAGC	1416-1438
AD-1565757.2	A-1991728.1	1588	UACCGUCAACUUUGCUUAUGA	1418-1438	A-2894528.1	2032	UCAUAAGCAAAGUUGACGGUAGC	1416-1438
AD-1565586.1	A-2894233.1	1589	UACCGUCAACUUUGCUUAUGA	1418-1438	A-2894234.1	2033	UCAUAAGCAAAGUUGACGGUAGC	1416-1438
AD-1565587.1	A-2324727.1	1590	ACCGUCAACUUUGCUUAUGAA	1419-1439	A-2894235.1	2034	UUCATAAGCAAAGUUGACGGUAG	1417-1439
AD-1565588.1	A-2894236.1	1591	ACCGUCAACUUUGCUUAUGAA	1419-1439	A-2894237.1	2035	UTCATAAGCAAAGUUGACGGUAG	1417-1439
AD-1565823.1	A-2894629.1	1592	CCGUCAACUUUGCUUAUGA	1420-1438	A-2894630.1	2036	UCAUAAGCAAAGUUGACGGUG	1418-1438
AD-1565589.1	A-2894238.1	1593	CCGUCAACUUUGCUUAUGACA	1420-1440	A-2894239.1	2037	UGUCAUAAGCAAAGUUGACGGUA	1418-1440
AD-1565590.1	A-2894240.1	1594	CGUCAACUUUGCUUAUGACAA	1421-1441	A-2894241.1	2038	UUGUCATAAGCAAAGUUGACGGU	1419-1441
AD-1565591.1	A-2894242.1	1595	GUCAACUUUGCUUAUGACACA	1422-1442	A-2894243.1	2039	UGUGTCAUAAGCAAAGUUGACGG	1420-1442
AD-1565758.1	A-2894244.1	1596	UCAACUUUGCUUAUGACACAA	1423-1443	A-2894529.1	2040	UUGUGUCAUAAGCAAAGUUGACG	1421-1443
AD-1565592.1	A-2894244.1	1597	UCAACUUUGCUUAUGACACAA	1423-1443	A-2894245.1	2041	UUGTGTCAUAAGCAAAGUUGACG	1421-1443
AD-1565594.1	A-2894247.1	1598	CAACUUUGCUUAUGACACAGA	1424-1444	A-2894248.1	2042	UCUGTGTCAUAAGCAAAGUUGAC	1422-1444
AD-1565759.1	A-2894530.1	1599	AACUUUGCUUAUGACACAGGA	1425-1445	A-2894531.1	2043	UCCUGUGUCAUAAGCAAAGUUGA	1423-1445
AD-1565595.1	A-2894249.1	1600	ACUUUGCUUAUGACACAGGCA	1426-1446	A-2894250.1	2044	UGCCTGTGUCAUAAGCAAAGUUG	1424-1446
AD-1565760.1	A-2894532.1	1601	CUUUGCUUAUGACACAGGCAA	1427-1447	A-2894533.1	2045	UUGCCUGUGUCAUAAGCAAAGUU	1425-1447
AD-1565761.1	A-2894534.1	1602	ACAGGCACAGGUAUCAGCAAA	1440-1460	A-2894535.1	2046	UUUGCUGAUACCUGUGCCUGUGU	1438-1460

AD-1565596.1	A-2894251.1	1603	AUAAGUACAGCAGCAUGAUUA	1489-1509	A-2894252.1	2047	UAAUCATGCUGCUGUACUUAUAG	1487-1509
AD-1565597.1	A-2894253.1	1604	UAAGUACAGCAGCAUGAUUGA	1490-1510	A-2894254.1	2048	UCAATCAUGCUGCUGUACUUAUA	1488-1510
AD-1565762.1	A-2894255.1	1605	AAGUACAGCAGCAUGAUUGAA	1491-1511	A-2894536.1	2049	UUCAAUCAUGCUGCUGUACUUAU	1489-1511
AD-1565598.1	A-2894255.1	1606	AAGUACAGCAGCAUGAUUGAA	1491-1511	A-2894256.1	2050	UUCAATCAUGCUGCUGUACUUAU	1489-1511
AD-1565599.1	A-2894255.1	1607	AAGUACAGCAGCAUGAUUGAA	1491-1511	A-2894257.1	2051	UUCAATCAUGCUGCUGUACUUAU	1489-1511
AD-1565600.1	A-2894258.1	1608	AGUACAGCAGCAUGAUUGACA	1492-1512	A-2894259.1	2052	UGUCAATCAUGCUGCUGUACUUA	1490-1512
AD-1565601.1	A-2894260.1	1609	AGUACAGCAGCAUGAUUGACA	1492-1512	A-2894261.1	2053	UGUCAATCAUGCUGCUGUACUUA	1490-1512
AD-1565602.1	A-2894262.1	1610	GUACAGCAGCAUGAUUGACUA	1493-1513	A-2894263.1	2054	UAGUCAAUCAUGCUGCUGUACUU	1491-1513
AD-1565827.1	A-2894637.1	1611	GUACAGCAGCAUGAUUGACUA	1493-1513	A-1804098.1	2055	UAGUCAAUCAUGCUGCUGUACUU	1491-1513
AD-1565828.1	A-2894262.1	1612	GUACAGCAGCAUGAUUGACUA	1493-1513	A-2894638.1	2056	UAGUCAAUCAUGCUGCUGUACUU	1491-1513
AD-1565829.1	A-2894262.1	1613	GUACAGCAGCAUGAUUGACUA	1493-1513	A-2894639.1	2057	UAGUCAAUCAUGCUGCUGUACUU	1491-1513
AD-1565830.1	A-2894262.1	1614	GUACAGCAGCAUGAUUGACUA	1493-1513	A-2894640.1	2058	UAGUCAAUCAUGCUGCUGUACUU	1491-1513
AD-1565831.1	A-2894262.1	1615	GUACAGCAGCAUGAUUGACUA	1493-1513	A-2894641.1	2059	UAGUCAAUCAUGCUGCUGUACUU	1491-1513
AD-1565833.1	A-2894644.1	1616	GUACUGCAGCAUGAUUGACUA	1493-1513	A-2894645.1	2060	UAGUCAAUCAUGCUGCAGUACUU	1491-1513
AD-1565834.1	A-2894646.1	1617	GUAGAGCAGCAUGAUUGACUA	1493-1513	A-2894647.1	2061	UAGUCAAUCAUGCUGCUCUACUU	1491-1513
AD-1565835.1	A-2894648.1	1618	GUUCAGCAGCAUGAUUGACUA	1493-1513	A-2894649.1	2062	UAGUCAAUCAUGCUGCUGAACUU	1491-1513
AD-1565602.2	A-2894262.1	1619	GUACAGCAGCAUGAUUGACUA	1493-1513	A-2894263.1	2063	UAGUCAAUCAUGCUGCUGUACUU	1491-1513

AD-1565603.1	A-2894264.1	1620	UACAGCAGCAUGAUUGACUAA	1494-1514	A-2894265.1	2064	UUAGTCAAUCAUGCUGCUGUACU	1492-1514
AD-1565832.1	A-2894642.1	1621	ACAGCAGCAUGAUUGACUA	1495-1513	A-2894643.1	2065	UAGUCAAUCAUGCUGCUGUGC	1493-1513
AD-1565763.1	A-2894266.1	1622	ACAGCAGCAUGAUUGACUACA	1495-1515	A-2894537.1	2066	UGUAGUCAAUCAUGCUGCUGUAC	1493-1515
AD-1565604.1	A-2894266.1	1623	ACAGCAGCAUGAUUGACUACA	1495-1515	A-2894267.1	2067	UGUAGTCAAUCAUGCUGCUGUAC	1493-1515
AD-1565605.1	A-2894266.1	1624	ACAGCAGCAUGAUUGACUACA	1495-1515	A-2894268.1	2068	UGUAGTCAAUCAUGCUGCUGUAC	1493-1515
AD-1565606.1	A-2894269.1	1625	CAGCAGCAUGAUUGACUACAA	1496-1516	A-2894270.1	2069	UUGUAGTCAAUCAUGCUGCUGUA	1494-1516
AD-1565764.1	A-2894538.1	1626	AGCAGCAUGAUUGACUACAAA	1497-1517	A-2894539.1	2070	UUUGTAGUCAAUCAUGCUGCUGU	1495-1517
AD-1565607.1	A-2894271.1	1627	GCAGCAUGAUUGACUACAACA	1498-1518	A-2894272.1	2071	UGUUGUAGUCAAUCAUGCUGCUG	1496-1518
AD-1565608.1	A-2894273.1	1628	CAGCAUGAUUGACUACAACCA	1499-1519	A-2894274.1	2072	UGGUTGTAGUCAAUCAUGCUGCU	1497-1519
AD-1565609.1	A-2894275.1	1629	GCAUGAUUGACUACAACCCCA	1501-1521	A-2894276.1	2073	UGGGGUTGUAGUCAAUCAUGCUG	1499-1521
AD-1565766.1	A-2894279.1	1630	AGCUCUUUGCCUGGGACAACA	1531-1551	A-2894542.1	2074	UGUUGUCCCAGGCAAAGAGCUUC	1529-1551
AD-1565611.1	A-2894279.1	1631	AGCUCUUUGCCUGGGACAACA	1531-1551	A-2894280.1	2075	UGUTGTCCCAGGCAAAGAGCUUC	1529-1551
AD-1565767.1	A-2894281.1	1632	GCCUGGGACAACUUGAACAUA	1539-1559	A-2894543.1	2076	UAUGTUCAAGUUGUCCCAGGCAA	1537-1559
AD-1565612.1	A-2894281.1	1633	GCCUGGGACAACUUGAACAUA	1539-1559	A-2894282.1	2077	UAUGUTCAAGUUGUCCCAGGCAA	1537-1559
AD-1565613.1	A-2894281.1	1634	GCCUGGGACAACUUGAACAUA	1539-1559	A-2894283.1	2078	UAUGUTCAAGUUGUCCCAGGCAA	1537-1559
AD-1565614.1	A-2894284.1	1635	CUGGGACAACUUGAACAUGGA	1541-1561	A-2894285.1	2079	UCCATGTUCAAGUUGUCCCAGGC	1539-1561
AD-1565768.1	A-2894544.1	1636	UGGGACAACUUGAACAUGGUA	1542-1562	A-2894545.1	2080	UACCAUGUUCAAGUUGUCCCAGG	1540-1562

AD-1565615.1	A-2894286.1	1637	GGGACAACUUGAACAUGGUCA	1543-1563	A-2894287.1	2081	UGACCATGUUCAAGUUGUCCCAG	1541-1563
AD-1565616.1	A-2894288.1	1638	GGGACAACUUGAACAUGGUCA	1543-1563	A-2894289.1	2082	UGACCATGUUCAAGUUGUCCCAG	1541-1563
AD-1565617.1	A-2894290.1	1639	GGACAACUUGAACAUGGUCAA	1544-1564	A-2894291.1	2083	UUGACCAUGUUCAAGUUGUCCCA	1542-1564
AD-1565769.1	A-2894292.1	1640	GACAACUUGAACAUGGUCACA	1545-1565	A-2894546.1	2084	UGUGACCAUGUUCAAGUUGUCCC	1543-1565
AD-1565618.1	A-2894292.1	1641	GACAACUUGAACAUGGUCACA	1545-1565	A-2894293.1	2085	UGUGACCAUGUUCAAGUUGUCCC	1543-1565
AD-1565770.1	A-2894294.1	1642	ACAACUUGAACAUGGUCACUA	1546-1566	A-2894547.1	2086	UAGUGACCAUGUUCAAGUUGUCC	1544-1566
AD-1565619.1	A-2894294.1	1643	ACAACUUGAACAUGGUCACUA	1546-1566	A-2894295.1	2087	UAGTGACCAUGUUCAAGUUGUCC	1544-1566
AD-1565620.1	A-2894296.1	1644	CAACUUGAACAUGGUCACUUA	1547-1567	A-2894297.1	2088	UAAGTGACCAUGUUCAAGUUGUC	1545-1567
AD-1565771.1	A-2894548.1	1645	AACUUGAACAUGGUCACUUAA	1548-1568	A-2894549.1	2089	UUAAGUGACCAUGUUCAAGUUGU	1546-1568
AD-1565621.1	A-1577540.1	1646	ACUUGAACAUGGUCACUUAUA	1549-1569	A-2894298.1	2090	UAUAAGTGACCAUGUUCAAGUUG	1547-1569
AD-1565772.1	A-1577508.1	1647	CUUGAACAUGGUCACUUAUGA	1550-1570	A-2894550.1	2091	UCAUAAGUGACCAUGUUCAAGUU	1548-1570
AD-1565836.1	A-1577508.1	1648	CUUGAACAUGGUCACUUAUGA	1550-1570	A-2894300.1	2092	UCAUAAGUGACCAUGUUCAAGUU	1548-1570
AD-1565837.1	A-1577508.1	1649	CUUGAACAUGGUCACUUAUGA	1550-1570	A-2894650.1	2093	UCATAAGUGACCAUGUUCAAGUU	1548-1570
AD-1565838.1	A-1577508.1	1650	CUUGAACAUGGUCACUUAUGA	1550-1570	A-2894651.1	2094	UCAUAAGUGACCAUGUUCAAGUU	1548-1570
AD-1565840.1	A-2894654.1	1651	CUUGUACAUGGUCACUUAUGA	1550-1570	A-2894655.1	2095	UCAUAAGUGACCAUGUACAAGUU	1548-1570
AD-1565841.1	A-2894656.1	1652	CUUCAACAUGGUCACUUAUGA	1550-1570	A-2894657.1	2096	UCAUAAGUGACCAUGUUGAAGUU	1548-1570
AD-1565842.1	A-2894658.1	1653	CUAGAACAUGGUCACUUAUGA	1550-1570	A-2894659.1	2097	UCAUAAGUGACCAUGUUCUAGUU	1548-1570

AD-822899.17	A-1577508.1	1654	CUUGAACAUGGUCACUUAUGA	1550-1570	A-1577509.1	2098	UCAUAAGUGACCAUGUUCAAGUU	1548-1570
AD-1565772.2	A-1577508.1	1655	CUUGAACAUGGUCACUUAUGA	1550-1570	A-2894550.1	2099	UCAUAAGUGACCAUGUUCAAGUU	1548-1570
AD-1565622.1	A-2894299.1	1656	CUUGAACAUGGUCACUUAUGA	1550-1570	A-2894300.1	2100	UCAUAAGUGACCAUGUUCAAGUU	1548-1570
AD-1565843.1	A-1577562.1	1657	UUGAACAUGGUCACUUAUGAA	1551-1571	A-2894660.1	2101	UUCATAAGUGACCAUGUUCAAGU	1549-1571
AD-1565844.1	A-1577562.1	1658	UUGAACAUGGUCACUUAUGAA	1551-1571	A-2894661.1	2102	UUCATAAGUGACCAUGUUCAAGU	1549-1571
AD-1565845.1	A-1577562.1	1659	UUGAACAUGGUCACUUAUGAA	1551-1571	A-2894662.1	2103	UTCATAAGUGACCAUGUUCAAGU	1549-1571
AD-1565846.1	A-2894663.1	1660	UUGAACAUGGUCACUUAUGAA	1551-1571	A-2894662.1	2104	UTCATAAGUGACCAUGUUCAAGU	1549-1571
AD-1565848.1	A-2894666.1	1661	UUGAUCAUGGUCACUUAUGAA	1551-1571	A-2894667.1	2105	UUCATAAGUGACCAUGAUCAAGU	1549-1571
AD-1565849.1	A-2894668.1	1662	UUGUACAUGGUCACUUAUGAA	1551-1571	A-2894669.1	2106	UUCATAAGUGACCAUGUACAAGU	1549-1571
AD-1565850.1	A-2894670.1	1663	UUCAACAUGGUCACUUAUGAA	1551-1571	A-2894671.1	2107	UUCATAAGUGACCAUGUUGAAGU	1549-1571
AD-822926.4	A-1577562.1	1664	UUGAACAUGGUCACUUAUGAA	1551-1571	A-1577563.1	2108	UUCAUAAGUGACCAUGUUCAAGU	1549-1571
AD-1565623.1	A-2894301.1	1665	UUGAACAUGGUCACUUAUGAA	1551-1571	A-2894302.1	2109	UTCATAAGUGACCAUGUUCAAGU	1549-1571
AD-1565839.1	A-2894652.1	1666	UGAACAUGGUCACUUAUGA	1552-1570	A-2894653.1	2110	UCAUAAGUGACCAUGUUCAGG	1550-1570
AD-1565624.1	A-2894303.1	1667	UGAACAUGGUCACUUAUGACA	1552-1572	A-2894304.1	2111	UGUCAUAAGUGACCAUGUUCAAG	1550-1572
AD-1565847.1	A-2894664.1	1668	GAACAUGGUCACUUAUGAA	1553-1571	A-2894665.1	2112	UUCATAAGUGACCAUGUUCGG	1551-1571
AD-1565625.1	A-2894305.1	1669	GAACAUGGUCACUUAUGACAA	1553-1573	A-2894306.1	2113	UUGUCATAAGUGACCAUGUUCAA	1551-1573
AD-1565626.1	A-2894307.1	1670	AACAUGGUCACUUAUGACAUA	1554-1574	A-2894308.1	2114	UAUGTCAUAAGUGACCAUGUUCA	1552-1574

AD-1565773.1	A-2894309.1	1671	ACAUGGUCACUUAUGACAUCA	1555-1575	A-2894551.1	2115	UGAUGUCAUAAGUGACCAUGUUC	1553-1575
AD-1565627.1	A-2894309.1	1672	ACAUGGUCACUUAUGACAUCA	1555-1575	A-2894310.1	2116	UGATGTCAUAAGUGACCAUGUUC	1553-1575
AD-1565628.1	A-2894309.1	1673	ACAUGGUCACUUAUGACAUCA	1555-1575	A-2894311.1	2117	UGAUGTCAUAAGUGACCAUGUUC	1553-1575
AD-1565629.1	A-2894312.1	1674	CAUGGUCACUUAUGACAUCAA	1556-1576	A-2894313.1	2118	UUGATGTCAUAAGUGACCAUGUU	1554-1576
AD-1565774.1	A-2894552.1	1675	AUGGUCACUUAUGACAUCAAA	1557-1577	A-2894553.1	2119	UUUGAUGUCAUAAGUGACCAUGU	1555-1577
AD-1565630.1	A-2894314.1	1676	UGGUCACUUAUGACAUCAAGA	1558-1578	A-2894315.1	2120	UCUUGATGUCAUAAGUGACCAUG	1556-1578
AD-1565631.1	A-2894316.1	1677	GGUCACUUAUGACAUCAAGCA	1559-1579	A-2894317.1	2121	UGCUTGAUGUCAUAAGUGACCAU	1557-1579
AD-1565775.1	A-2894554.1	1678	GUCACUUAUGACAUCAAGCUA	1560-1580	A-2894555.1	2122	UAGCTUGAUGUCAUAAGUGACCA	1558-1580
AD-1565632.1	A-2894318.1	1679	GCAGAAGGAGAUGCUCAGGGA	1619-1639	A-2894319.1	2123	UCCCTGAGCAUCUCCUUCUGCCA	1617-1639
AD-1565776.1	A-2894556.1	1680	AAGGGAGAGCCAGCCAGCCAA	1659-1679	A-2894557.1	2124	UUGGCUGGCUGGCUCUCCCUUCA	1657-1679
AD-1565777.1	A-2894558.1	1681	GAUGAACAUGGUCACCAUCUA	1727-1747	A-2894559.1	2125	UAGATGGUGACCAUGUUCAUCCU	1725-1747
AD-1565634.1	A-2894322.1	1682	GCAUUUAUGGGAUGUUUAAUA	1816-1836	A-2894323.1	2126	UAUUAAACAUCCCAUAAAUGCUG	1814-1836
AD-1565778.1	A-2894324.1	1683	UGUUUAAUGACAUAGUUCAAA	1828-1848	A-2894560.1	2127	UUUGAACUAUGUCAUUAAACAUC	1826-1848
AD-1565635.1	A-2894324.1	1684	UGUUUAAUGACAUAGUUCAAA	1828-1848	A-2894325.1	2128	UUUGAACUAUGUCAUUAAACAUC	1826-1848
AD-1565636.1	A-2894324.1	1685	UGUUUAAUGACAUAGUUCAAA	1828-1848	A-2894326.1	2129	UUUGAACTAUGUCAUUAAACAUC	1826-1848
AD-1565637.1	A-2894327.1	1686	GUUUAAUGACAUAGUUCAAGA	1829-1849	A-2894328.1	2130	UCUUGAACUAUGUCAUUAAACAU	1827-1849
AD-1565638.1	A-2894329.1	1687	GUUUAAUGACAUAGUUCAAGA	1829-1849	A-2894330.1	2131	UCUUGAACUAUGUCAUUAAACAU	1827-1849

AD-1565639.1	A-2894331.1	1688	UUUAAUGACAUAGUUCAAGUA	1830-1850	A-2894332.1	2132	UACUTGAACUAUGUCAUUAAACA	1828-1850
AD-1565779.1	A-2894561.1	1689	UUAAUGACAUAGUUCAAGUUA	1831-1851	A-2894562.1	2133	UAACTUGAACUAUGUCAUUAAAC	1829-1851
AD-1565640.1	A-2894333.1	1690	UAAUGACAUAGUUCAAGUUUA	1832-1852	A-2894334.1	2134	UAAACUTGAACUAUGUCAUUAAA	1830-1852
AD-1565641.1	A-2894335.1	1691	UAAUGACAUAGUUCAAGUUUA	1832-1852	A-2894336.1	2135	UAAACUTGAACTAUGUCAUUAAA	1830-1852
AD-1565642.1	A-2894337.1	1692	AAUGACAUAGUUCAAGUUUUA	1833-1853	A-2894338.1	2136	UAAAACTUGAACUAUGUCAUUAA	1831-1853
AD-1565643.1	A-2894339.1	1693	AAUGACAUAGUUCAAGUUUUA	1833-1853	A-2894340.1	2137	UAAAACTUGAACUAUGUCAUUAA	1831-1853
AD-1565644.1	A-2894341.1	1694	AUGACAUAGUUCAAGUUUUCA	1834-1854	A-2894342.1	2138	UGAAAACUUGAACUAUGUCAUUA	1832-1854
AD-1565645.1	A-2894343.1	1695	UGACAUAGUUCAAGUUUUCUA	1835-1855	A-2894344.1	2139	UAGAAAACUUGAACUAUGUCAUU	1833-1855
AD-1565646.1	A-2894345.1	1696	GACAUAGUUCAAGUUUUCUUA	1836-1856	A-2894346.1	2140	UAAGAAAACUUGAACUAUGUCAU	1834-1856
AD-1565851.1	A-2894672.1	1697	ACAUAGUUCAAGUUUUCUUGA	1837-1857	A-1806645.1	2141	UCAAGAAAACUUGAACUAUGUCA	1835-1857
AD-1565852.1	A-2894347.1	1698	ACAUAGUUCAAGUUUUCUUGA	1837-1857	A-2894673.1	2142	UCAAGAAAACUTGAACUAUGUCG	1835-1857
AD-1565853.1	A-2894347.1	1699	ACAUAGUUCAAGUUUUCUUGA	1837-1857	A-2894674.1	2143	UCAAGAAAACUUGAACUAUGUCG	1835-1857
AD-1565854.1	A-2894347.1	1700	ACAUAGUUCAAGUUUUCUUGA	1837-1857	A-2894675.1	2144	UCAAGAAAACUTGAACUAUGUCG	1835-1857
AD-1565857.1	A-2894679.1	1701	ACAUUGUUCAAGUUUUCUUGA	1837-1857	A-2894680.1	2145	UCAAGAAAACUUGAACAAUGUCG	1835-1857
AD-1565858.1	A-2894681.1	1702	ACAAAGUUCAAGUUUUCUUGA	1837-1857	A-2894682.1	2146	UCAAGAAAACUUGAACUUUGUCG	1835-1857
AD-1565859.1	A-2894683.1	1703	ACUUAGUUCAAGUUUUCUUGA	1837-1857	A-2894684.1	2147	UCAAGAAAACUUGAACUAAGUCG	1835-1857
AD-1565647.1	A-2894347.1	1704	ACAUAGUUCAAGUUUUCUUGA	1837-1857	A-2894348.1	2148	UCAAGAAAACUTGAACUAUGUCA	1835-1857

AD-1565648.1	A-2894349.1	1705	CAUAGUUCAAGUUUUCUUGUA	1838-1858	A-2894350.1	2149	UACAAGAAAACTUGAACUAUGUC	1836-1858
AD-1565855.1	A-2894676.1	1706	AUAGUUCAAGUUUUCUUGA	1839-1857	A-2894677.1	2150	UCAAGAAAACUUGAACUAUGU	1837-1857
AD-1565856.1	A-2894676.1	1707	AUAGUUCAAGUUUUCUUGA	1839-1857	A-2894678.1	2151	UCAAGAAAACUTGAACUAUGU	1837-1857
AD-1565649.1	A-2894351.1	1708	AUAGUUCAAGUUUUCUUGUGA	1839-1859	A-2894352.1	2152	UCACAAGAAAACUUGAACUAUGU	1837-1859
AD-1565650.1	A-2894353.1	1709	UAGUUCAAGUUUUCUUGUGAA	1840-1860	A-2894354.1	2153	UUCACAAGAAAACUUGAACUAUG	1838-1860
AD-1565651.1	A-2894355.1	1710	UAGUUCAAGUUUUCUUGUGAA	1840-1860	A-2894356.1	2154	UTCACAAGAAAACUUGAACUAUG	1838-1860
AD-1565652.1	A-2894357.1	1711	AGUUCAAGUUUUCUUGUGAUA	1841-1861	A-2894358.1	2155	UAUCACAAGAAAACUUGAACUAU	1839-1861
AD-1565653.1	A-2894359.1	1712	AGUUCAAGUUUUCUUGUGAUA	1841-1861	A-2894360.1	2156	UAUCACAAGAAAACUUGAACUAU	1839-1861
AD-1565780.1	A-2894361.1	1713	GUUCAAGUUUUCUUGUGAUUA	1842-1862	A-2894563.1	2157	UAAUCACAAGAAAACUUGAACUA	1840-1862
AD-1565654.1	A-2894361.1	1714	GUUCAAGUUUUCUUGUGAUUA	1842-1862	A-2894362.1	2158	UAATCACAAGAAAACUUGAACUA	1840-1862
AD-1565655.1	A-2894363.1	1715	UUCAAGUUUUCUUGUGAUUUA	1843-1863	A-2894364.1	2159	UAAATCACAAGAAAACUUGAACU	1841-1863
AD-1565781.1	A-2894365.1	1716	UCAAGUUUUCUUGUGAUUUGA	1844-1864	A-2894564.1	2160	UCAAAUCACAAGAAAACUUGAAC	1842-1864
AD-1565656.1	A-2894365.1	1717	UCAAGUUUUCUUGUGAUUUGA	1844-1864	A-2894366.1	2161	UCAAATCACAAGAAAACUUGAAC	1842-1864
AD-1565657.1	A-2894365.1	1718	UCAAGUUUUCUUGUGAUUUGA	1844-1864	A-2894367.1	2162	UCAAATCACAAGAAAACUUGAAC	1842-1864
AD-1565658.1	A-2894368.1	1719	UCAAGUUUUCUUGUGAUUUGA	1844-1864	A-2894369.1	2163	UCAAAUCACAAGAAAACUUGAAC	1842-1864
AD-1565659.1	A-2894370.1	1720	CAAGUUUUCUUGUGAUUUGGA	1845-1865	A-2894371.1	2164	UCCAAATCACAAGAAAACUUGAA	1843-1865
AD-1565660.1	A-2894372.1	1721	AAGUUUUCUUGUGAUUUGGGA	1846-1866	A-2894373.1	2165	UCCCAAAUCACAAGAAAACUUGA	1844-1866

AD-1565782.1	A-2894565.1	1722	UGAAAACCAUUGCUCUUGCAA	1897-1917	A-2894566.1	2166	UUGCAAGAGCAAUGGUUUUCAGG	1895-1917
AD-1565661.1	A-2894374.1	1723	GAAAACCAUUGCUCUUGCAUA	1898-1918	A-2894375.1	2167	UAUGCAAGAGCAAUGGUUUUCAG	1896-1918
AD-1565662.1	A-2894376.1	1724	GAAAACCAUUGCUCUUGCAUA	1898-1918	A-2894377.1	2168	UAUGCAAGAGCAAUGGUUUUCAG	1896-1918
AD-1565663.1	A-2894378.1	1725	AAAACCAUUGCUCUUGCAUGA	1899-1919	A-2894379.1	2169	UCAUGCAAGAGCAAUGGUUUUCA	1897-1919
AD-1565664.1	A-2894380.1	1726	AAAACCAUUGCUCUUGCAUGA	1899-1919	A-2894381.1	2170	UCAUGCAAGAGCAAUGGUUUUCA	1897-1919
AD-1565783.1	A-2894382.1	1727	AAACCAUUGCUCUUGCAUGUA	1900-1920	A-2894567.1	2171	UACATGCAAGAGCAAUGGUUUUC	1898-1920
AD-1565665.1	A-2894382.1	1728	AAACCAUUGCUCUUGCAUGUA	1900-1920	A-2894383.1	2172	UACAUGCAAGAGCAAUGGUUUUC	1898-1920
AD-1565784.1	A-2894568.1	1729	AACCAUUGCUCUUGCAUGUUA	1901-1921	A-2894569.1	2173	UAACAUGCAAGAGCAAUGGUUUU	1899-1921
AD-1565666.1	A-2894384.1	1730	ACCAUUGCUCUUGCAUGUUAA	1902-1922	A-2894385.1	2174	UUAACATGCAAGAGCAAUGGUUU	1900-1922
AD-1565667.1	A-2894386.1	1731	CCAUUGCUCUUGCAUGUUACA	1903-1923	A-2894387.1	2175	UGUAACAUGCAAGAGCAAUGGUU	1901-1923
AD-1565785.1	A-2894388.1	1732	CAUUGCUCUUGCAUGUUACAA	1904-1924	A-2894570.1	2176	UUGUAACAUGCAAGAGCAAUGGU	1902-1924
AD-1565668.1	A-2894388.1	1733	CAUUGCUCUUGCAUGUUACAA	1904-1924	A-2894389.1	2177	UUGTAACAUGCAAGAGCAAUGGU	1902-1924
AD-1565669.1	A-2894390.1	1734	AUUGCUCUUGCAUGUUACAUA	1905-1925	A-2894391.1	2178	UAUGTAACAUGCAAGAGCAAUGG	1903-1925
AD-1565670.1	A-2894392.1	1735	UUGCUCUUGCAUGUUACAUGA	1906-1926	A-2894393.1	2179	UCAUGUAACAUGCAAGAGCAAUG	1904-1926
AD-1565860.1	A-2894685.1	1736	UUGCUCUUGCAUGUUACAUGA	1906-1926	A-1804746.1	2180	UCAUGUAACAUGCAAGAGCAAUG	1904-1926
AD-1565861.1	A-2894686.1	1737	UUGCACUUGCAUGUUACAUGA	1906-1926	A-2894687.1	2181	UCAUGUAACAUGCAAGUGCAAUG	1904-1926
AD-1565862.1	A-2894688.1	1738	UUGGUCUUGCAUGUUACAUGA	1906-1926	A-2894689.1	2182	UCAUGUAACAUGCAAGACCAAUG	1904-1926

AD-1565863.1	A-2894690.1	1739	UUCCUCUUGCAUGUUACAUGA	1906-1926	A-2894691.1	2183	UCAUGUAACAUGCAAGAGGAAUG	1904-1926
AD-1565864.1	A-2894692.1	1740	UUGCUCUUGCAUGUUACAUGA	1906-1926	A-2894393.1	2184	UCAUGUAACAUGCAAGAGCAAUG	1904-1926
AD-1565866.1	A-2894685.1	1741	UUGCUCUUGCAUGUUACAUGA	1906-1926	A-2894695.1	2185	UCAUGUAACAUGCAAGAGCAAUG	1904-1926
AD-1565670.2	A-2894392.1	1742	UUGCUCUUGCAUGUUACAUGA	1906-1926	A-2894393.1	2186	UCAUGUAACAUGCAAGAGCAAUG	1904-1926
AD-1565671.1	A-2894394.1	1743	UGCUCUUGCAUGUUACAUGGA	1907-1927	A-2894395.1	2187	UCCATGTAACAUGCAAGAGCAAU	1905-1927
AD-1565865.1	A-2894693.1	1744	GCUCUUGCAUGUUACAUGA	1908-1926	A-2894694.1	2188	UCAUGUAACAUGCAAGAGCGG	1906-1926
AD-1565786.1	A-2894571.1	1745	GCUCUUGCAUGUUACAUGGUA	1908-1928	A-2894572.1	2189	UACCAUGUAACAUGCAAGAGCAA	1906-1928
AD-1565672.1	A-2894396.1	1746	CUCUUGCAUGUUACAUGGUUA	1909-1929	A-2894397.1	2190	UAACCATGUAACAUGCAAGAGCA	1907-1929
AD-1565673.1	A-2894396.1	1747	CUCUUGCAUGUUACAUGGUUA	1909-1929	A-2894398.1	2191	UAACCATGUAACAUGCAAGAGCA	1907-1929
AD-1565674.1	A-2894399.1	1748	UCUUGCAUGUUACAUGGUUAA	1910-1930	A-2894400.1	2192	UUAACCAUGUAACAUGCAAGAGC	1908-1930
AD-1565787.1	A-1577564.1	1749	CUUGCAUGUUACAUGGUUACA	1911-1931	A-2894573.1	2193	UGUAACCAUGUAACAUGCAAGAG	1909-1931
AD-1565675.1	A-1577564.1	1750	CUUGCAUGUUACAUGGUUACA	1911-1931	A-2894401.1	2194	UGUAACCAUGUAACAUGCAAGAG	1909-1931
AD-1565788.1	A-1577566.1	1751	UUGCAUGUUACAUGGUUACCA	1912-1932	A-2894574.1	2195	UGGUAACCAUGUAACAUGCAAGA	1910-1932
AD-1565676.1	A-1577566.1	1752	UUGCAUGUUACAUGGUUACCA	1912-1932	A-2894402.1	2196	UGGTAACCAUGUAACAUGCAAGA	1910-1932
AD-1565677.1	A-2894403.1	1753	UUGCAUGUUACAUGGUUACCA	1912-1932	A-2894404.1	2197	UGGUAACCAUGTAACAUGCAAGA	1910-1932
AD-1565678.1	A-2894405.1	1754	UGCAUGUUACAUGGUUACCAA	1913-1933	A-2894406.1	2198	UUGGTAACCAUGUAACAUGCAAG	1911-1933
AD-1565679.1	A-1577580.1	1755	GCAUGUUACAUGGUUACCACA	1914-1934	A-2894407.1	2199	UGUGGUAACCAUGUAACAUGCAA	1912-1934

AD-1565680.1	A-2894408.1	1756	GCAUGUUACAUGGUUACCACA	1914-1934	A-2894409.1	2200	UGUGGUAACCATGUAACAUGCAA	1912-1934
AD-1565681.1	A-2894410.1	1757	CAUGUUACAUGGUUACCACAA	1915-1935	A-2894411.1	2201	UUGUGGTAACCAUGUAACAUGCA	1913-1935
AD-1565789.1	A-2894575.1	1758	AUGUUACAUGGUUACCACAAA	1916-1936	A-2894576.1	2202	UUUGTGGUAACCAUGUAACAUGC	1914-1936
AD-1565790.1	A-2894577.1	1759	UGUUACAUGGUUACCACAAGA	1917-1937	A-2894578.1	2203	UCUUGUGGUAACCAUGUAACAUG	1915-1937
AD-1565682.1	A-2894412.1	1760	CUCCUCUGGCCAGCAUCGAAA	1976-1996	A-2894413.1	2204	UTUCGATGCUGGCCAGAGGAGCU	1974-1996
AD-1565791.1	A-2894579.1	1761	AUAUAAGUAAGAUGCAUUUAA	1995-2015	A-2894580.1	2205	UUAAAUGCAUCUUACUUAUAUUC	1993-2015
AD-1565683.1	A-2894414.1	1762	UAUAAGUAAGAUGCAUUUACA	1996-2016	A-2894415.1	2206	UGUAAATGCAUCUUACUUAUAUU	1994-2016
AD-1565684.1	A-2894416.1	1763	AUAAGUAAGAUGCAUUUACUA	1997-2017	A-2894417.1	2207	UAGUAAAUGCATCUUACUUAUAU	1995-2017
AD-1565685.1	A-2894418.1	1764	UAAGUAAGAUGCAUUUACUAA	1998-2018	A-2894419.1	2208	UTAGTAAAUGCAUCUUACUUAUA	1996-2018
AD-1565867.1	A-2894420.1	1765	AAGUAAGAUGCAUUUACUACA	1999-2019	A-1804926.1	2209	UGUAGUAAAUGCAUCUUACUUAU	1997-2019
AD-1565868.1	A-2894420.1	1766	AAGUAAGAUGCAUUUACUACA	1999-2019	A-2894696.1	2210	UGUAGUAAAUGCAUCUUACUUGU	1997-2019
AD-1565869.1	A-2894420.1	1767	AAGUAAGAUGCAUUUACUACA	1999-2019	A-2894697.1	2211	UGUAGUAAAUGCAUCUUACUUGU	1997-2019
AD-1565870.1	A-2894420.1	1768	AAGUAAGAUGCAUUUACUACA	1999-2019	A-2894698.1	2212	UGUAGUAAAUGCAUCUUACUUGU	1997-2019
AD-1565871.1	A-2894422.1	1769	AAGUAAGAUGCAUUUACUACA	1999-2019	A-2894697.1	2213	UGUAGUAAAUGCAUCUUACUUGU	1997-2019
AD-1565872.1	A-2894699.1	1770	AAGUAAGATGCAUUUACUACA	1999-2019	A-2894700.1	2214	UGUAGUAAAUGCAUCUUACUUGU	1997-2019
AD-1565873.1	A-2894422.1	1771	AAGUAAGAUGCAUUUACUACA	1999-2019	A-2894700.1	2215	UGUAGUAAAUGCAUCUUACUUGU	1997-2019
AD-1565874.1	A-2894701.1	1772	AAGUUAGAUGCAUUUACUACA	1999-2019	A-2894702.1	2216	UGUAGUAAAUGCAUCUAACUUGU	1997-2019

AD-1565875.1	A-2894703.1	1773	AAGAAAGAUGCAUUUACUACA	1999-2019	A-2894704.1	2217	UGUAGUAAAUGCAUCUUUCUUGU	1997-2019
AD-1565876.1	A-2894705.1	1774	AACUAAGAUGCAUUUACUACA	1999-2019	A-2894706.1	2218	UGUAGUAAAUGCAUCUUAGUUGU	1997-2019
AD-1565686.1	A-2894420.1	1775	AAGUAAGAUGCAUUUACUACA	1999-2019	A-2894421.1	2219	UGUAGUAAAUGCAUCUUACUUAU	1997-2019
AD-1565687.1	A-2894422.1	1776	AAGUAAGAUGCAUUUACUACA	1999-2019	A-2894423.1	2220	UGUAGUAAAUGCAUCUUACUUAU	1997-2019
AD-1565688.1	A-2894424.1	1777	AGUAAGAUGCAUUUACUACAA	2000-2020	A-2894425.1	2221	UUGUAGTAAAUGCAUCUUACUUA	1998-2020
AD-1565877.1	A-2894707.1	1778	GUAAGAUGCAUUUACUACA	2001-2019	A-2894708.1	2222	UGUAGUAAAUGCAUCUUACUU	1999-2019
AD-1565689.1	A-2894426.1	1779	UUGGCUUCUAAUGCUUCAGAA	2021-2041	A-2894427.1	2223	UUCUGAAGCAUUAGAAGCCAACU	2019-2041
AD-1565792.1	A-2894428.1	1780	CUUCUAAUGCUUCAGAUAGAA	2025-2045	A-2894581.1	2224	UUCUAUCUGAAGCAUUAGAAGCC	2023-2045
AD-1565690.1	A-2894428.1	1781	CUUCUAAUGCUUCAGAUAGAA	2025-2045	A-2894429.1	2225	UUCTATCUGAAGCAUUAGAAGCC	2023-2045
AD-1565691.1	A-2894428.1	1782	CUUCUAAUGCUUCAGAUAGAA	2025-2045	A-2894430.1	2226	UUCUATCTGAAGCAUUAGAAGCC	2023-2045

Example 2. *In vitro* screening of MYOC siRNA

Experimental Methods

Cell culture and transfections:

Human Trabecular Meshwork Cells (HTMC) Cell Transfections

HTMC cells (ATCC) were transfected by adding 4.9 µl of Opti-MEM plus 0.1 µl of RNAiMAX per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 µl of siRNA duplexes per well, with 4 replicates of each siRNA duplex, into a 384-well plate, and incubated at room temperature for 15 minutes. Forty µl of DMEM:F12 Medium (ThermoFisher) containing ~5 x10^3 cells were then added to the siRNA-transfection mixture. Cells were incubated for 24 hours prior to RNA purification. Experiments were performed at 10 nM, 1 nM, and 0.1 nM.

Total RNA isolation using DYNABEADS mRNA Isolation Kit:

RNA was isolated using an automated protocol on a BioTek-EL406 platform using DYNABEADs (Invitrogen, cat#61012). Briefly, 70 µl of Lysis/Binding Buffer and 10 µl of lysis buffer containing 3 µl of magnetic beads were added to the plate with cells. Plates were incubated on an electromagnetic shaker for 10 minutes at room temperature and then magnetic beads were captured and the supernatant was removed. Bead-bound RNA was then washed 2 times with 150 µl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150 µl Elution Buffer, re-captured and supernatant removed.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813):

Ten µl of a master mix containing 1 µl 10X Buffer, 0.4 µl 25X dNTPs, 1 µl 10x Random primers, 0.5 µl Reverse Transcriptase, 0.5 µl RNase inhibitor and 6.6 µl of H2O per reaction was added to RNA isolated above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2 h 37°C.

Real time PCR:

Two µl of cDNA and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) were added to either 0.5 µl of Human GAPDH TaqMan Probe (4326317E) and 0.5 µl MYOC Human probe per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was

done in a LightCycler480 Real Time PCR system (Roche). Each duplex was tested at least two times and data were normalized to cells transfected with a non-targeting control siRNA. To calculate relative fold change, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with a non-targeting control siRNA.

Results

The results of the multi-dose screen in human trabecular meshwork cells (HTMC) with exemplary human MYOC siRNAs are shown in Table 3 (correspond to siRNAs in Table 2A), The multi-dose experiments were performed at 10 nM, 1 nM, and 0.1 nM final duplex concentrations and the data are expressed as percent message remaining relative to non-targeting control.

Of the exemplary siRNA duplexes evaluated in Table 3 below, 174 achieved a knockdown of MYOC of ≥ 90%, 347 achieved a knockdown of MYOC of ≥ 70%, 392 achieved a knockdown of MYOC of ≥ 50%, 424 achieved a knockdown of MYOC of ≥ 20%, and 433 achieved a knockdown of MYOC of ≥ 10% in HTMC cells when administered at the 10 nM concentration.

Of the exemplary siRNA duplexes evaluated in Table 3 below, 142 achieved a knockdown of MYOC of ≥ 90%, 341 achieved a knockdown of MYOC of ≥ 70%, 391 achieved a knockdown of MYOC of ≥ 50%, 424 achieved a knockdown of MYOC of ≥ 20%, and 431 achieved a knockdown of MYOC of ≥ 10% in HTMC cells when administered at the 1 nM concentration.

Of the exemplary siRNA duplexes evaluated in Table 3 below, 57 achieved a knockdown of MYOC of ≥ 90%, 277 achieved a knockdown of MYOC of ≥ 70%, 361 achieved a knockdown of MYOC of ≥ 50%, 416 achieved a knockdown of MYOC of ≥ 20%, and 425 achieved a knockdown of MYOC of ≥ 10% in HTMC cells when administered at the 0.1 nM concentration.

Of the exemplary siRNA duplexes evaluated in Table 3 below, AD-1565448.1, AD-1193175.5, AD-1565798.1, AD-1565452.1, AD-1565453.1, AD-1565454.1, AD-1565456.1, AD-1565492.1, AD-1565493.1, AD-1565503.1, AD-1073418.5, AD-1565804.1, AD-1565806.1, AD-1244366.3, AD-1565589.1, AD-1565837.1, AD-1565624.1, AD-1565626.1 showed superior knockdown of MYOC in HTMC cells.

Table 3. MYOC endogenous *in vitro* multi-dose screen with one set of exemplary human MYOC siRNAs (*the number following the decimal point in a duplex name merely refers to a batch production number)

Duplex Name*	10 nM		1 nM		0.1 nM	
	% message remaining	St. Dev.	% message remaining	St. Dev.	% message remaining	St. Dev.
AD-1565444.1	13.58	1.84	14.71	7.84	26.73	7.48
AD-1565445.1	10.87	6.23	13.16	4.98	25.60	5.38
AD-1565692.1	16.41	2.08	18.61	0.75	25.71	4.17
AD-1565446.1	14.37	7.51	6.94	1.71	14.17	3.72
AD-1565447.1	10.38	3.76	10.19	3.32	23.50	6.92
AD-1565448.1	9.24	5.79	4.13	2.66	5.97	2.52
AD-1565693.1	3.99	1.51	6.10	1.34	12.35	2.47
AD-1565449.1	7.20	2.64	3.83	1.28	7.08	3.96
AD-1565450.1	8.78	4.45	6.72	1.51	12.08	2.48
AD-1565694.1	4.18	2.71	9.79	5.26	8.35	1.68
AD-1565695.1	7.35	3.15	6.28	1.55	9.28	3.23
AD-1193175.5	2.63	0.72	2.84	1.11	9.95	4.58
AD-1565793.1	5.77	0.80	2.63	0.70	9.78	3.84
AD-1565795.1	3.59	2.06	6.15	1.52	8.48	3.62
AD-1565796.1	1.04	0.24	3.44	1.14	5.75	1.09
AD-1565797.1	2.72	0.83	3.58	2.60	8.08	3.77
AD-1565798.1	3.95	2.69	2.43	1.56	3.53	1.68
AD-1565799.1	4.64	3.39	3.82	0.78	4.90	2.65
AD-1565451.1	5.31	1.89	4.64	1.66	10.31	5.68
AD-1565452.1	6.08	2.03	7.09	1.62	7.98	5.26
AD-1565696.1	5.58	2.54	9.52	3.27	8.31	3.16
AD-1565794.1	6.22	1.99	4.53	2.58	6.90	3.64
AD-1565453.1	6.30	1.27	8.30	1.28	10.41	6.07
AD-1565454.1	19.20	4.92	6.07	1.17	9.34	1.31
AD-1565455.1	16.24	3.57	6.52	3.62	13.11	3.35
AD-1565456.1	10.95	4.22	4.41	1.79	9.13	3.28
AD-1565457.1	16.55	3.00	5.13	2.63	13.24	6.40
AD-1565697.1	6.35	1.94	9.23	3.77	10.72	0.63
AD-1565698.1	5.42	2.65	12.12	1.42	6.99	1.98
AD-1565458.1	11.01	3.72	4.33	2.88	11.84	3.86
AD-1565459.1	42.85	5.80	36.24	26.02	45.00	15.33
AD-1565699.1	8.28	2.74	10.34	4.26	18.07	7.91
AD-1565700.1	7.73	4.21	11.72	4.13	9.75	1.96
AD-1565460.1	8.30	1.22	8.69	2.89	12.07	3.01
AD-1565461.1	14.00	3.46	5.33	2.92	16.28	3.37

AD-1565462.1	21.75	3.70	20.28	2.83	31.62	4.90
AD-1565701.1	8.93	5.10	9.40	3.76	13.18	1.17
AD-1565463.1	71.43	19.78	76.54	8.03	82.89	9.94
AD-1565464.1	16.39	4.06	10.09	2.80	31.39	3.54
AD-1565465.1	20.94	5.18	12.50	2.79	45.66	3.05
AD-1565466.1	82.31	6.76	75.73	28.49	92.65	18.60
AD-1565467.1	102.55	23.01	84.98	22.44	116.91	27.98
AD-1565702.1	116.28	14.85	85.92	14.00	76.00	18.38
AD-1565468.1	121.69	34.80	86.60	5.71	79.45	23.26
AD-1565469.1	46.82	18.05	34.92	10.42	53.10	9.35
AD-1565703.1	40.06	8.59	41.08	11.27	50.91	16.81
AD-1565704.1	23.10	2.72	32.11	12.42	49.32	8.69
AD-1565705.1	20.82	4.90	14.51	3.79	54.80	11.30
AD-1565470.1	58.45	4.61	77.51	21.21	78.93	12.11
AD-1565471.1	8.36	2.25	14.98	3.23	29.42	6.67
AD-1565472.1	67.13	22.48	107.96	18.35	81.66	15.72
AD-1565473.1	17.14	6.70	12.35	2.42	52.91	13.13
AD-1565706.1	12.69	1.44	17.98	1.45	33.72	10.29
AD-1565474.1	7.35	1.92	11.57	0.74	12.54	0.65
AD-1565707.1	14.28	5.67	12.45	3.33	16.18	4.97
AD-1565475.1	104.91	17.03	92.52	25.04	68.96	7.03
AD-1565476.1	13.25	5.61	10.71	3.39	13.33	2.98
AD-1565477.1	20.62	5.16	21.71	6.04	39.44	2.72
AD-1565708.1	20.35	7.28	34.51	0.60	46.23	16.06
AD-1565478.1	113.51	34.51	84.19	13.48	93.61	8.41
AD-1565479.1	120.66	38.76	113.47	21.10	107.18	23.97
AD-1565709.1	96.03	16.16	82.56	20.98	34.55	10.86
AD-1565480.1	9.45	3.51	15.47	2.78	25.03	4.12
AD-1565481.1	11.63	2.84	8.17	1.49	24.18	8.77
AD-1565482.1	55.07	21.96	62.38	10.34	76.05	18.42
AD-1565483.1	42.47	1.01	32.88	6.07	40.92	10.70
AD-1565484.1	41.71	8.56	35.08	6.28	47.77	12.25
AD-1565710.1	14.95	7.13	25.67	5.90	22.93	2.01
AD-1565711.1	23.82	14.98	23.65	4.48	19.78	3.54
AD-1565485.1	25.24	2.83	19.83	6.64	25.74	2.78
AD-1565486.1	10.05	1.20	14.31	1.72	31.42	7.60
AD-1565712.1	5.20	2.02	19.24	6.00	20.42	4.82
AD-1565713.1	9.27	2.63	19.11	2.21	25.72	3.81
AD-1565487.1	46.68	5.49	28.42	4.77	77.47	6.44
AD-1565714.1	5.67	1.97	5.35	2.39	11.46	2.28
AD-1565488.1	9.36	2.02	11.25	2.36	20.20	1.78

AD-1565715.1	3.35	1.88	5.60	2.17	18.53	6.10
AD-1565716.1	3.87	1.95	8.41	5.17	10.69	3.07
AD-1565489.1	9.86	1.57	14.64	3.55	19.00	5.75
AD-1565717.1	3.41	0.66	2.37	0.59	12.97	3.81
AD-1565718.1	2.99	0.86	3.53	2.40	8.04	3.91
AD-1565490.1	24.04	8.60	11.67	1.80	31.96	10.18
AD-1565719.1	6.39	5.62	2.51	0.45	8.90	2.14
AD-1565491.1	88.01	24.84	75.92	22.03	84.59	11.40
AD-1565720.1	8.86	1.70	4.23	2.27	13.61	2.34
AD-1565721.1	2.67	0.90	4.76	0.47	12.89	4.11
AD-1565492.1	9.24	3.51	4.60	2.16	9.33	1.80
AD-1565493.1	5.01	2.24	6.54	2.52	25.69	11.06
AD-1565722.1	16.33	5.69	19.52	3.24	66.71	10.30
AD-1565723.1	4.09	1.77	10.11	5.93	10.33	2.92
AD-1565494.1	40.03	5.07	32.09	5.50	64.47	10.72
AD-1565495.1	9.19	5.03	12.26	3.30	29.55	9.26
AD-1565724.1	3.97	1.66	3.08	1.67	5.45	1.50
AD-1565496.1	10.35	2.39	20.15	3.54	23.78	8.91
AD-1565725.1	10.58	5.58	4.90	2.19	16.16	7.18
AD-1565497.1	12.46	5.89	10.46	3.87	16.17	5.75
AD-1565498.1	12.18	4.54	8.54	1.66	12.85	5.36
AD-1565499.1	28.70	6.47	25.63	9.41	33.18	6.95
AD-1565500.1	5.40	1.94	4.31	1.08	7.40	2.30
AD-1565726.1	4.06	1.69	2.85	0.29	9.86	5.60
AD-1565501.1	38.18	13.02	39.37	15.62	49.41	13.10
AD-1565502.1	10.38	6.35	10.28	5.46	32.08	11.21
AD-1565503.1	5.08	2.84	7.82	2.01	7.77	0.71
AD-1565801.1	18.47	3.93	10.99	7.48	22.01	4.67
AD-1565802.1	3.76	1.21	4.21	1.61	7.43	1.97
AD-1565803.1	7.26	4.22	7.65	1.94	12.10	3.11
AD-1565804.1	1.84	0.46	1.46	0.81	4.34	0.27
AD-1565805.1	1.81	1.74	1.62	1.12	7.02	3.33
AD-1073418.5	1.66	0.30	2.82	1.23	7.78	4.94
AD-1565504.1	3.08	1.15	3.44	1.47	7.77	2.53
AD-1565504.2	5.57	0.51	6.32	3.44	12.16	4.91
AD-1565505.1	6.00	3.10	9.11	0.70	19.63	5.31
AD-1565800.1	2.31	0.80	3.23	1.74	8.90	5.48
AD-1565506.1	23.85	10.31	28.07	3.84	54.83	10.10
AD-1565727.1	3.70	2.94	4.10	2.15	6.37	2.91
AD-1565507.1	21.31	7.92	27.51	3.72	45.97	12.71
AD-1565728.1	5.43	1.60	3.89	1.30	13.39	1.84

AD-1565508.1	16.45	8.67	8.99	1.68	34.15	11.20
AD-1565509.1	7.04	2.82	6.61	2.08	12.83	2.54
AD-1565730.1	3.38	1.27	6.47	1.28	12.36	3.43
AD-1565510.1	14.18	2.68	24.57	2.78	27.00	1.39
AD-1565511.1	50.88	7.77	59.88	11.33	85.05	19.40
AD-1565512.1	5.77	0.64	16.79	8.26	18.40	4.16
AD-1565731.1	5.79	1.45	4.79	1.98	9.21	1.74
AD-1565513.1	7.85	3.02	8.39	4.73	15.10	2.47
AD-1565514.1	8.18	4.29	8.45	1.99	14.87	2.07
AD-1565732.1	10.04	3.21	20.83	10.19	28.95	5.75
AD-1565515.1	31.33	13.22	25.47	9.82	66.58	12.24
AD-1565516.1	32.42	6.12	22.34	7.88	52.38	10.81
AD-1565733.1	10.20	4.94	7.05	2.67	29.37	6.82
AD-1565517.1	89.04	28.81	134.10	30.29	77.81	27.77
AD-1565734.1	8.69	5.99	8.33	2.02	28.19	7.79
AD-1565518.1	113.31	25.68	99.25	17.54	105.41	19.72
AD-1565735.1	8.25	2.86	7.32	2.99	17.49	2.04
AD-1565736.1	89.87	11.16	82.82	8.36	64.20	3.72
AD-1565519.1	33.76	6.02	27.44	10.42	58.44	19.24
AD-1565520.1	9.40	1.00	20.63	6.01	29.93	7.27
AD-1565521.1	16.54	5.72	10.87	2.71	24.04	8.83
AD-1244360.3	0.93	0.59	2.05	1.46	6.06	1.47
AD-1565522.1	2.11	0.17	2.21	0.63	10.71	6.20
AD-1565806.1	2.01	1.23	1.99	0.78	6.54	4.21
AD-1565807.1	1.14	0.28	2.06	0.28	9.34	3.20
AD-1565808.1	6.46	2.75	13.23	2.10	30.00	3.49
AD-1565809.1	31.44	9.01	31.65	16.95	61.12	8.34
AD-1565810.1	73.75	16.63	55.04	14.78	90.94	25.33
AD-1565812.1	4.64	0.12	8.16	2.01	16.45	9.69
AD-1565813.1	3.88	2.31	5.72	3.22	12.47	5.05
AD-1565814.1	3.74	1.35	2.28	1.09	11.58	10.04
AD-1565522.2	3.48	2.31	5.47	1.39	8.65	3.67
AD-1565523.1	8.60	5.54	16.19	3.18	24.63	10.25
AD-1565811.1	1.86	0.79	3.34	1.00	6.85	1.49
AD-1565524.1	72.95	22.18	71.70	9.91	94.59	16.44
AD-1565525.1	6.68	1.44	29.00	11.53	32.55	7.03
AD-1565526.1	64.82	8.95	81.81	23.93	94.24	1.92
AD-1565737.1	4.73	1.10	5.96	1.36	27.49	7.25
AD-1565527.1	35.16	10.68	57.77	13.50	44.78	5.90
AD-1565738.1	33.81	11.97	37.71	4.91	40.38	9.49
AD-1565528.1	4.57	0.93	13.08	6.54	16.65	3.80

AD-1565739.1	13.69	2.79	7.63	0.94	17.67	1.57
AD-1565529.1	12.48	6.69	12.41	3.01	24.60	4.68
AD-1565740.1	46.59	11.69	24.59	13.81	29.99	8.28
AD-1565530.1	3.85	1.11	16.59	6.50	24.15	14.29
AD-1565741.1	4.79	2.45	5.19	2.76	11.67	1.29
AD-1565531.1	2.88	0.62	8.86	2.91	29.15	16.15
AD-1565532.1	47.20	12.54	54.02	12.23	60.18	10.88
AD-1565533.1	52.58	14.14	52.92	6.95	68.08	20.69
AD-1565534.1	16.47	2.43	36.07	8.10	38.80	1.21
AD-1565535.1	19.47	1.42	18.84	0.59	88.34	12.82
AD-1565536.1	5.75	2.08	26.98	12.23	32.41	10.44
AD-1565537.1	2.45	1.00	14.01	3.91	15.90	7.45
AD-1565742.1	8.77	3.85	14.00	8.58	28.85	4.45
AD-1565538.1	41.11	17.44	17.75	3.59	36.81	13.99
AD-1565539.1	6.54	1.80	23.36	8.41	35.51	12.28
AD-1565540.1	10.90	3.12	33.16	11.52	49.84	15.31
AD-1565743.1	78.79	17.64	56.91	8.13	67.87	7.64
AD-1565541.1	89.10	26.43	118.75	23.82	89.25	13.47
AD-1565542.1	38.04	7.88	75.90	21.39	33.19	9.91
AD-1565543.1	30.20	9.87	29.14	4.70	41.52	4.90
AD-1565544.1	9.42	2.27	28.40	7.23	39.24	12.42
AD-1565744.1	24.73	6.76	22.51	8.57	25.91	5.69
AD-1565545.1	47.11	6.45	68.77	12.27	59.03	19.68
AD-1565745.1	6.33	1.91	4.99	2.25	10.10	3.22
AD-1565546.1	19.20	3.77	28.33	6.33	52.11	19.47
AD-1565547.1	5.54	1.02	15.31	5.28	33.54	9.36
AD-1565548.1	8.24	3.75	14.70	2.67	40.16	5.50
AD-1565746.1	8.80	2.96	7.57	2.68	13.62	1.74
AD-1565549.1	5.83	2.43	10.39	0.49	20.05	4.04
AD-1565550.1	20.30	6.68	20.60	4.03	50.94	7.85
AD-1565551.1	5.55	2.48	14.71	3.54	40.87	9.99
AD-1244365.3	2.81	0.70	6.43	2.73	14.36	7.50
AD-1565552.1	4.23	2.73	4.99	1.74	23.97	7.92
AD-1565553.1	3.13	1.08	4.01	2.83	14.81	3.86
AD-1565815.1	20.58	6.87	17.87	5.67	55.24	13.57
AD-1565816.1	9.35	5.93	6.40	1.94	17.06	5.64
AD-1565818.1	71.18	34.11	91.54	39.75	102.07	7.11
AD-1565819.1	8.53	2.68	16.42	9.17	37.73	10.42
AD-1565820.1	4.30	1.60	16.39	15.44	31.97	6.06
AD-1565552.2	8.71	4.40	17.83	6.52	34.81	10.71
AD-1565553.2	9.88	2.16	15.76	3.45	18.52	1.32

AD-1565554.1	86.10	20.06	92.44	20.16	90.05	11.05
AD-1565817.1	8.82	3.58	9.94	1.98	27.04	8.06
AD-1565555.1	60.03	7.25	79.65	1.93	94.66	18.30
AD-1565747.1	3.71	0.52	4.30	0.82	5.49	1.97
AD-1565556.1	6.54	1.27	18.17	7.56	24.57	9.17
AD-1565557.1	6.55	0.71	11.80	1.61	14.15	4.83
AD-1565558.1	34.90	6.82	45.74	16.31	51.71	5.43
AD-1565559.1	7.56	3.35	16.90	6.13	35.70	6.70
AD-1565560.1	12.01	4.77	20.12	8.08	42.42	14.79
AD-1565561.1	14.87	2.55	41.04	16.82	56.34	19.15
AD-1565562.1	31.27	1.55	39.84	11.11	68.61	16.49
AD-1565748.1	4.73	2.30	7.31	2.75	6.16	3.13
AD-1565563.1	11.56	3.31	14.52	3.50	32.01	6.14
AD-1565564.1	45.30	5.78	31.11	13.84	62.81	24.12
AD-1565565.1	7.88	3.48	17.14	3.33	37.36	12.76
AD-1565566.1	8.76	0.08	25.95	2.71	16.79	4.03
AD-1565567.1	36.72	12.97	31.96	7.23	27.27	12.04
AD-1565568.1	12.21	5.78	15.35	4.76	17.90	8.52
AD-1565569.1	10.87	4.71	14.51	4.01	25.63	7.40
AD-1565749.1	5.31	1.88	6.58	1.58	14.50	3.78
AD-1565570.1	9.58	4.30	9.96	2.16	23.30	4.89
AD-1565571.1	8.78	4.04	10.58	1.49	31.02	14.75
AD-1565750.1	6.27	1.49	5.63	0.86	9.31	2.00
AD-1565573.1	13.43	7.22	17.01	1.73	32.17	10.08
AD-1565751.1	13.71	7.32	10.60	1.99	26.81	4.95
AD-1565574.1	58.13	13.35	39.02	9.73	100.79	15.98
AD-1565575.1	67.71	7.53	62.51	3.58	86.20	24.09
AD-1565576.1	10.63	2.87	18.40	9.43	34.88	5.78
AD-1565752.1	6.45	2.12	7.36	3.77	9.02	2.33
AD-1565577.1	18.86	4.83	15.88	7.49	44.31	13.98
AD-1565578.1	10.01	3.05	15.75	5.12	21.61	4.88
AD-1565579.1	36.46	2.52	13.87	3.07	22.87	6.37
AD-1565753.1	4.80	2.52	4.20	0.60	5.88	2.28
AD-1565580.1	50.99	16.10	54.79	19.39	47.79	8.33
AD-1565754.1	5.40	1.31	5.57	1.86	20.02	4.99
AD-1565581.1	76.36	0.65	50.01	18.97	112.59	14.54
AD-1565582.1	68.84	13.42	59.94	12.89	54.61	10.15
AD-1565755.1	14.04	3.54	17.44	4.58	30.93	9.58
AD-1565583.1	75.79	12.48	58.61	13.48	91.02	22.93
AD-1565584.1	16.03	6.74	31.39	5.39	53.28	7.92
AD-1565756.1	10.12	1.85	13.28	1.55	18.52	5.93

AD-1565585.1	65.26	7.83	76.22	19.35	138.12	8.35
AD-1073420.3	4.68	3.06	8.04	5.93	14.60	5.21
AD-1244366.3	2.80	0.55	2.75	1.99	8.93	3.10
AD-1565757.1	2.58	1.56	3.05	0.76	9.14	2.74
AD-1565821.1	5.45	1.74	4.48	0.95	18.08	6.93
AD-1565822.1	2.64	1.05	3.40	1.03	11.58	3.46
AD-1565824.1	10.07	4.80	5.40	1.41	14.69	3.10
AD-1565825.1	3.43	0.99	4.41	0.69	14.18	3.87
AD-1565826.1	2.60	0.31	5.27	2.04	11.44	3.39
AD-1565757.2	5.70	4.74	3.31	1.11	6.26	1.50
AD-1565586.1	10.54	3.26	9.72	4.39	20.36	3.90
AD-1565587.1	10.26	1.67	23.30	4.07	23.21	6.92
AD-1565588.1	5.50	3.07	8.28	3.79	14.45	5.15
AD-1565823.1	2.90	1.50	5.22	2.56	12.75	5.20
AD-1565589.1	7.82	4.23	10.17	3.33	14.98	7.64
AD-1565590.1	17.34	3.89	25.42	6.05	39.12	6.12
AD-1565591.1	9.81	6.12	16.24	6.75	14.32	3.13
AD-1565758.1	5.05	1.81	6.30	2.45	9.65	2.97
AD-1565592.1	10.85	4.12	20.95	5.38	22.85	8.66
AD-1565594.1	71.45	12.79	106.92	35.47	73.36	15.85
AD-1565759.1	8.02	3.35	10.76	2.33	13.69	2.12
AD-1565595.1	13.55	3.92	40.56	9.53	66.88	17.43
AD-1565760.1	7.84	3.28	13.72	4.96	27.50	7.10
AD-1565761.1	5.55	2.42	6.63	1.87	16.59	1.03
AD-1565596.1	60.69	7.73	48.61	17.04	79.55	24.61
AD-1565597.1	17.28	4.78	27.20	12.46	18.26	5.10
AD-1565762.1	13.16	1.54	10.59	2.12	13.86	3.34
AD-1565598.1	59.38	11.46	56.84	17.50	52.34	9.18
AD-1565599.1	79.70	12.67	150.03	21.77	122.89	36.87
AD-1565600.1	82.87	13.09	57.05	6.94	88.22	21.48
AD-1565601.1	25.18	2.27	22.89	9.55	44.49	5.77
AD-1565602.1	3.97	1.58	6.23	0.60	30.83	8.44
AD-1565827.1	3.03	1.64	4.22	2.36	11.52	3.63
AD-1565828.1	3.65	0.82	2.60	0.33	21.71	9.56
AD-1565829.1	3.60	2.48	2.89	0.44	9.09	3.82
AD-1565830.1	2.98	1.54	5.47	1.73	9.98	5.35
AD-1565831.1	4.47	1.82	7.16	2.87	14.17	5.18
AD-1565833.1	49.82	24.88	57.44	5.52	82.58	21.84
AD-1565834.1	44.76	9.00	21.17	7.13	49.24	18.58
AD-1565835.1	21.43	3.51	28.41	7.50	53.98	13.43
AD-1565602.2	8.60	3.72	16.07	8.09	21.32	2.93

AD-1565603.1	15.64	6.79	20.38	8.40	15.80	3.00
AD-1565832.1	2.84	0.85	4.48	1.91	9.88	4.28
AD-1565763.1	31.78	11.13	55.15	17.04	43.31	1.78
AD-1565604.1	74.65	13.66	64.27	3.82	76.86	24.52
AD-1565605.1	114.63	37.42	90.22	23.73	51.30	6.16
AD-1565606.1	10.93	4.65	19.08	6.65	16.70	4.41
AD-1565764.1	4.24	0.65	4.49	2.04	5.61	2.08
AD-1565607.1	27.14	6.06	36.08	7.73	64.66	4.95
AD-1565608.1	14.35	6.24	31.28	7.21	44.69	9.15
AD-1565609.1	9.04	3.66	20.95	7.58	25.35	5.78
AD-1565766.1	7.96	1.91	9.92	1.72	19.25	1.48
AD-1565611.1	15.58	4.66	25.13	8.99	27.02	3.88
AD-1565767.1	7.79	5.06	13.20	3.78	14.68	3.73
AD-1565612.1	27.72	4.59	35.45	10.64	49.10	6.99
AD-1565613.1	32.54	7.98	63.94	9.99	64.04	15.15
AD-1565614.1	14.27	6.39	25.57	1.52	43.23	30.00
AD-1565768.1	4.44	1.76	5.23	1.23	14.17	5.16
AD-1565615.1	55.18	8.55	79.04	23.74	78.22	6.99
AD-1565616.1	15.40	4.47	35.68	7.48	51.99	10.44
AD-1565617.1	3.84	2.17	11.03	1.79	34.82	1.45
AD-1565769.1	5.91	3.78	3.71	0.60	6.10	2.19
AD-1565618.1	7.81	3.67	7.98	3.83	22.24	8.77
AD-1565770.1	17.14	1.76	14.94	0.31	51.38	16.55
AD-1565619.1	65.85	13.72	60.46	19.23	51.34	12.11
AD-1565620.1	4.02	3.39	4.75	3.11	18.39	8.53
AD-1565771.1	5.16	1.04	6.21	1.84	11.84	4.85
AD-1565621.1	83.75	17.64	64.18	12.51	110.01	8.38
AD-1565772.1	2.83	0.68	4.28	1.68	12.04	3.51
AD-1565836.1	3.31	1.54	6.47	2.88	13.20	3.79
AD-1565837.1	4.13	1.20	4.14	0.94	14.83	4.25
AD-1565838.1	6.38	1.77	5.75	2.18	15.94	4.66
AD-1565840.1	7.19	3.59	5.53	1.80	32.45	6.46
AD-1565841.1	4.72	1.31	8.47	2.00	18.97	8.19
AD-1565842.1	3.04	1.48	4.60	3.10	14.00	3.53
AD-822899.17	3.51	1.21	5.57	2.82	12.90	3.86
AD-1565772.2	2.85	0.87	4.95	1.83	13.34	9.42
AD-1565622.1	9.09	3.35	11.85	1.77	21.74	5.30
AD-1565843.1	9.58	4.09	8.54	1.89	15.97	7.50
AD-1565844.1	2.75	1.37	4.20	1.48	8.94	4.30
AD-1565845.1	27.92	5.48	26.58	6.38	57.51	9.61
AD-1565846.1	48.68	10.51	49.20	29.92	71.91	11.76

AD-1565848.1	94.29	22.52	111.68	12.87	111.38	29.52
AD-1565849.1	37.68	9.68	17.51	5.53	45.10	16.47
AD-1565850.1	57.87	9.01	36.20	10.32	63.68	16.98
AD-822926.4	7.22	4.66	4.35	1.48	17.62	7.83
AD-1565623.1	8.25	3.03	11.68	4.46	14.42	3.89
AD-1565839.1	6.07	2.22	5.99	5.16	11.18	3.84
AD-1565624.1	6.10	1.35	8.89	1.90	9.85	2.62
AD-1565847.1	9.04	3.53	6.91	2.93	38.22	4.27
AD-1565625.1	60.76	1.13	51.43	8.88	33.00	0.71
AD-1565626.1	8.87	3.65	8.36	3.19	9.48	1.76
AD-1565773.1	6.64	1.30	13.77	6.02	38.32	6.58
AD-1565627.1	7.68	2.90	8.60	2.57	9.19	3.34
AD-1565628.1	27.35	5.83	27.90	9.21	42.72	13.86
AD-1565629.1	12.25	4.89	42.80	12.64	37.72	0.21
AD-1565774.1	17.59	5.54	5.12	0.90	18.54	7.94
AD-1565630.1	12.95	1.09	29.20	7.37	36.41	6.87
AD-1565631.1	14.87	4.36	29.86	5.00	30.09	6.35
AD-1565775.1	12.20	0.98	10.60	2.86	11.61	3.39
AD-1565632.1	34.01	6.07	33.10	1.65	43.01	7.59
AD-1565776.1	59.47	10.06	46.53	13.29	76.25	10.64
AD-1565777.1	3.25	3.60	4.24	1.29	7.38	4.40
AD-1565634.1	19.64	6.63	24.71	9.68	12.30	3.61
AD-1565778.1	18.08	3.67	20.05	8.75	19.26	5.00
AD-1565635.1	24.90	4.36	27.27	3.30	17.01	6.09
AD-1565636.1	18.08	5.60	26.04	6.67	19.12	5.80
AD-1565637.1	24.19	7.75	22.69	7.34	22.00	7.85
AD-1565638.1	20.13	4.33	21.73	5.82	23.78	7.73
AD-1565639.1	19.98	6.07	17.46	5.27	17.52	4.96
AD-1565779.1	16.57	5.59	23.11	7.46	18.57	2.30
AD-1565640.1	24.23	7.16	24.86	4.78	22.95	5.65
AD-1565641.1	14.78	4.59	20.27	7.35	15.40	4.17
AD-1565642.1	22.92	7.06	27.36	1.86	22.58	4.00
AD-1565643.1	21.33	5.08	25.45	4.54	21.06	4.94
AD-1565644.1	21.50	4.66	28.68	4.46	21.57	3.60
AD-1565645.1	20.71	1.23	30.21	6.14	24.39	5.16
AD-1565646.1	25.16	2.74	21.62	2.13	26.27	4.91
AD-1565851.1	32.51	12.05	25.85	3.74	28.17	4.26
AD-1565852.1	23.35	2.52	24.85	2.61	25.45	1.40
AD-1565853.1	20.79	5.56	15.58	2.51	23.92	2.07
AD-1565854.1	21.35	2.18	16.74	4.57	37.31	10.98
AD-1565857.1	89.72	24.46	60.67	10.16	72.79	19.16

AD-1565858.1	32.39	5.99	20.22	3.18	34.87	11.29
AD-1565859.1	44.64	4.32	28.84	7.42	41.43	14.25
AD-1565647.1	33.50	6.27	23.93	7.48	20.42	3.95
AD-1565648.1	17.96	3.05	16.97	2.55	15.90	1.48
AD-1565855.1	24.15	5.54	18.07	0.61	37.07	7.91
AD-1565856.1	32.83	3.41	20.30	3.88	46.01	8.44
AD-1565649.1	21.70	5.38	31.01	4.14	14.39	2.35
AD-1565650.1	14.11	2.91	16.32	3.70	13.62	10.74
AD-1565651.1	15.70	3.79	23.56	8.66	14.00	4.41
AD-1565652.1	28.65	5.13	32.11	6.96	41.78	11.65
AD-1565653.1	18.91	3.29	22.20	8.89	16.74	4.17
AD-1565780.1	21.89	7.89	22.48	5.59	25.73	7.52
AD-1565654.1	28.76	5.80	27.06	9.35	36.78	10.64
AD-1565655.1	14.26	1.84	16.84	2.93	22.57	3.51
AD-1565781.1	22.73	5.86	23.64	7.88	26.26	4.73
AD-1565656.1	8.05	3.17	18.34	3.69	20.89	1.60
AD-1565657.1	37.82	7.32	34.46	4.71	46.82	15.41
AD-1565658.1	20.16	5.46	22.60	1.17	33.74	16.69
AD-1565659.1	21.80	5.01	32.48	6.03	27.19	5.57
AD-1565660.1	18.96	4.48	25.75	6.04	33.55	9.37
AD-1565782.1	19.49	5.05	13.48	3.51	23.54	5.97
AD-1565661.1	23.24	4.21	30.64	8.86	21.49	5.88
AD-1565662.1	22.44	1.52	29.91	6.54	23.11	6.46
AD-1565663.1	19.51	3.82	19.55	2.35	22.06	6.15
AD-1565664.1	20.84	2.90	14.97	3.73	16.29	4.16
AD-1565783.1	19.12	7.35	16.94	6.39	19.67	8.30
AD-1565665.1	17.64	0.62	29.45	9.88	29.19	7.74
AD-1565784.1	16.20	5.45	15.66	5.02	13.24	3.05
AD-1565666.1	17.38	4.44	22.81	7.48	24.53	5.40
AD-1565667.1	17.79	4.54	26.44	5.86	16.24	2.00
AD-1565785.1	18.10	7.14	10.86	5.66	17.76	6.07
AD-1565668.1	41.49	8.93	43.99	2.72	37.17	6.96
AD-1565669.1	24.91	3.24	25.11	4.53	14.49	3.34
AD-1565670.1	14.17	6.35	21.96	9.65	29.29	2.34
AD-1565860.1	12.24	3.88	14.80	4.75	36.53	4.17
AD-1565861.1	19.87	5.39	14.91	8.45	25.82	7.66
AD-1565862.1	16.77	5.82	23.11	4.79	28.52	8.22
AD-1565863.1	20.42	7.47	23.10	8.82	22.00	7.25
AD-1565864.1	23.64	12.69	17.75	5.07	21.37	4.31
AD-1565866.1	21.29	7.65	17.97	4.76	25.56	4.55
AD-1565670.2	20.00	4.48	19.21	5.61	15.66	2.61

AD-1565671.1	34.15	10.48	35.84	7.21	26.67	12.46
AD-1565865.1	22.15	7.26	22.19	5.76	29.14	5.04
AD-1565786.1	19.30	7.79	15.01	4.01	23.94	1.39
AD-1565672.1	18.40	4.74	28.01	8.90	16.08	3.92
AD-1565673.1	11.46	5.01	19.74	4.14	18.41	3.39
AD-1565674.1	22.27	7.50	26.45	5.36	22.47	7.98
AD-1565787.1	20.36	2.47	9.49	3.21	18.01	2.96
AD-1565675.1	28.40	5.05	34.29	3.63	29.59	10.48
AD-1565788.1	15.79	4.25	15.72	4.64	16.69	0.95
AD-1565676.1	22.39	5.86	24.17	3.94	28.08	5.73
AD-1565677.1	22.77	5.35	26.27	7.04	22.49	7.99
AD-1565678.1	14.89	2.15	17.14	5.83	19.52	5.04
AD-1565679.1	17.93	3.69	18.38	3.92	21.25	4.78
AD-1565680.1	16.07	3.75	20.49	2.95	19.02	1.58
AD-1565681.1	66.05	9.80	45.76	2.54	76.24	17.73
AD-1565789.1	17.89	6.69	17.09	1.75	15.21	4.11
AD-1565790.1	15.85	0.71	12.83	5.40	24.07	8.61
AD-1565682.1	28.99	9.62	28.53	6.54	34.55	13.61
AD-1565791.1	13.75	1.62	12.50	3.96	34.69	3.47
AD-1565683.1	33.85	9.82	28.85	2.71	27.77	9.17
AD-1565684.1	46.44	16.14	47.20	12.01	92.00	26.93
AD-1565685.1	44.46	12.33	36.23	5.10	58.69	23.40
AD-1565867.1	24.71	5.92	23.04	2.85	31.52	3.79
AD-1565868.1	33.54	8.52	31.27	12.38	37.03	4.99
AD-1565869.1	27.19	9.45	33.17	9.74	30.69	5.68
AD-1565870.1	22.23	4.78	21.55	2.08	29.63	9.89
AD-1565871.1	22.89	4.89	24.65	0.94	21.11	1.91
AD-1565872.1	28.60	9.58	27.43	10.76	23.01	5.72
AD-1565873.1	15.65	4.07	30.34	7.91	29.58	6.70
AD-1565874.1	77.37	12.83	106.84	16.49	58.43	2.43
AD-1565875.1	77.77	23.99	44.29	12.88	55.96	13.56
AD-1565876.1	114.72	42.05	55.55	13.12	52.88	7.61
AD-1565686.1	23.56	4.75	40.73	8.81	43.94	3.80
AD-1565687.1	23.34	1.87	21.53	3.56	22.50	4.26
AD-1565688.1	27.59	6.13	27.27	2.35	24.05	3.29
AD-1565877.1	21.23	2.76	37.13	7.45	45.66	6.89
AD-1565689.1	26.48	4.22	22.79	6.21	24.67	3.21
AD-1565792.1	10.48	2.13	8.23	1.24	21.07	8.93
AD-1565690.1	28.90	2.94	27.49	4.31	22.85	4.91
AD-1565691.1	30.69	8.84	33.68	2.31	34.86	6.66

Example 3. Validation of Human MYOC siRNAs in a Mouse Glaucoma Model

To test human MYOC siRNAs *in vivo*, a glaucoma mouse model was used: synergistic activation mediator (SAM) mice comprising a humanized *MYOC* locus comprising a Y437H mutation (SAM-MYOC mice). In these mice, a SAM guide RNA targeting the *MYOC* promoter (SAM gRNA) can be used to increase expression of the humanized *MYOC* comprising the Y437H mutation, resulting in increased intraocular pressure (IOP). The SAM-MYOC mice were generated by crossing mice comprising genomically integrated dCas9 synergistic activation mediator (SAM) system components (dCas9-VP64 and MCP-p65-HSF1) as one transcript driven by the endogenous *Rosa26* promoter (described in US 2019/0284572 and WO 2019/183123, each of which is herein incorporated by reference) with mice comprising a humanized *MYOC* locus comprising a Y437H mutation. In these mice, the mouse *Myoc* sequence from the start codon to the stop codon was replaced with the corresponding human *MYOC* sequence. The inserted human *MYOC* sequence comprises a Y437H mutation, which is a mutation associated with elevated IOP and development of glaucoma. Injection of AAV2.Y3F or lentivirus encoding the SAM guide RNA targeting the *MYOC* promoter resulted in increased humanized *MYOC* Y437H expression in the limbal ring (trabecular meshwork (TM), iris, and ciliary body (CB)), and this correlated with increased IOP, validating the SAM-MYOC mice as a suitable MYOC disease model with increased IOP (data not shown).

siRNAs targeting human *MYOC* were tested to determine if they could lower the high intraocular pressure (IOP) observed in SAM mice comprising a humanized *MYOC* locus comprising a Y437H mutation (SAM-MYOC mice) following treatment with the SAM gRNA. The experimental setup is shown in **Figure 1A**. The SAM gRNA was administered via intracameral (IC) injection at day 0, and baseline IOP was measured. IOP was then measured over the following five weeks. At week five, MYOC siRNA AD-822899 (1 µL, 15 µg dose) was administered via intravitreal (IVT) injection, and IOP was measured at various timepoints over the subsequent weeks. There were three treatment groups: (1) naïve control mice; (2) SAM-gRNA-treated mice treated with human *MYOC* siRNA; and (3) SAM-gRNA-treated mice treated with luciferase siRNA. As shown in **Figure 1B**, the human *MYOC* siRNA lowered IOP in the SAM-MYOC mice treated with the SAM gRNA, reversing and returning the IOP to baseline levels starting at D7 after siRNA injection, whereas the luciferase siRNA had no effect on IOP.

Several additional siRNAs targeting human *MYOC* were then tested at a lower dose to determine if they could lower the IOP observed in the SAM-MYOC mice following treatment with lentiviral SAM gRNA. Mice were bilaterally injected with SAM gRNA and siRNAs. The experimental setup is shown in **Figure 2A**. SAM gRNA was administered via intracameral (IC) injection at day 0, and baseline IOP was measured. IOP was then measured over the following five weeks. At week five, MYOC siRNAs AD-822899, AD-1565804, AD-1565837, AD-1193175, or AD-1565503 (1 µL, 7.5 µg dose) were administered via intravitreal (IVT) injection, and IOP was measured at various timepoints over the subsequent weeks. mRNA knockdown in the limbal ring was tested by qPCR, and RNASCOPE® analysis was done. The control groups included naïve control mice, PBS-treated mice, and LV-SAM gRNA-treated mice. As shown in **Figure 2B**, each human *MYOC* siRNA lowered IOP in the SAM-MYOC mice treated with SAM gRNA, reversing and returning the IOP to baseline levels soon after siRNA injection. As shown in **Figure 2C**, each human *MYOC* siRNA decreased human *MYOC* mRNA expression relative to the LV-SAM-gRNA group as measured by qPCR in a sample from the limbal ring. RNASCOPE® analysis confirmed that the siRNAs mediated knockdown of human *MYOC* mRNA in the SAM-MYOC mice (**Figure 2D**).

Human *MYOC* siRNAs AD-1565804 or AD-1565837 were then tested at even lower doses to determine if they could lower the IOP observed in the SAM-MYOC mice following treatment with lentiviral SAM-g4. Mice were bilaterally injected with SAM gRNA and siRNA. The experimental setup is shown in **Figure 3A**. SAM-g4 was administered via intracameral (IC) injection at day 0, and baseline IOP was measured. IOP was then measured over the following five weeks. At week five, MYOC siRNAs AD-1565804 or AD-1565837 (1 µL, 3.75 µg dose or 1.87 µg dose) were administered via intravitreal (IVT) injection, and IOP was measured at various timepoints over the subsequent weeks. mRNA knockdown in the limbal ring was tested by qPCR, and RNASCOPE® analysis was done. The control groups included naïve control mice, PBS-treated mice, and LV-SAM-gRNA-treated mice. As shown in **Figure 3B**, each human *MYOC* siRNA lowered IOP in the SAM-MYOC mice treated with SAM gRNA at each dose tested, reversing and returning the IOP to baseline levels soon after siRNA injection. As shown in **Figure 3C**, each human *MYOC* siRNA decreased human *MYOC* mRNA expression relative to the LV-SAM gRNA group as measured by qPCR in a sample from the limbal ring at each dose tested.

Example 4. Validation of Human MYOC siRNAs in a Non-Human Primate (NHP) Glaucoma Model

siRNAs targeting *MYOC* were tested to determine if they could lower the high intraocular pressure (IOP) observed in a non-human primate (NHP) glaucoma model. The study design is shown in Table 4. The animals received a PBS control or various doses of the duplex AD-1565837 or the duplex AD-1565804.

OE's and intraocular pressure (IOP) were measured pre-study, on days 3, 8, 15, 29, and 57, and once during week 12. Electroretinography (ERG) was performed pre-study, mid-study (week 6), and at termination (week 12). Aqueous humor (AH) was collected pre-dose, week 4, week 8 and at termination. At termination, the following eye samples were collected from the right eye: AH, vitreous humor (VH), cornea, iris, trabecular meshwork (TM), ciliary body (CB), sclera (limbal ring without TM), retina, and all remaining tissue in posterior segment of the eye. Blood was collected twice at pre-dose and termination. Hematology, coagulation, and clinical chemistry studies were perfomed on the blood samples.

Tissues were collected for histopathology at termination including the right eye, optic nerve and extra-ocular and peri-ocular tissues: Brain, right (cerebral hemisphere); Esophagus, proximal; Stomach, esophageal-gastric junction; Eyelid, upper with palpebral conjunctivae, right; Eyelid, lower with palpebral conjunctivae, right; Heart, apex; Jejunum; Kidney, right cortex; Liver, right median lobe; Lacrimal Gl, right; Deep Cervical, lymph node, right; Mandibular, lymph node, right; Sciatic nerve, right; Muscle, rectus femoris; Muscle, diaphragm; Muscle, extraocular (lateral and medial rectus, right); Ovary, right; Mandibular salivary gland, right; Spinal cord, cervical; Spinal cord, thoracic; Spinal cord, lumbar; Thyroid, right; Tongue; Tonsil, right; Trachea; and Uterus.

Table 4: NHP DC Selection Study Design

Group	Test Article	Dose Level (µg/eye)	No. Monkeys	Total
1	PBS	0	3	31
2	AD-1565837	10	3	
3		30	3	

4		100	3	
5		300	3	
6		1000*	2	
7	AD-1565804	10	3	
8		30	3	
9		100	3	
10		300	3	
11		1000*	2	

* Animals treated with highest dose: both eyes will be examined with histopathology read outs.

MYOC protein knock down in the TM was analyzed at day 85. The knock down results are shown in **Figure 4.** About a 50% reduction in MYOC protein in the TM was seen with the AD-1565837 duplex, while minimal knockdown was observed with the AD-1565804 duplex. MYOC protein in the aqueous humor was analyzed at days -35, 22, 50, and day 85 (terminal collection). As depicted in **Figure 5**, MYOC protein knockdown of about 50% was observed at day 50, but not at day 85. MYOC protein knock down in the vitreous humor, iris, ciliary body, and sclera was analyzed at day 85 (terminal collection). The results are shown in **Figure 6A** for vitreous humor and ciliary body, and in **Figure 6B** for iris and sclera. At day 85, no knock down was observed in the MYOC mRNA for either duplex evaluated (**Figure 7**).

WE CLAIM:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of myocilin (MYOC), wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from one of the antisense sequences listed in any one of Tables 2A and 2B, and wherein the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from a sense sequence listed in any one of Tables 2A and 2B that corresponds to the antisense sequence.

2. The dsRNA agent of claim 1, wherein the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1565804 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1565804.

3. The dsRNA agent of claim 1, wherein the antisense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the antisense strand nucleotide sequence of duplex AD-1565837 and the sense strand comprises a nucleotide sequence comprising at least 15 contiguous nucleotides, with 0, 1, 2, or 3 mismatches, from the sense strand nucleotide sequence of duplex AD-1565837.

4. The dsRNA agent of any one of claims 1-3, wherein at least one of the sense strand and the antisense strand is conjugated to one or more lipophilic moieties.

5. The dsRNA agent of claim 4, wherein the lipophilic moiety is conjugated via a linker or carrier.

6. The dsRNA agent of claim 4 or 5, wherein one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand.

7. The dsRNA agent of any one of claims 1-3, wherein at least one of the sense strand and the antisense strand is conjugated to one or more of an arginine-glycine-aspartic acid (RGD)-peptide or RGD peptide mimetic.

8. The dsRNA agent of any one of claims 4-6, wherein the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound.

9. The dsRNA agent of claim 8, wherein the lipophilic moiety contains a saturated or unsaturated C16 hydrocarbon chain.

10. The dsRNA agent of any one of claims 4-6, wherein 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.

11. The dsRNA agent of any one of claims 4-6, wherein 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.

12. The double-stranded iRNA agent of any one of claims 4-6, wherein the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

13. The dsRNA agent of any one of claims 1-12, wherein the dsRNA agent comprises at least one modified nucleotide.

14. The dsRNA agent of claim 13, wherein no more than five of the sense strand nucleotides and not more than five of the nucleotides of the antisense strand are unmodified nucleotides.

15. The dsRNA agent of claim 13, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

16. The dsRNA agent of any one of claims 13-15, wherein 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 phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a glycol modified nucleotide, and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

17. The dsRNA agent of any of any one of claims 1-16, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

18. The dsRNA agent of any one of claims1-17, wherein the double stranded region is 15-30 nucleotide pairs in length.

19. The dsRNA agent of claim 18, wherein the double stranded region is 17-23 nucleotide pairs in length.

20. The dsRNA agent of any one of claims 1-19, wherein each strand has 19-30 nucleotides.

21. The dsRNA agent of any one of claims 1-20, wherein the agent comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

22. The dsRNA agent of any one of claims 4-21, further comprising a targeting ligand, *e.g.*, a ligand that targets an ocular tissue.

23. The dsRNA agent of claim 22, wherein the ocular tissue is a trabecular meshwork tissue, a ciliary body, a retinal tissue, a retinal pigment epithelium (RPE) or choroid tissue, e.g., a choroid vessel.

24. The dsRNA agent of any one of claims 1-23, further comprising a phosphate or phosphate mimic at the 5'-end of the antisense strand.

25. The dsRNA agent of claim 24, wherein the phosphate mimic is a 5'-vinyl phosphonate (VP).

26. The dsRNA of any one of claims 1-25, wherein the dsRNA agent targets a hotspot region of an mRNA encoding MYOC.

27. A dsRNA agent that targets a hotspot region of a myocilin (MYOC) mRNA.

28. A cell containing the dsRNA agent of any one of claims 1-27.

29. A pharmaceutical composition for inhibiting expression of a MYOC, comprising the dsRNA agent of any one of claims 1-27.

30. A method of inhibiting expression of MYOC in a cell, the method comprising:

(a) contacting the cell with the dsRNA agent of any one of claims 1-27, or a pharmaceutical composition of claim 29; and

(b) maintaining the cell produced in step (a) for a time sufficient to reduce levels of MYOC mRNA, MYOC protein, or both of MYOC mRNA and protein, thereby inhibiting expression of MYOC in the cell.

31. The method of claim 30, wherein the cell is within a subject.

32. The method of claim 31, wherein the subject is a human.

33. The method of claim 32, wherein the subject has been diagnosed with a MYOC-associated disorder, *e.g.*, glaucoma (*e.g.*, primary open angle glaucoma (POAG), angle closure glaucoma, congenital glaucoma, and secondary glaucoma).

34. A method of treating a subject diagnosed with a MYOC-associated disorder comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-27 or a pharmaceutical composition of claim 29, thereby treating the disorder.

35. The method of claim 34, wherein the MYOC-associated disorder is glaucoma.

36. The method of claim 35, wherein glaucoma is primary open angle glaucoma (POAG).

37. The method of any one of claims 34-36, wherein treating comprises amelioration of at least one sign or symptom of the disorder.

38. The method of any one of claims 34-37, wherein the treating comprises (a) inhibiting or reducing the expression or activity of MYOC; (b) reducing the level of misfolded MYOC protein; (c) reducing trabecular meshwork cell death; (d) decreasing intraocular pressure; or (e) increasing visual acuity.

39. The method of any one of claims 31-38, wherein the dsRNA agent is administered to the subject intraocularly, intravenously, or topically.

40. The method of claim 39, wherein the intraocular administration comprises intravitreal administration (e.g., intravitreal injection), transscleral administration (e.g.,

transscleral injection), subconjunctival administration (e.g., subconjunctival injection), retrobulbar administration (e.g., retrobulbar injection), intracameral administration (e.g., intracameral injection), or subretinal administration (e.g., subretinal injection).

41. The method of any one of claims 31-40, further comprising administering to the subject an additional agent or therapy suitable for treatment or prevention of an MYOC-associated disorder (*e.g.*, laser trabeculoplasty surgery, trabeculectomy surgery, a minimally invasive glaucoma surgery, placement of a drainage tube in the eye, oral medication, or eye drops).

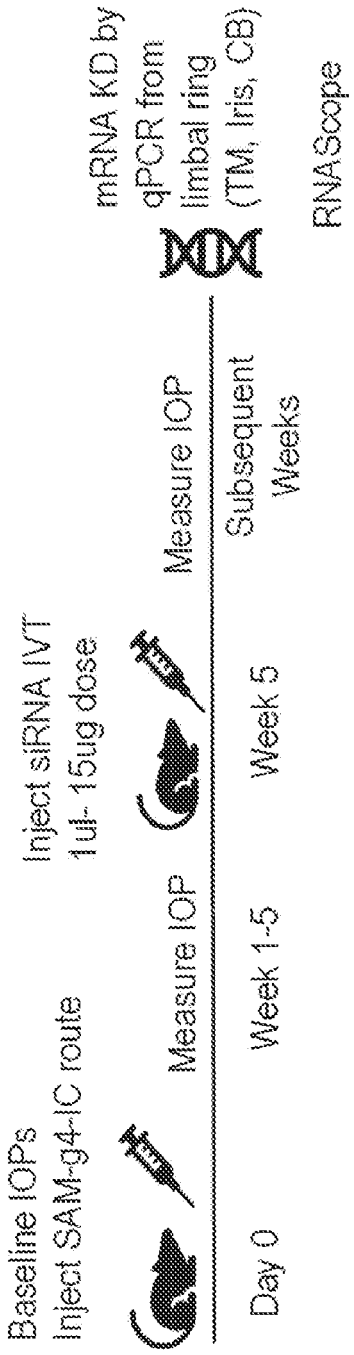

Baseline IOPs
Inject SAM-g4-IC route
Inject siRNA IVT
1ul- 15ug dose
mRNA KD by
qPCR from
limbal ring
(TM, Iris, CB)
RNAScope
Measure IOP
Measure IOP
Day 0
Week 1-5
Week 5
Subsequent
Weeks

FIGURE 1A

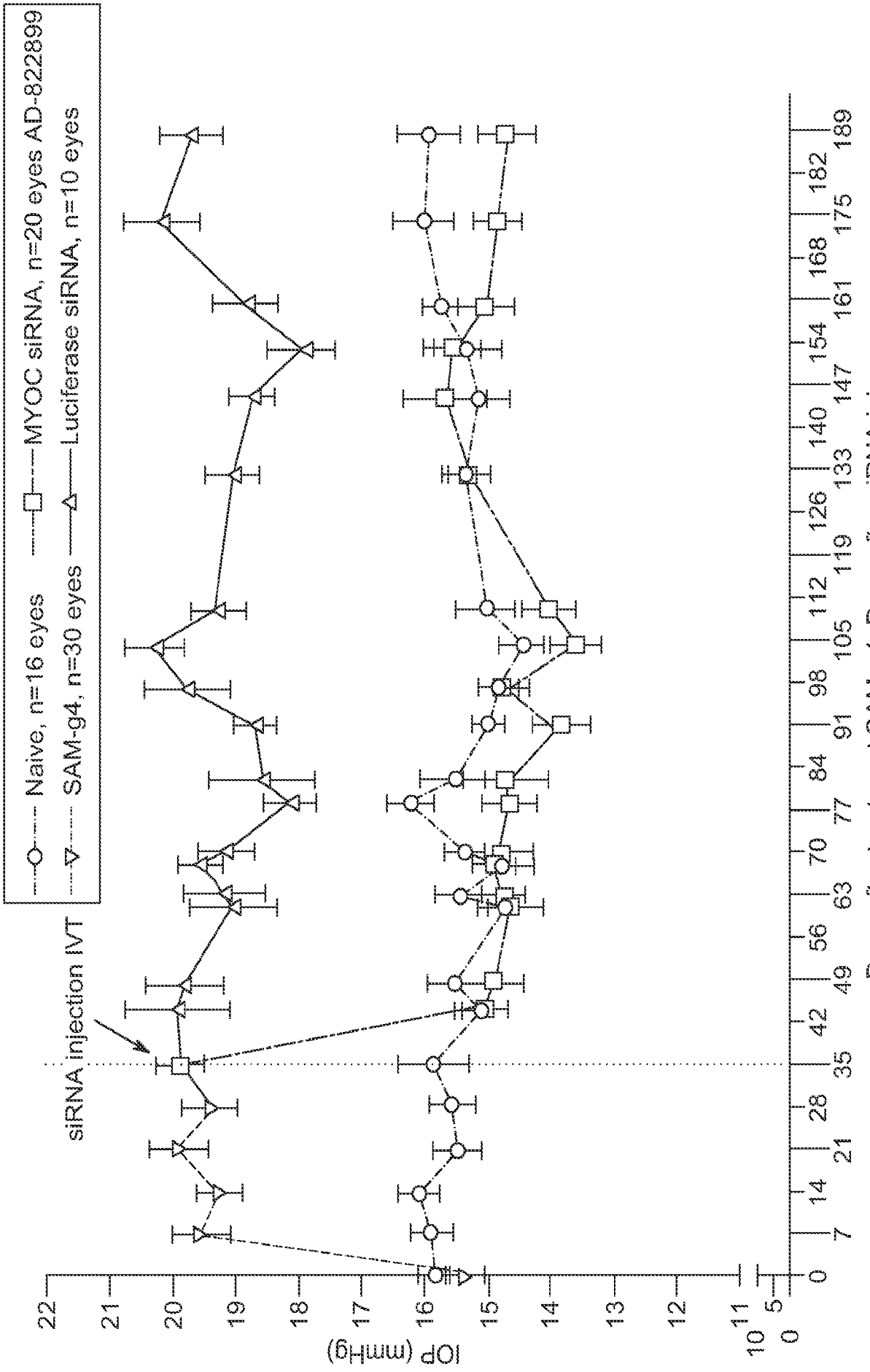

Naive, n=16 eyes
MYOC siRNA, n=20 eyes AD-822899
SAM-g4, n=30 eyes
Luciferase siRNA, n=10 eyes
siRNA injection IVT
IOP (mmHg)
Days after treatment SAM-g4; Days after siRNA inj

FIGURE. 1B

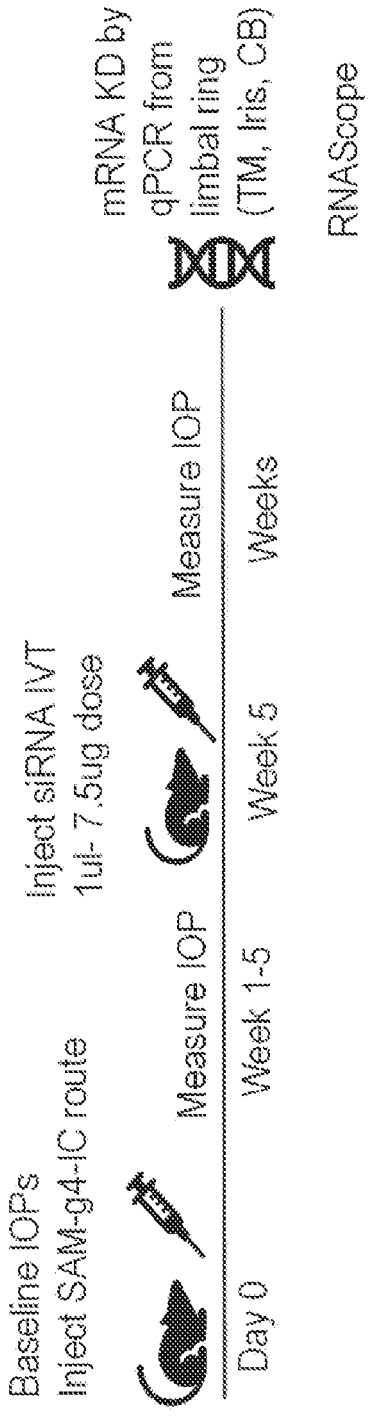

Baseline IOPs
Inject SAM-g4-IC route
Measure IOP
Week 1-5
Inject siRNA IVT
1ul- 7.5ug dose
Week 5
Measure IOP
Weeks
mRNA KD by
qPCR from
limbal ring
(TM, Iris, CB)
RNAScope
Day 0

FIGURE 2A

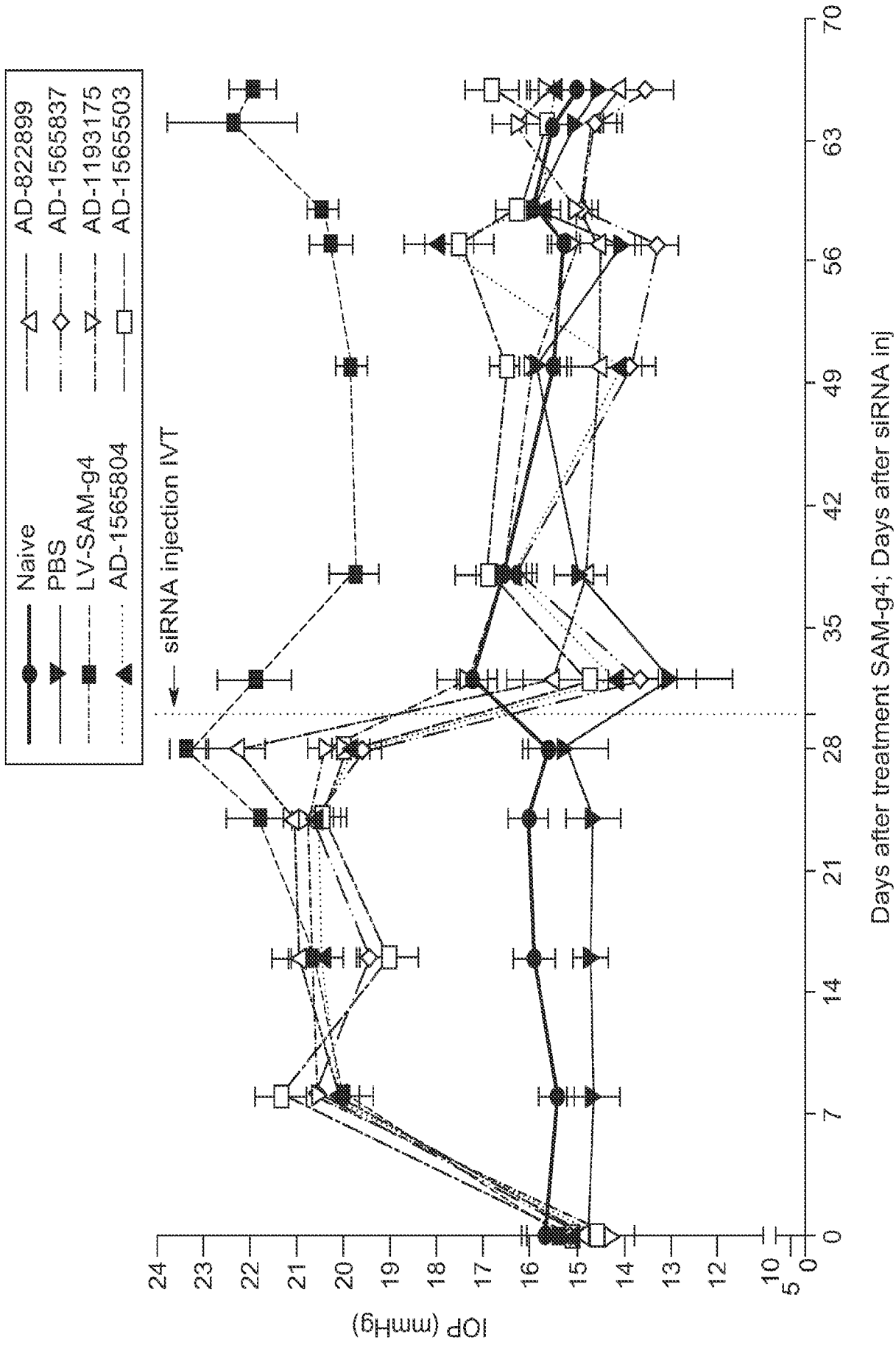

Naive
PBS
LV-SAM-g4
AD-1565804
AD-822899
AD-1565837
AD-1193175
AD-1565503
siRNA injection IVT
IOP (mmHg)
Days after treatment SAM-g4; Days after siRNA inj

FIGURE. 2B

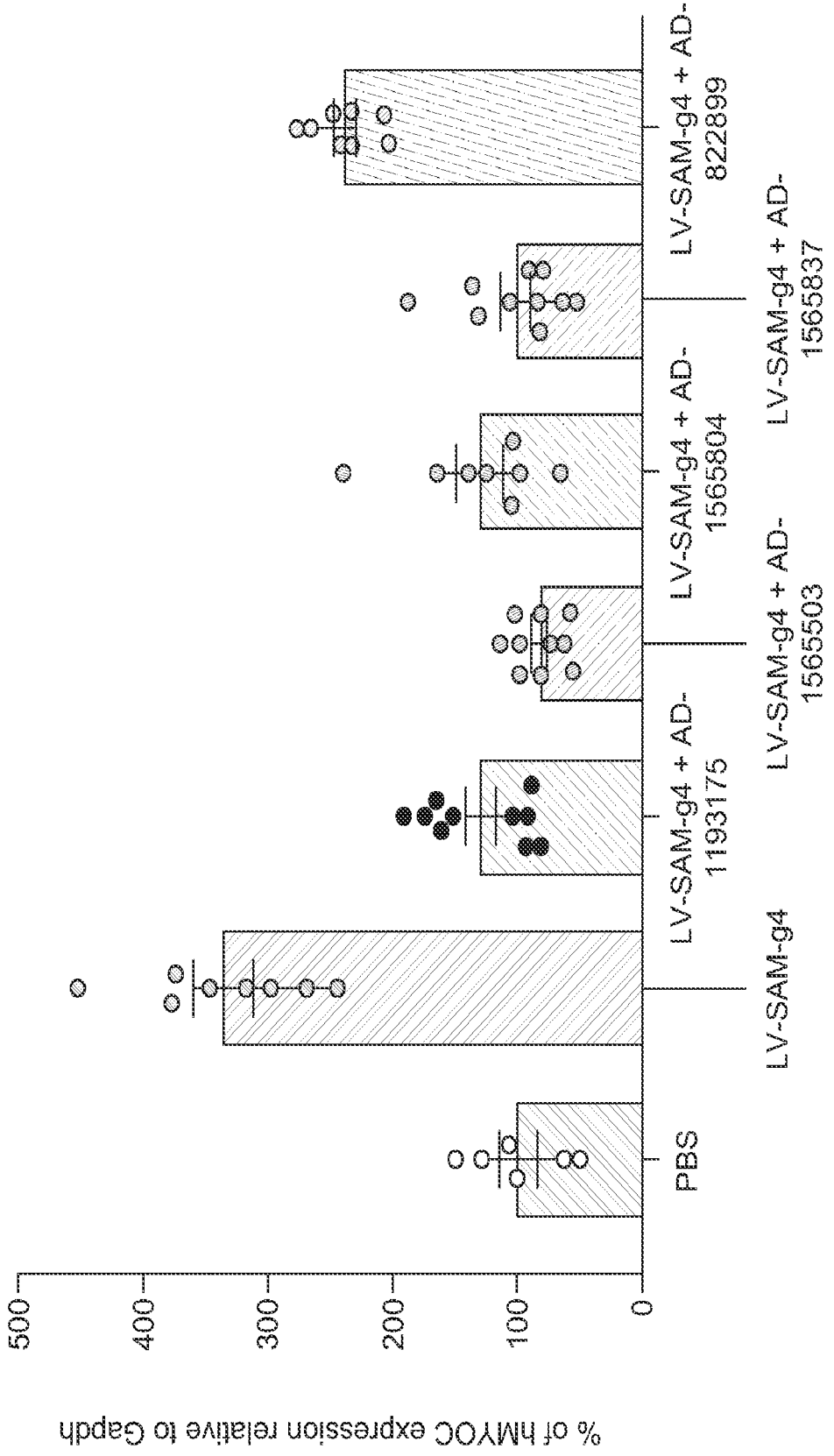

% of hMYOC expression relative to Gapdh
500
400
300
200
100
0
PBS
LV-SAM-g4
LV-SAM-g4 + AD-1193175
LV-SAM-g4 + AD-1565503
LV-SAM-g4 + AD-1565804
LV-SAM-g4 + AD-1565837
LV-SAM-g4 + AD-822899

FIGURE. 2C

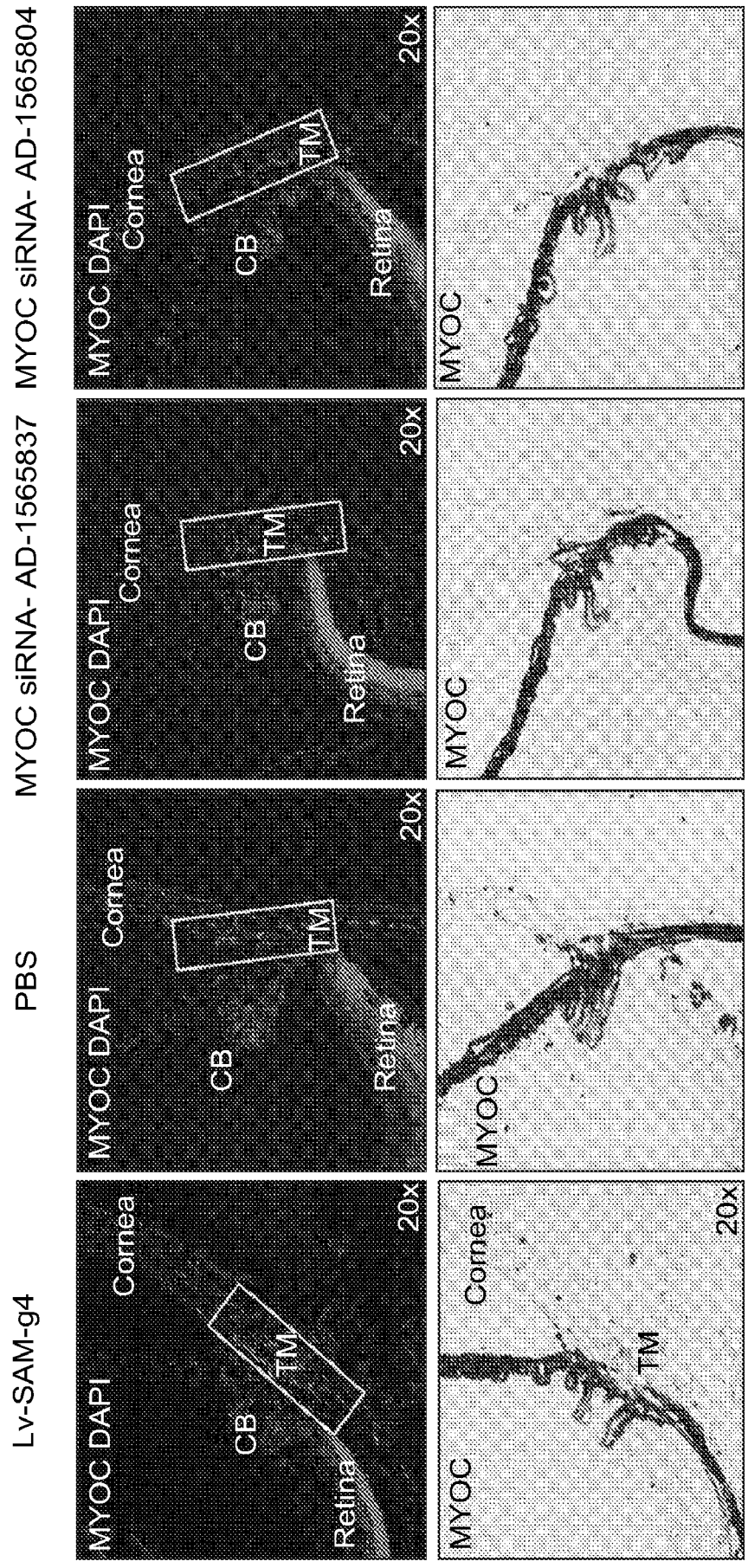

Lv-SAM-g4
PBS
MYOC siRNA- AD-1565837
MYOC siRNA- AD-1565804
MYOC DAPI
Cornea
CB
TM
Retina
20x
MYOC

FIGURE. 2D

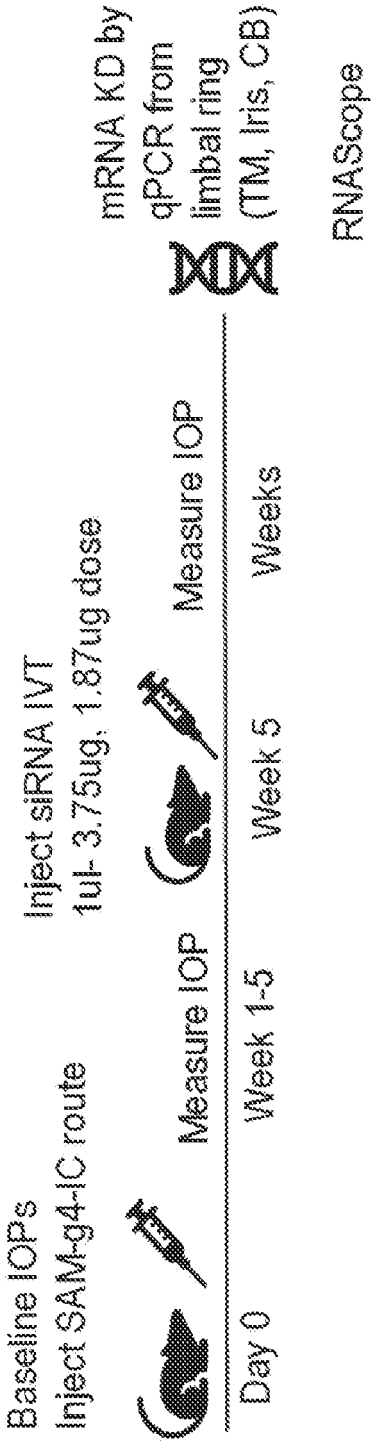

Baseline IOPs
Inject SAM-g4-IC route
Measure IOP
Week 1-5
Inject siRNA IVT
1ul- 3.75ug, 1.87ug dose
Week 5
Measure IOP
Weeks
mRNA KD by
qPCR from
limbal ring
(TM, Iris, CB)
RNAScope
Day 0

FIGURE 3A

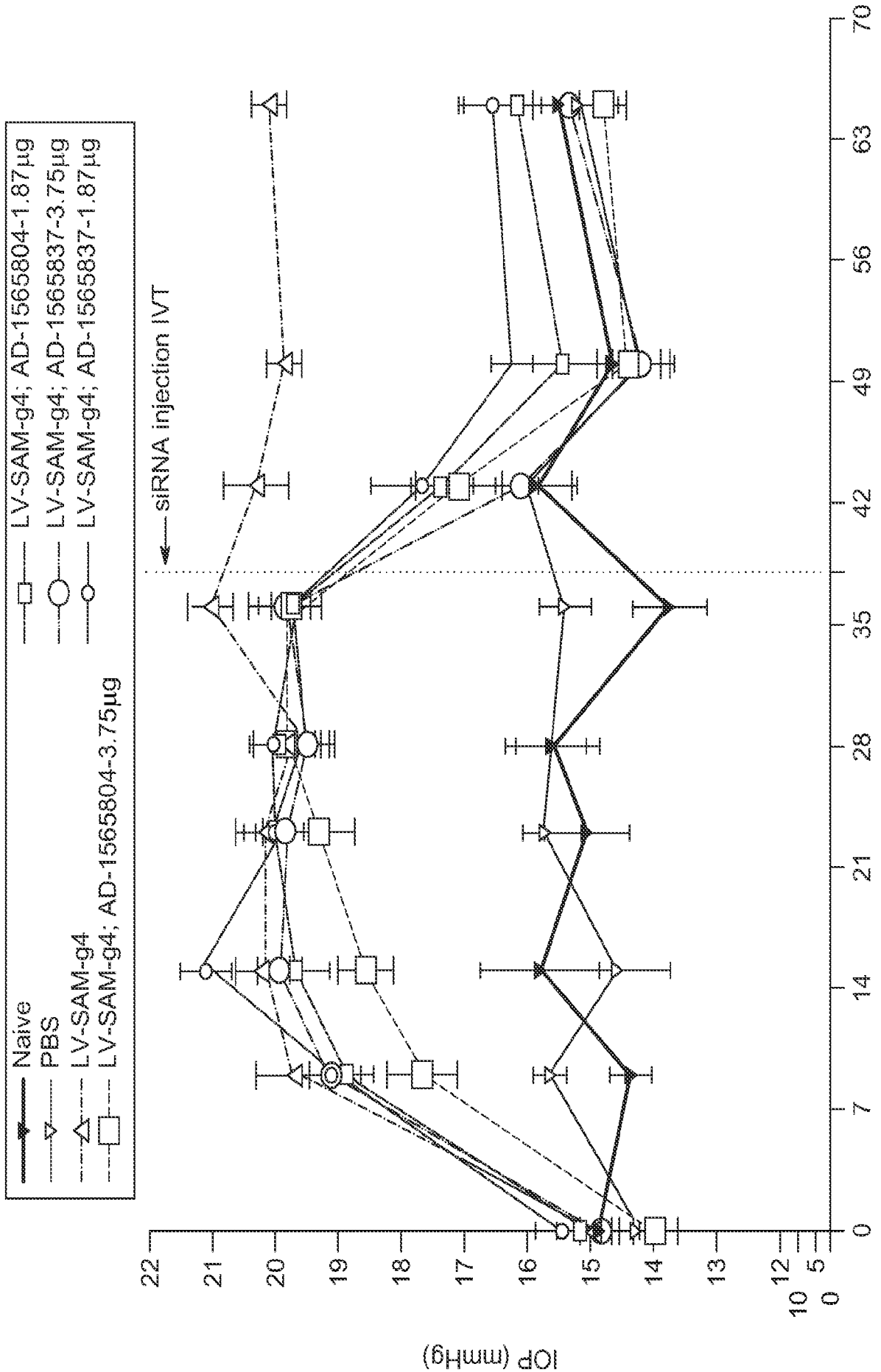

Naive
PBS
LV-SAM-g4
LV-SAM-g4; AD-1565804-3.75µg
LV-SAM-g4; AD-1565804-1.87µg
LV-SAM-g4; AD-1565837-3.75µg
LV-SAM-g4; AD-1565837-1.87µg
siRNA injection IVT
IOP (mmHg)
Days after treatment
0
5
10
12
13
14
15
16
17
18
19
20
21
22
0
7
14
21
28
35
42
49
56
63
70

FIGURE. 3B

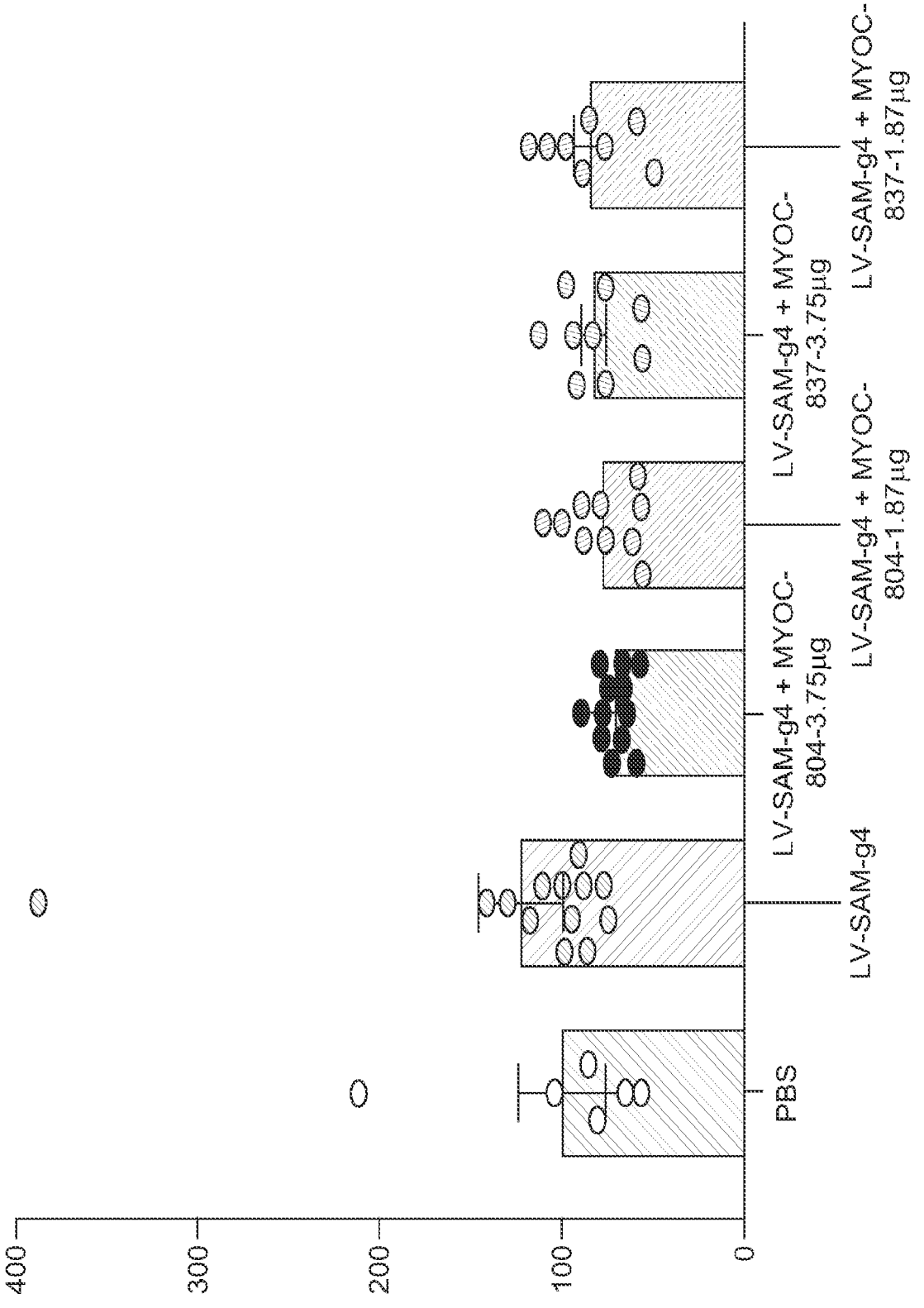

% of hMYOC expression relative to Gapdh
400
300
200
100
0
PBS
LV-SAM-g4
LV-SAM-g4 + MYOC-804-3.75µg
LV-SAM-g4 + MYOC-804-1.87µg
LV-SAM-g4 + MYOC-837-3.75µg
LV-SAM-g4 + MYOC-837-1.87µg

FIGURE. 3C

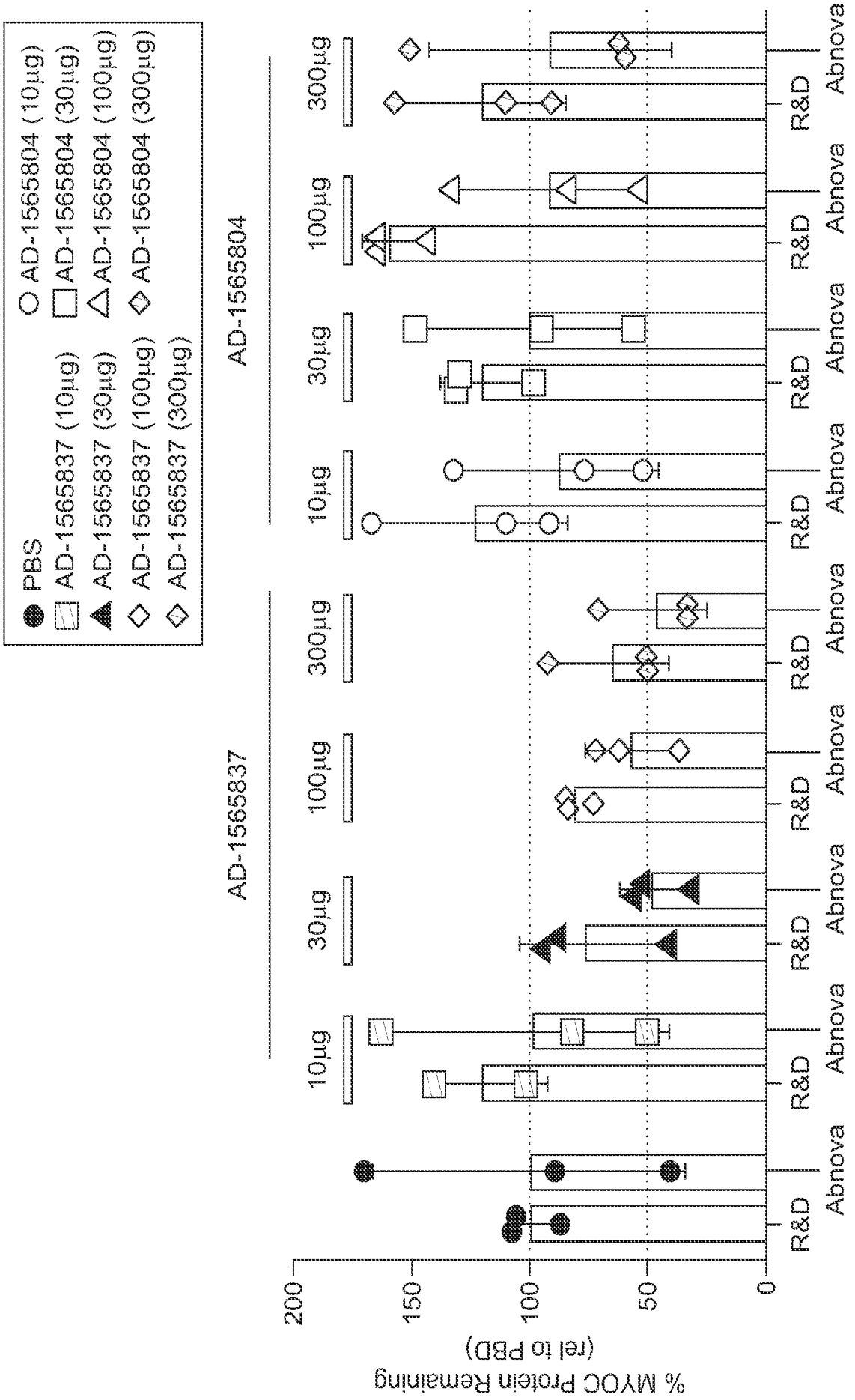

PBS
AD-1565837 (10µg)
AD-1565837 (30µg)
AD-1565837 (100µg)
AD-1565837 (300µg)
AD-1565804 (10µg)
AD-1565804 (30µg)
AD-1565804 (100µg)
AD-1565804 (300µg)
AD-1565837
AD-1565804
10µg
30µg
100µg
300µg
R&D
Abnova
% MYOC Protein Remaining
(rel to PBD)
200
150
100
50
0

FIGURE. 4

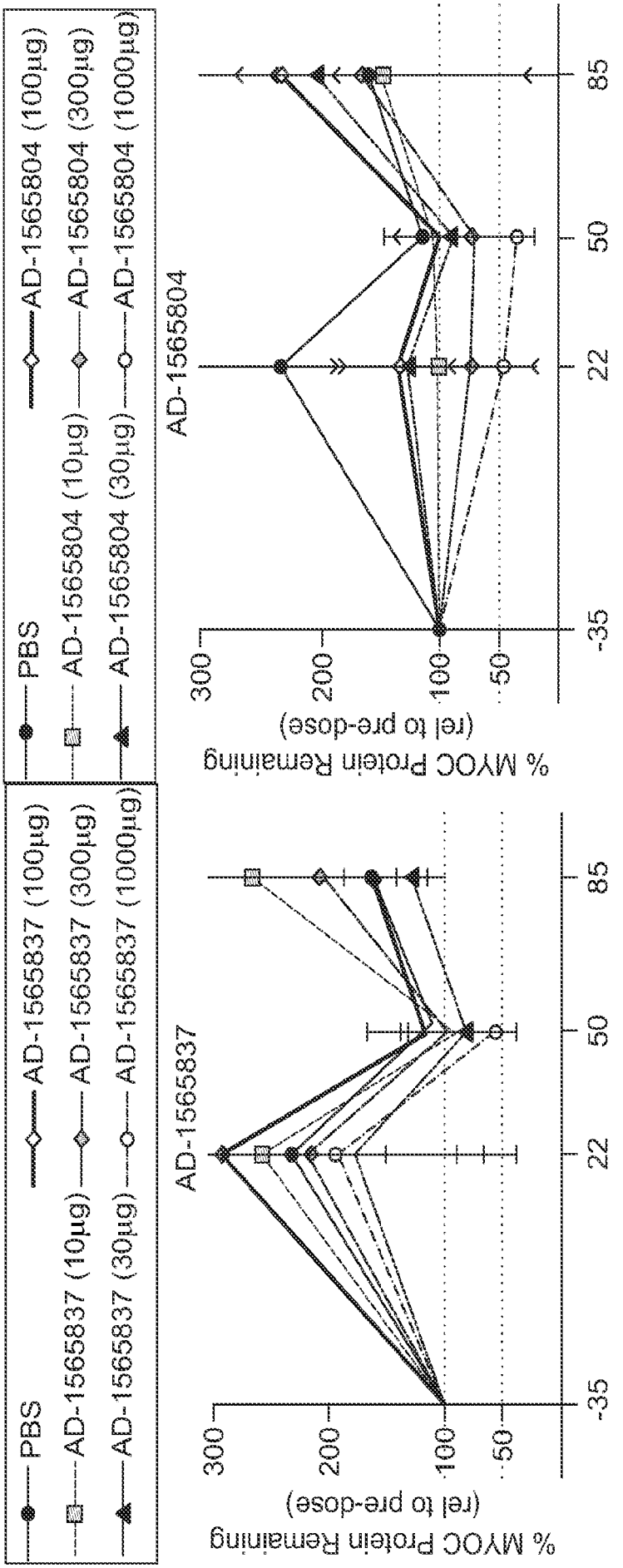

PBS
AD-1565837 (100µg)
AD-1565837 (10µg)
AD-1565837 (300µg)
AD-1565837 (30µg)
AD-1565837 (1000µg)
AD-1565804 (100µg)
AD-1565804 (10µg)
AD-1565804 (300µg)
AD-1565804 (30µg)
AD-1565804 (1000µg)
AD-1565837
AD-1565804
% MYOC Protein Remaining (rel to pre-dose)
300
200
100
50
-35
22
50
85
Timepoint (days)
Abnova RbPAb + Abnova Ms PAb

FIGURE. 5

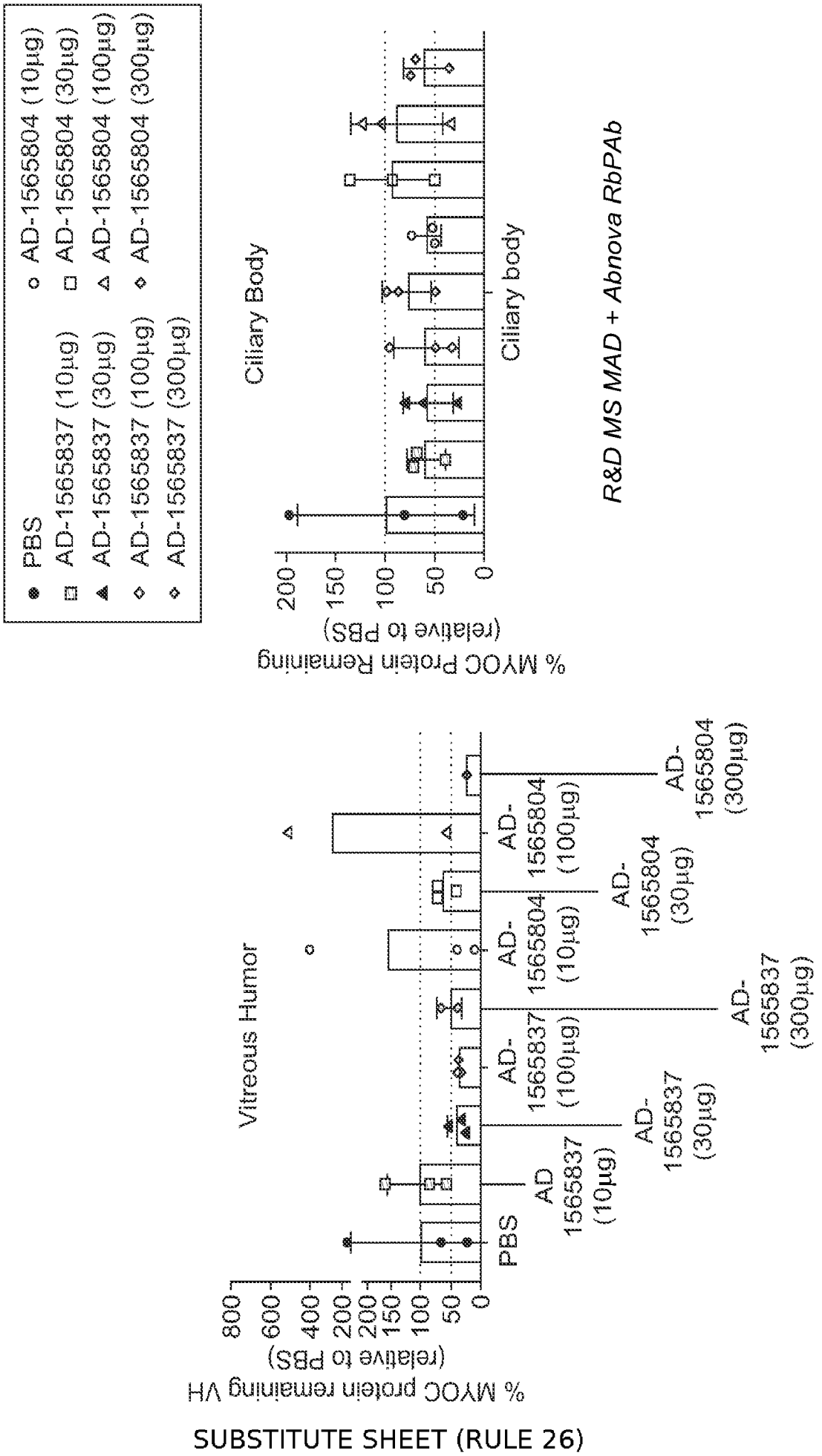

PBS
AD-1565837 (10µg)
AD-1565837 (30µg)
AD-1565837 (100µg)
AD-1565837 (300µg)
AD-1565804 (10µg)
AD-1565804 (30µg)
AD-1565804 (100µg)
AD-1565804 (300µg)
Ciliary Body
% MYOC Protein Remaining (relative to PBS)
Ciliary body
R&D MS MAD + Abnova RbPAb
Vitreous Humor
% MYOC protein remaining VH (relative to PBS)
PBS
AD 1565837 (10µg)
AD-1565837 (30µg)
AD-1565837 (100µg)
AD-1565837 (300µg)
AD-1565804 (10µg)
AD-1565804 (30µg)
AD-1565804 (100µg)
AD-1565804 (300µg)

FIGURE. 6A

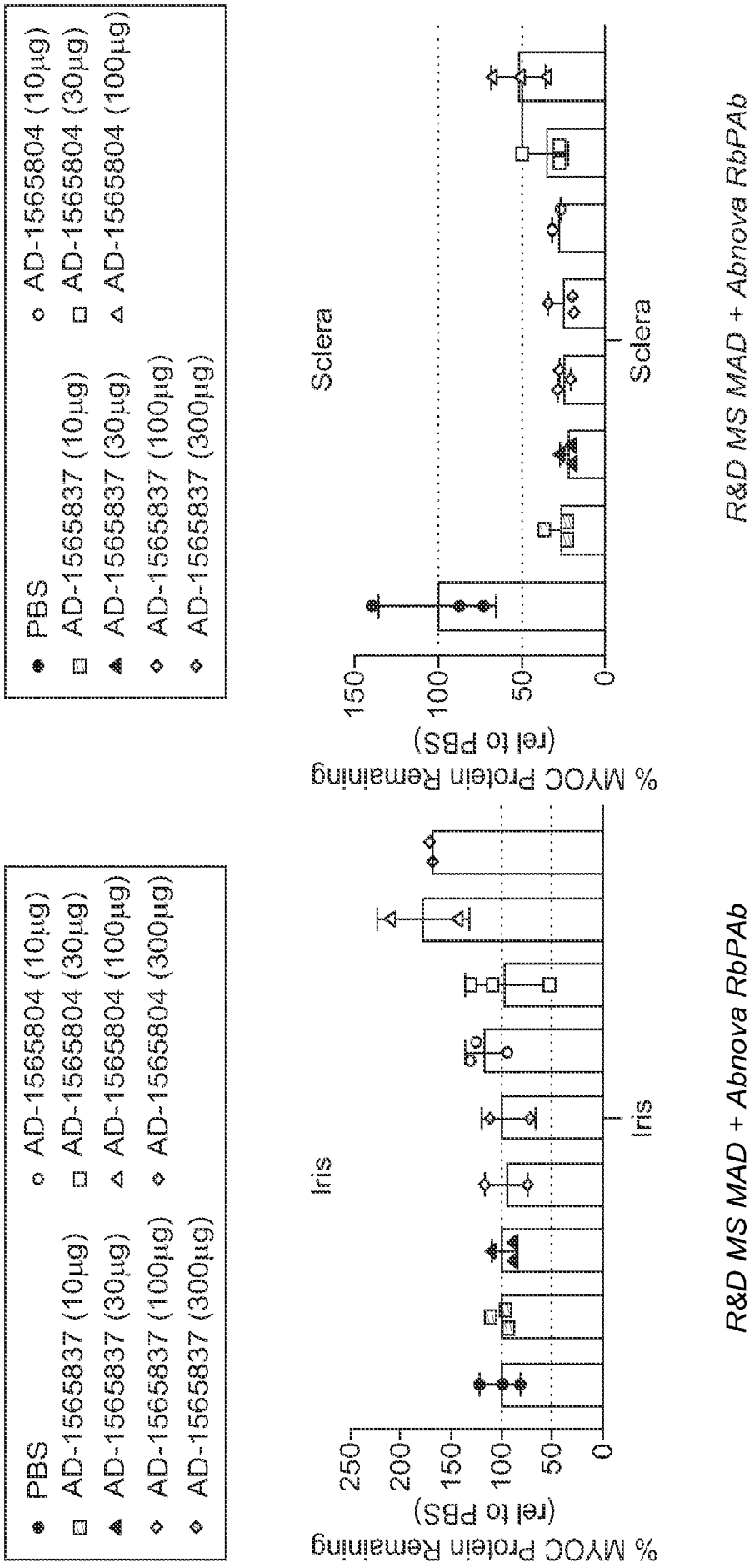

PBS
AD-1565837 (10µg)
AD-1565837 (30µg)
AD-1565837 (100µg)
AD-1565837 (300µg)
AD-1565804 (10µg)
AD-1565804 (30µg)
AD-1565804 (100µg)
AD-1565804 (300µg)
Iris
Sclera
% MYOC Protein Remaining (rel to PBS)
0
50
100
150
200
250
R&D MS MAD + Abnova RbPAb

FIGURE. 6B

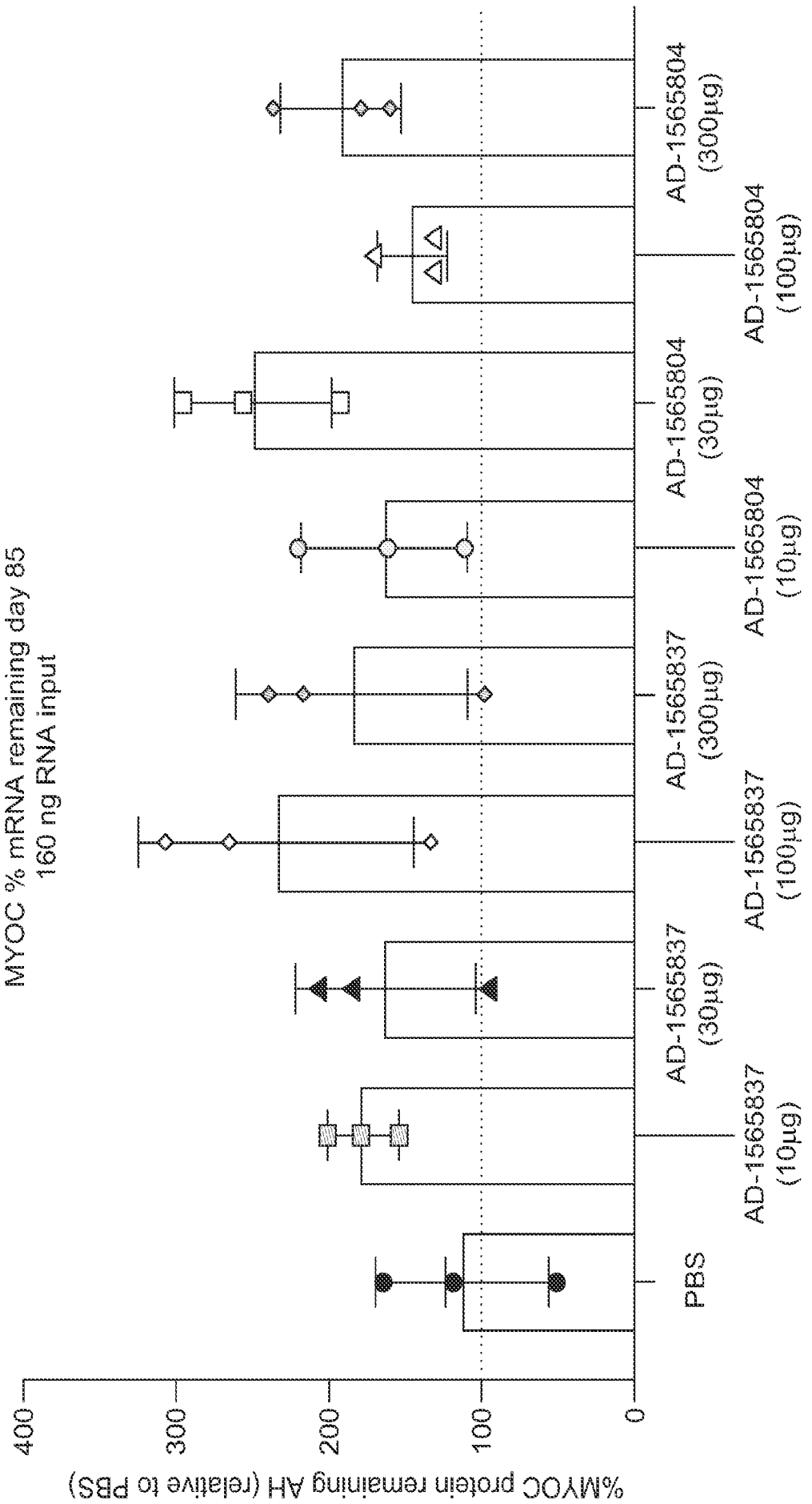

MYOC % mRNA remaining day 85
160 ng RNA input
%MYOC protein remaining AH (relative to PBS)
400
300
200
100
0
PBS
AD-1565837 (10µg)
AD-1565837 (30µg)
AD-1565837 (100µg)
AD-1565837 (300µg)
AD-1565804 (10µg)
AD-1565804 (30µg)
AD-1565804 (100µg)
AD-1565804 (300µg)

FIGURE. 7