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(54) Title: PATATIN-LIKE PHOSPHOLIPASE DOMAIN CONTAINING 3 (PNPLA3) IRNA COMPOSITIONS AND METHODS OF USE THEREOF

(57) Abstract: The present invention relates to RNAi agents, e.g., double stranded RNA (dsRNA) agents, targeting the Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene. The invention also relates to methods of using such RNAi agents to inhibit expression of a PNPLA3 gene and to methods of preventing and treating an PNPLA3-associated disorder, e.g., Nonalcoholic Fatty Liver Disease (NAFLD).

WO 2022/256395 A1

PATATIN-LIKE PHOSPHOLIPASE DOMAIN CONTAINING 3 (PNPLA3) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

Related Applications

This application claims the benefit of priority to U.S. Provisional Application No. 63/195,769, filed on June 2, 2021; and U.S. Provisional Application No. 63/232,797, filed on August 13, 2021. The entire contents of each of the foregoing applications are 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 May 31, 2022, is named 121301-14820_SL.txt and is 457,724 bytes in size.

Background of the Invention

The accumulation of excess triglyceride in the liver is known as hepatic steatosis (or fatty liver), and is associated with adverse metabolic consequences, including insulin resistance and dyslipidemia. Fatty liver is frequently found in subjects having excessive alcohol intake and subjects having obesity, diabetes, or hyperlipidemia. However, in the absence of excessive alcohol intake (> 10 g/day), nonalcoholic fatty liver disease (NAFLD) can develop. NAFLD refers to a wide spectrum of liver diseases that can progress from simple fatty liver (steatosis), to nonalcoholic steatohepatitis (NASH), to cirrhosis (irreversible, advanced scarring of the liver). All of the stages of NAFLD have in common the accumulation of fat (fatty infiltration) in the liver cells (hepatocytes).

The NAFLD spectrum begins with and progress from its simplest stage, called simple fatty liver (steatosis). Simple fatty liver involves the accumulation of fat (triglyceride) in the liver cells with no inflammation (hepatitis) or scarring (fibrosis). The next stage and degree of severity in the NAFLD spectrum is NASH, which involves the accumulation of fat in the liver cells, as well as inflammation of the liver. The inflammatory cells destroy liver cells (hepatocellular necrosis), and NASH ultimately leads to scarring of the liver (fibrosis), followed by irreversible, advanced scarring (cirrhosis). Cirrhosis that is caused by NASH is the last and most severe stage in the NAFLD spectrum.

In 2008, a genomewide association study of individuals with proton magnetic resonance spectroscopy of the liver to evaluate hepatic fat content, a significant association was identified between hepatic fat content and the Patatin-like Phospholipase Domain Containing 3 (PNPLA3) gene (see, for example, Romeo *et al.* (2008) *Nat. Genet.*, 40(12):1461-1465). Studies with knock-in mice have demonstrated that expression of a sequence polymorphism (rs738409, I148M) in PNPLA3 causes NAFLD, and that the accumulation of catalytically inactive PNPLA3 on the surfaces of lipid droplets is associated with the accumulation of triglycerides in the liver (Smagris *et al.* (2015) *Hepatology*, 61:108-118). Specifically, the PNPLA3 I148M variant was associated with promoting

the development of fibrogenesis by activating the hedgehog (Hh) signaling pathway, leading to the activation and proflieration of hepatic stellate cells and excessive generation and deposition of extracellular matrix (Chen *et al*. (2015) *World J. Gastroenterol*., 21(3):794-802).

Currently, treatments for NAFLD are directed towards weight loss and treatment of any secondary conditions, such as insulin resistance or dyslipidemia. To date, no pharmacologic treatments for NAFLD have been approved. Therefore, there is a need for therapies for subjects suffering from NAFLD.

Summary of the Invention

The present invention provides iRNA compositions which affect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding Patatin-Like Phospholipase Domain Containing 3 (PNPLA3). The Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) may be within a cell, *e.g.,* a cell within a subject, such as a human subject.

In an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:2. In one embodiment, the dsRNA agent comprises at least one thermally destabilizing nucleotide modification, *e.g.,* an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), or a glycerol nucleic acid (GNA). In some embodiments, the antisense strand comprises the at least one thermally destabilizing nucleotide modification.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding Patatin-Like Phospholipase Domain Containing 3 (PNPLA3), and wherein the region of complementarity comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2, 3, 6, and 7.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide

sequence of nucleotides 187-209; 214-238; 219-245; 283-305; 351-379; 361-391; 395-419; 416-439; 472-494; 483-506; 570-598; 618-649; 631-654; 636-659; 640-662; 643-677; 676-710; 740-772; 782-805; 803-825; 810-842; 864-905; 905-927; 910-934; 919-942; 953-983; 1062-1087; 1069-1097; 1078-1108; 1094-112; 1164-1187; 1170-1199; 1180-1212; 1196-1224; 1234-1262; 1259-1297; 1278-1318; 1326-1351; 1382-1411; 1518-1545; 1543-1568; 1549-1574; 1575-1597; 1621-1643; 1644-1692; 1676-1700; 1712-1734; 1719-1745; 1733-1778; 1733-1760; 1739-1770; 1749-1778; 1829-1856; 1865-1890; 1900-1925; 2076-2098; 2121-2148; 2175-2208; or 2243-2265 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 687-709, 1182-1204, 1201-1223, 1738-1760, or 2186-2208 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In some embodiments, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1526902.2, AD-1526891.3, AD-1526820.3, AD-1526960.2, and AD-1526996.2.

In some embodiments, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1526902.2, AD-1526891.3, AD-1526820.3, and AD-1526960.2.

In some embodiments, the sense and the antisense strand comprise at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the sense and the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1526902, AD-1526891, AD-1526820, and AD-1526960.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3), wherein said dsRNA agent comprises a sense strand comprising or consisting of asascuugCfuAfCfCfcauuaggauuL96 (SEQ ID NO:723) and an antisense strand comprising or consisting of asAfsuccUfaaugggguAfgCfaaguusgsc (SEQ ID NO:1012), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Cf, and Uf are 2'-fluoro A, C and U; s is a phosphorothioate linkage; and L96 is a GalNAc3 ligand.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3), wherein said dsRNA agent comprises a sense strand comprising or consisting of

asusacauGfaGfCfAfagauuugcauL96 (SEQ ID NO:713) and an antisense strand comprising or consisting of asUfsgcaAfaucuugcUfcAfuguauscsc (SEQ ID NO:1001), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf, and Uf are 2'-fluoro A, G, C and U; s is a phosphorothioate linkage; and L96 is a GalNAc3 ligand.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3), wherein said dsRNA agent comprises a sense strand comprising or consisting of csgsuaccCfuUfCfAfuugaugccauL96 (SEQ ID NO:647) and an antisense strand comprising or consisting of asUfsggdCa(Tgn)caaugaAfgGfguacgsusu (SEQ ID NO:930), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf, and Uf are 2'-fluoro A, G, C and U; Tgn is thymidine-glycol nucleic acid (GNA) S-isomer; s is a phosphorothioate linkage; and L96 is a GalNAc3 ligand.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3), wherein said dsRNA agent comprises a sense strand comprising or consisting of uscsugagCfuGfAfGfuugguuuuauL96 (SEQ ID NO:774) and an antisense strand comprising or consisting of asUfsaaaAfccaacucAfgCfucagasgsg (SEQ ID NO:1070), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf, and Uf are 2'-fluoro A, G, C and U; s is a phosphorothioate linkage; and L96 is a GalNAc3 ligand.

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

In one embodiment, substantially all of the nucleotides of the sense strand; substantially all of the nucleotides of the antisense strand are modified nucleotides; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides.

In one embodiment, all of the nucleotides of the sense strand are modified nucleotides; all of the nucleotides of the antisense strand are modified nucleotides; or all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3’-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide (LNA), 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, 2’-hydroxly-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 thermally destabilizing

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

In one embodiment, the modified nucleotides are selected from the group consisting of LNA modified nucleotides, 1,5-anhydrohexitol (HNA) modified nucleotides, cyclohexenyl (CeNA) modified nucleotides, 2′-methoxyethyl modified nucleotides, 2′-O-alkyl modified nucleotides, 2′-O-allyl modified nucleotides, 2′-C- allyl modified nucleotides, 2′-fluoro modified nucleotides, 2′-deoxy modified nucleotides, 2'-hydroxyl modified nucleotides, 2'-O-methyl modified nucleotides, 2'-halo modified nucleotides, and glycol modified nucleotides; and combinations thereof.

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 comprising a 2' phosphate, *e.g.*, G2p, C2p, A2p, U2p, and a vinyl-phosphonate nucleotide; and combinations thereof.

In another embodiment, at least one of the modified nucleotides is a thermally destabilizing nucleotide modification.

In one embodiment, the thermally destabilizing nucleotide modification is selected from the group consisting of an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), and a glycerol nucleic acid (GNA).

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

In some embodiments, the modifications on the nucleotides are 2'-O-methyl, GNA and 2'fluoro modifications.

In some embodiments, the dsRNA agent further comprises at least one phosphorothioate internucleotide linkage. In some embodiments, the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages. In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand. In a related embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand. In another embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand.

The double stranded region may be 19-30 nucleotide pairs in length;19-25 nucleotide pairs in length;19-23 nucleotide pairs in length; 23-27 nucleotide pairs in length; or 21-23 nucleotide pairs in length; 19, 20, 21 nucleotides in length. The double stranded region may have 0, 1, 2, or 3 mismatches.

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

In one embodiment, the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

The region of complementarity may be at least 17 nucleotides in length; between 19 and 23 nucleotides in length; or 19 nucleotides in length.

In one embodiment, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides.

In one embodiment, the dsRNA agent further comprises a ligand.

In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

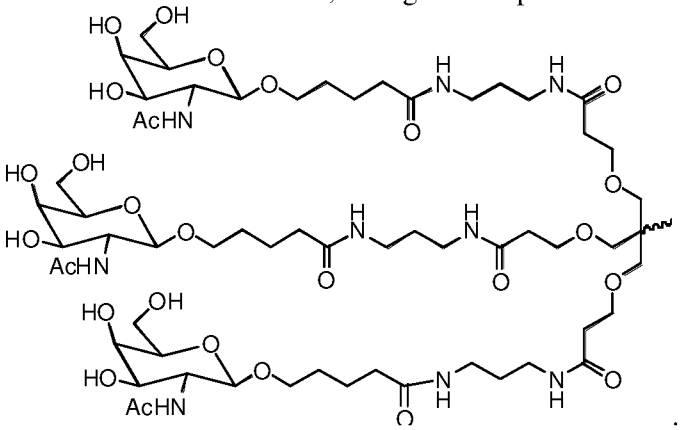
In one embodiment, the ligand cooomprises an N-acetylgalactosamine (GalNAc) derivative.

In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

In one embodiment, the ligand comprises one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one embodiment, the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one embodiment, the ligand comprises



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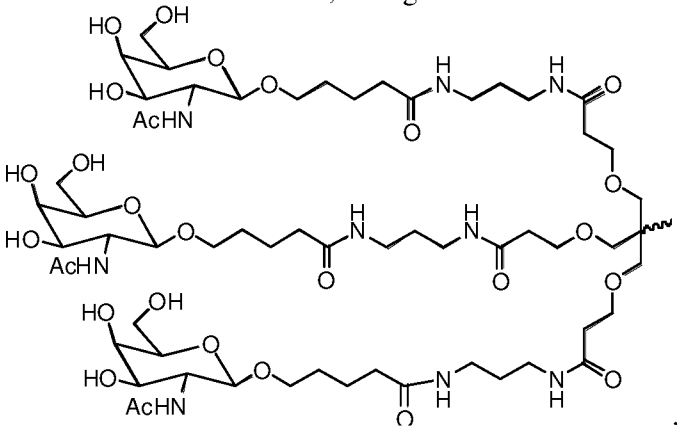
In one embodiment, the ligand is



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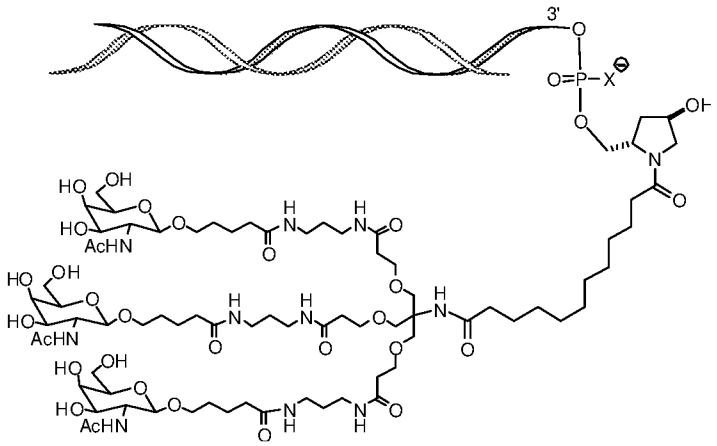
In one embodiment, the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

In one embodiment, the X is O.

In one embodiment, the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand, *e.g.,* the antisense strand or the sense strand.

In another embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand, *e.g.,* the antisense strand or the sense strand.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. In one embodiment, the strand is the antisense strand.

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

The present invention also provides cells containing any of the dsRNA agents of the invention and pharmaceutical compositions comprising any of the dsRNA agents of the invention.

The pharmaceutical composition of the invention may include dsRNA agent in an unbuffered solution, *e.g.,* saline or water, or the pharmaceutical composition of the invention may include the dsRNA agent is in a buffer solution, *e.g.,* a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof; or phosphate buffered saline (PBS).

In one aspect, the present invention provides a method of inhibiting expression of a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene in a cell. The method includes contacting the cell with any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby inhibiting expression of the PNPLA3 gene in the cell.

In one embodiment, the cell is within a subject, *e.g.,* a human subject, *e.g.,* a subject having a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder, such as a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD).

In one embodiment, contacting the cell with the dsRNA agent inhibits the expression of PNPLA3 by at least 50%, 60%, 70%, 80%, 90%, or 95%.

In one embodiment, inhibiting expression of PNPLA3 decreases PNPLA3 protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

In one aspect, the present invention provides a method of treating a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression. The method includes administering to the subject a therapeutically effective amount of any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby treating the subject having the disorder that would benefit from reduction in PNPLA3 expression.

In another aspect, the present invention provides a method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression. The method includes administering to the subject a prophylactically effective amount of any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in PNPLA3 expression.

In one embodiment, the disorder is a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder, *e.g.,* a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder is selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD).

In one embodiment, the PNPLA3-associated disorder is NAFLD.

In one embodiment, the subject is human.

In one embodiment, the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

In one embodiment, the dsRNA agent is administered to the subject subcutaneously.

In one embodiment, the methods of the invention include further determining the level of PNPLA3 in a sample(s) from the subject.

In one embodiment, the level of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in the subject sample(s) is a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) protein level in a blood or serum sample(s).

In certain embodiments, the methods of the invention further comprise administering to the subject an additional therapeutic agent. In a further embodiment, the additional therapeutic agent is selected from the group consisting of an HMG-CoA reductase inhibitor, a fibrate, a bile acid sequestrant, niacin, an antiplatelet agent, an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist, an acylCoA cholesterol acetyltransferase (ACAT) inhibitor, a cholesterol absorption inhibitor, a cholesterol ester transfer protein (CETP) inhibitor, a microsomal triglyceride transfer protein (MTTP) inhibitor, a cholesterol modulator, a bile acid modulator, a peroxisome proliferation activated receptor (PPAR) agonist, a gene-based therapy, a composite vascular

protectant, a glycoprotein IIb/IIIa inhibitor, aspirin or an aspirin- like compound, an IBAT inhibitor, a squalene synthase inhibitor, a monocyte chemoattractant protein (MCP)-I inhibitor, or fish oil.

The present invention also provides kits comprising any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, and optionally, instructions for use.

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

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic depicting the overall study design for the *in vivo* screening of the dsRNA agents targeting human PNPLA3.

Figure 2 is a graph showing human PNPLA3 mRNA levels in mice (n = 3 per group) subcutaneously administered a single 10 mg/kg dose of the indicated dsRNA duplexes, on day 14 post-dose. Human PNPLA3 mRNA levels are shown relative to control levels detected with PBS treatment.

Detailed Description of the Invention

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene. The gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The use of these iRNAs enables the targeted degradation of mRNAs of the corresponding gene (Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene) in mammals.

The iRNAs of the invention have been designed to target the human Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene, including portions of the gene that are conserved in the Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) orthologs of other mammalian species. Without intending to be limited by theory, it is believed that a combination or sub-combination of the foregoing properties and the specific target sites or the specific modifications in these iRNAs confer to the iRNAs of the invention improved efficacy, stability, potency, durability, and safety.

Accordingly, the present invention provides methods for treating and preventing an Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder, *e.g.*, fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD), using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a PNPLA3 gene.

The iRNAs of the invention include an RNA strand (the antisense strand) having a region which is up to about 30 nucleotides or less in length, *e.g.*, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of an PNPLA3 gene.

In certain embodiments, one or both of the strands of the double stranded RNAi agents of the invention is up to 66 nucleotides in length, *e.g.*, 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length, with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of an PNPLA3 gene. In some embodiments, such iRNA agents having longer length antisense strands preferably may include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

The use of iRNAs of the invention enables the targeted degradation of mRNAs of the corresponding gene (PNPLA3 gene) in mammals. Using *in vitro* assays, the present inventors have demonstrated that iRNAs targeting a PNPLA3 gene can potently mediate RNAi, resulting in significant inhibition of expression of a PNPLA3 gene. Thus, methods and compositions including these iRNAs are useful for treating a subject having a PNPLA3-associated disorder, *e.g.*, fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD).

Accordingly, the present invention provides methods and combination therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of an PNPLA3 gene, *e.g.*, a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disease, such as fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD), using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an PNPLA3 gene.

The present invention also provides methods for preventing at least one symptom in a subject having a disorder that would benefit from inhibiting or reducing the expression of a PNPLA3 gene, *e.g.*, fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD). For example, in a subject having NAFLLD, the methods of the present invention may reduce at least one symptom in the subject, *e.g.*, fatigue, weakness, weight loss, loss of apetite, nausea, abdominal pain, spider-like blood vessels, yellowing of the skin and eyes (jaundice), itching, fluid build up and swelling of the legs (edema), abdomen swelling (ascites), and mental confusion.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a PNPLA3 gene as well as compositions, uses, and methods for treating subjects that would benefit from inhibition and/or reduction of the expression of a PNPLA3 gene, *e.g.*, subjects susceptible to or diagnosed with a PNPLA3-associated disorder.

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are

recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

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

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to".

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise. For example, “sense strand or antisense strand” is understood as “sense strand or antisense strand or sense strand and antisense strand.”

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

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

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

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

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

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

As used herein, “Patatin-Like Phospholipase Domain Containing 3,” used interchangeably with the term “PNPLA3,” refers to the well-known gene that encodes a triacylglycerol lipase that mediates triacyl glycerol hydrolysis in adipocytes.

Exemplary nucleotide and amino acid sequences of PNPLA3 can be found, for example, at GenBank Accession No. NM_025225.2 (Homo sapiens PNPLA3; SEQ ID NO:1; reverse

complement, SEQ ID NO:2); GenBank Accession No. NM_054088.3 (Mus musculus PNPLA3; SEQ ID NO:3; reverse complement, SEQ ID NO:4); GenBank Accession No. NM_001282324.1 (Rattus norvegicus PNPLA3; SEQ ID NO:5; reverse complement, SEQ ID NO:6); GenBank Accession No. XM_005567051.1 (Macaca fascicularis PNPLA3, SEQ ID NO:7; reverse complement, SEQ ID NO:8); GenBank Accession No. XM_001109144.2 (Macaca mulatta PNPLA3, SEQ ID NO:9; reverse complement, SEQ ID NO:10); and GenBank Accession No. XM_005567052.1 (Macaca fascicularis PNPLA3, SEQ ID NO:11; reverse complement, SEQ ID NO:12).

Additional examples of PNPLA3 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, and the *Macaca* genome project web site.

Further information on PNPLA3 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=pnpla3.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term PNPLA3, as used herein, also refers to variations of the PNPLA3 gene including variants provided in the SNP database. Numerous seuqnce variations within the PNPLA3 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=pnpla3, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a PNPLA3 gene, including mRNA that is a product of RNA processing of a primary transcription product. The target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a PNPLA3 gene. In one embodiment, the target sequence is within the protein coding region of PNPLA3. It is understood that if the nucleotide sequence of a target sequence is provided as, *e.g.,* a cDNA sequence or the reverse complement of a cDNA sequence, *e.g.,* SEQ ID NOs:1-12, the "Ts" are "Us" in the corresponding mRNA sequence.

The target sequence may be from about 19-36 nucleotides in length, *e.g.*, preferably about 19-30 nucleotides in length. For example, the target sequence can be about 19-30 nucleotides, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. In certain embodiments, the target sequence is 19-23 nucleotides in length, optionally 21-23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

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.

"G," "C," "A," "T," and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that

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

The terms "iRNA", "RNAi agent," "iRNA agent,", "RNA interference agent" as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The iRNA modulates, *e.g.,* inhibits, the expression of a PNPLA3 gene in a cell, *e.g.,* a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNA that interacts with a target RNA sequence, *e.g.,* a PNPLA3 target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev*. 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev*. 15:188). Thus, in one aspect the invention relates to a single stranded RNA (siRNA) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.,* a PNPLA3 gene. Accordingly, the term "siRNA" is also used herein to refer to an iRNA as described above.

In certain embodiments, the RNAi agent may be a single-stranded siRNA (ssRNAi) that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents bind to the RISC endonuclease, Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are generally 15-30 nucleotides and are chemically modified. The design and testing of single-stranded 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 may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima *et al.*, (2012) *Cell* 150:883-894.

In certain embodiments, an "iRNA" for use in the compositions, uses, and methods of the invention is a double stranded RNA and is referred to herein as a "double stranded RNA agent," "double stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA", refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, *i.e.,* a PNPLA3 gene. In some embodiments of the invention, a double stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.,* an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

In general, the majority of nucleotides of each strand of a dsRNA molecule are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide or a modified nucleotide. In addition, as used in this specification, an "iRNA" may include ribonucleotides with chemical modifications; an iRNA may include substantial modifications at multiple nucleotides. As used herein, the term "modified nucleotide" refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, or modified nucleobase, or any combination thereof. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.,* a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the invention include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by "iRNA" or "RNAi agent" for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide if present within an RNAi agent can be considered to constitute a modified nucleotide.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 19 to 36 base pairs in length, *e.g.*, about 19-30 base pairs in length, for example, about 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, such as about 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. In certain embodiments, the duplex region is 19-21 base pairs in length, *e.g.*, 21 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 23 or more unpaired nucleotides. In some embodiments, the hairpin loop can be 10 or fewer

nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 nucleotides.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not be, but can be covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs. In one embodiment of the RNAi agent, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In certain embodiments, an iRNA agent of the invention is a dsRNA, each strand of which comprises 19-23 nucleotides, that interacts with a target RNA sequence, *e.g.*, a PNPLA3 gene, to direct cleavage of the target RNA.

In some embodiments, an iRNA of the invention is a dsRNA of 24-30 nucleotides that interacts with a target RNA sequence, *e.g.*, a PNPLA3 target mRNA sequence, to direct the cleavage of the target RNA.

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

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

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

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotides, *e.g.,* a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In certain embodiments, the overhang on the sense strand or the antisense strand, or both, can include extended lengths longer than 10 nucleotides, *e.g.*, 1-30 nucleotides, 2-30 nucleotides, 10-30 nucleotides, 10-25 nucleotides, 10-20 nucleotides, or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5'end of the antisense strand of the duplex. In certain embodiments, one or more of the nucleotides in the extended overhang is replaced with a nucleoside thiophosphate. In certain embodiments, the overhang includes a self-complementary portion such that the overhang is capable of forming a hairpin structure that is stable under physiological conditions.

"Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the double stranded RNA agent, *i.e.*, no nucleotide overhang. A "blunt ended" double stranded RNA agent is double stranded over its entire length, *i.e.*, no nucleotide overhang at either end of the molecule. The RNAi agents of the invention include RNAi agents with no nucleotide overhang at one end (*i.e.*, agents with one overhang and one blunt end) or with no nucleotide overhangs at either end. Most often such a molecule will be double-stranded over its entire length.

The term "antisense strand" or "guide strand" refers to the strand of an iRNA, *e.g.*, a dsRNA, which includes a region that is substantially complementary to a target sequence, *e.g.,* a PNPLA3 mRNA.

As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, *e.g.,* a PNPLA3 nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, or 3 nucleotides of the 5'- or 3'-end of the iRNA. In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the antisense strand. In some embodiments, the antisense strand of the double stranded RNA agent of the invention includes no more than 4 mismatches with the target mRNA, *e.g.*, the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the target mRNA. In some embodiments, the antisense strand double stranded RNA agent of the invention includes no more than 4 mismatches with the sense strand, *e.g.*, the antisense strand

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

Thus, an RNAi agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, an RNAi agent as described herein contains no more than 3 mismatches (*i.e.,* 3, 2, 1, or 0 mismatches). In one embodiment, an RNAi agent as described herein contains no more than 2 mismatches. In one embodiment, an RNAi agent as described herein contains no more than 1 mismatch. In one embodiment, an RNAi agent as described herein contains 0 mismatches. In certain embodiments, if the antisense strand of the RNAi agent contains mismatches to the target sequence, the mismatch can optionally be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, in such embodiments, for a 23 nucleotide RNAi agent, the strand which is complementary to a region of a PNPLA3 gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of a PNPLA3 gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a PNPLA3 gene is important, especially if the particular region of complementarity in a PNPLA3 gene is known to have polymorphic sequence variation within the population.

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.

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

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

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 can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50ºC or 70ºC for 12-16 hours followed by washing (see, *e.g.*, "Molecular Cloning: A Laboratory Manual, Sambrook, *et al.* (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

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 can form one or more, but generally not more than 5, 4, 3, or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.*, inhibition of gene expression *via* a RISC pathway. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as "fully complementary" for the purposes described herein.

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

The terms "complementary," "fully complementary" and "substantially complementary" herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a double stranded RNA 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 PNPLA3 gene). For example, a polynucleotide is complementary to at least a part of a PNPLA3 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding a PNPLA3 gene.

Accordingly, in some embodiments, the antisense polynucleotides disclosed herein are fully complementary to the target PNPLA3 sequence. In other embodiments, the antisense polynucleotides

disclosed herein are substantially complementary to the target PNPLA3 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of SEQ ID NOs:1, 3, 5, 7, 9, or 11, or a fragment of any one of SEQ ID NOs:1, 3, 5, 7, 9, or 11, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target PNPLA3 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 187-209; 214-238; 219-245; 283-305; 351-379; 361-391; 395-419; 416-439; 472-494; 483-506; 570-598; 618-649; 631-654; 636-659; 640-662; 643-677; 676-710; 740-772; 782-805; 803-825; 810-842; 864-905; 905-927; 910-934; 919-942; 953-983; 1062-1087; 1069-1097; 1078-1108; 1094-112; 1164-1187; 1170-1199; 1180-1212; 1196-1224; 1234-1262; 1259-1297; 1278-1318; 1326-1351; 1382-1411; 1518-1545; 1543-1568; 1549-1574; 1575-1597; 1621-1643; 1644-1692; 1676-1700; 1712-1734; 1719-1745; 1733-1778; 1733-1760; 1739-1770; 1749-1778; 1829-1856; 1865-1890; 1900-1925; 2076-2098; 2121-2148; 2175-2208; or 2243-2265 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target PNPLA3 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 687-709, 1182-1204, 1201-1223, 1738-1760, or 2186-2208 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target PNPLA3 sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of any one of Tables 2, 3, 6, and 7, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2, 3, 6, and 7, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In one embodiment, an RNAi agent of the disclosure includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is the same as a target PNPLA3 sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NOs: 2, 4, 6, 8, 10, or 12, or a fragment of any one of SEQ ID NOs:2, 4, 6, 8, 10, or 12, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In some embodiments, an iRNA of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target PNPLA3

sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the antisense strand nucleotide sequences in any one of any one of Tables 2, 3, 6, and 7, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 2, 3, 6, and 7, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In some embodiments, the sense and antisense strands are selected from any one of duplexes AD-1526902.2, AD-1526891.3, AD-1526820.3, AD-1526960.2, and AD-1526996.2.

In some embodiments, the sense and antisense strands are selected from any one of duplexes AD-1526902.2, AD-1526891.3, AD-1526820.3, and AD-1526960.2.

In some embodiments, the sense and antisense strands are selected from duplex AD-1526902.

In some embodiments, the sense and antisense strands are selected from duplex AD-1526891.

In some embodiments, the sense and antisense strands are selected from duplex AD-1526820.

In some embodiments, the sense and antisense strands are selected from duplex AD-1526960.

In general, an "iRNA" includes ribonucleotides with chemical modifications. Such modifications may include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a dsRNA molecule, are encompassed by "iRNA" for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide if present within an RNAi agent can be considered to constitute a modified nucleotide.

In an aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense oligonucleotide molecule that inhibits a target mRNA *via* an antisense inhibition mechanism. The single-stranded antisense oligonucleotide molecule is complementary to a sequence within the target mRNA. The single-stranded antisense oligonucleotides can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. *et al.*, (2002) *Mol Cancer Ther* 1:347-355. The single-stranded antisense oligonucleotide molecule may be about 14 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense oligonucleotide molecule may comprise a sequence that is at least about 14, 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

The phrase "contacting a cell with an iRNA," such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an iRNA includes contacting a cell *in vitro* with the iRNA or contacting a cell *in vivo* with the iRNA. The contacting may be done directly or indirectly. Thus, for example, the iRNA may be put into physical contact with the cell by the individual performing the method, or alternatively, the iRNA 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 iRNA. Contacting a cell *in vivo* may be done, for example, by injecting the iRNA into or near the tissue

where the cell is located, or by injecting the iRNA into another area, *e.g.*, the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the iRNA may contain or be coupled to a ligand, *e.g.*, GalNAc, that directs the iRNA to a site of interest, *e.g.*, the liver. 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 iRNA and subsequently transplanted into a subject.

In certain embodiments, contacting a cell with an iRNA includes "introducing" or "delivering the iRNA into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an iRNA can occur through unaided diffusion or active cellular processes, or by auxiliary agents or devices. Introducing an iRNA into a cell may be *in vitro* or *in vivo*. For example, for *in vivo* introduction, iRNA can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or are known in the art.

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.*, an iRNA or a plasmid from which an iRNA 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, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, or a mouse), or a bird that expresses the target gene, either endogenously or heterologously. In an embodiment, the subject is a human, such as a human being treated or assessed for a disease or disorder that would benefit from reduction in PNPLA3 expression; a human at risk for a disease or disorder that would benefit from reduction in PNPLA3 expression; a human having a disease or disorder that would benefit from reduction in PNPLA3 expression; or human being treated for a disease or disorder that would benefit from reduction in PNPLA3 expression as described herein. In some embodiments, the subject is a female human. In other embodiments, the subject is a male human. In one embodiment, the subject is an adult subject. In another embodiment, the subject is a pediatric subject.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result, such as reducing at least one sign or symptom of a PNPLA3-associated disorder in a subject. Treatment also includes a reduction of one or more sign or symptoms associated with unwanted PNPLA3 expression; diminishing the extent of unwanted PNPLA3 activation or stabilization; amelioration or palliation of unwanted PNPLA3 activation or stabilization. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.The term "lower" in the context of the level of PNPLA3 in a subject or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 10%, 15%, 20%, 25%, 30%, %, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more. In certain embodiments, a decrease is at least 20%. In certain embodiments, the decrease is at least 50% in a

disease marker, *e.g.*, protein or gene expression level. "Lower" in the context of the level of PNPLA3 in a subject is preferably down to a level accepted as within the range of normal for an individual without such disorder. In certain embodiments, "lower" is the decrease in the difference between the level of a marker or symptom for a subject suffering from a disease and a level accepted within the range of normal for an individual, *e.g.*, the level of decrease in bodyweight between an obese individual and an individual having a weight accepted within the range of normal.

As used herein, "prevention" or "preventing," when used in reference to a disease, disorder or condition thereof, may be treated or ameliorated by a reduction in expression of an PNPLA3 gene, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, *e.g.,* a symptom of unwanted or excessive PNPLA3 expression, such as the presence of elevated levels of proteins in the hedgehog signaling pathway, fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD). The likelihood of developing, *e.g.,* NAFLD, is reduced, for example, when an individual having one or more risk factors for NAFLD either fails to develop NAFLD or develops NAFLD with less severity relative to a population having the same risk factors and not receiving treatment as described herein. The failure to develop a disease, disorder or condition, or the reduction in the development of a symptom associated with such a disease, disorder or condition (*e.g.*, by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed symptoms delayed (*e.g.*, by days, weeks, months or years) is considered effective prevention.

As used herein, the term "Patatin-Like Phospholipase Domain Containing 3-associated disease" or "PNPLA3-associated disease," is a disease or disorder that is caused by, or associated with PNPLA3 gene expression or PNPLA3 protein production. The term "PNPLA3-associated disease" includes a disease, disorder or condition that would benefit from a decrease in PNPLA3 gene expression, replication, or protein activity. Non-limiting examples of PNPLA3-associated diseases include, for example, fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the lvier, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD). In another embodiment, the PNPLA3-associated disease is nonalcoholic fatty liver disease (NAFLD). In another embodiment, the PNPLA3-associated disease is nonalcoholic steatohepatitis (NASH). In another embodiment, the PNPLA3-associated disease is liver cirrhosis. In another embodiment, the PNPLA3-associated disease is insulin resistance. In another embodiment, the PNPLA3-associated disease is not insulin resistance. In one embodiment, the PNPLA3-associated disease is obesity.

In one embodiment, a PNPLA3-associated disease is nonalcoholic fatty liver disease (NAFLD). As used herein, "nonalcoholic fatty liver disease," used interchangeably with the term "NAFLD," refers to a disease defined by the presence of macrovascular steatosis in the presence of less than 20 gm of alcohol ingestion per day. NAFLD is the most common liver disease in the United States, and is commonly associated with insulin resistance/type 2 diabetes mellitus and obesity. NAFLD is manifested by steatosis, steatohepatitis, cirrhosis, and sometimes hepatocellaular

carcinoma. For a review of NAFLD, see Tolman and Dalpiaz (2007) *Ther. Clin. Risk. Manag.*, 3(6):1153-1163 the entire contents of which are incorporated herein by reference.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a PNPLA3-associated disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating, or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a PNPLA3-associated disorder, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylactically effective amount" also includes an amount of an RNAi agent that produces some desired effect at a reasonable benefit/risk ratio applicable to any treatment. The iRNA employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

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

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. Such carriers are known in the art. Pharmaceutically acceptable carriers include carriers for administration by injection.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs, or

localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from the liver (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver, such as, *e.g.*, hepatocytes). In some embodiments, a "sample derived from a subject" refers to urine obtained from the subject. A "sample derived from a subject" can refer to blood or blood derived serum or plasma from the subject.

II. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of a PNPLA3 gene. In preferred embodiments, the iRNA includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of an PNPLA3 gene in a cell, such as a cell within a subject, *e.g.,* a mammal, such as a human susceptible to developing a PNPLA3-associated disorder, *e.g.*, hypertriglyceridemia. The dsRNAi agent 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 a PNPLA3 gene. The region of complementarity is about 19-30 nucleotides in length (*e.g.,* about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, or 19 nucleotides in length). Upon contact with a cell expressing the PNPLA3 gene, the iRNA inhibits the expression of the PNPLA3 gene (*e.g.,* a human, a primate, a non-primate, or a rat PNPLA3 gene) by at least about 50% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting or flow cytometric techniques. In preferred embodiments, inhibition of expression is determined by the qPCR method provided in the examples herein with the siRNA at, *e.g.,* a 10 nM concentration, in an appropriate organism cell line provided therein. In preferred embodiments, inhibition of expression *in vivo* is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.*, a mouse or an AAV-infected mouse expressing the human target gene, *e.g.*, when administered as single dose, *e.g.,* at 3 mg/kg at the nadir of RNA expression.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of a PNPLA3 gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is 15 to 30 base pairs in length, *e.g.,* 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-

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

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

In some embodiments, the duplex structure is 19 to 30 base pairs in length. Similarly, the region of complementarity to the target sequence is 19 to 30 nucleotides in length.

In some embodiments, the dsRNA is about 19 to about 23 nucleotides in length, or about 25 to about 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well-known in the art that dsRNAs longer than about 21-23 nucleotides in length may serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 19 to about 30 base pairs, *e.g.*, about 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target PNPLA3 gene expression is not generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.,* 1-4, 2-4, 1-3, 2-3, 1, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide overhang can have superior inhibitory properties relative to their blunt-ended counterparts. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand, or any

combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end, or both ends of an antisense or sense strand of a dsRNA.

A dsRNA can be synthesized by standard methods known in the art. Double stranded RNAi compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide strands comprising unnatural or modified nucleotides can be easily prepared. Similarly, single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In an aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand is selected from the group of sequences provided in any one of Tables 2, 3, 6, and 7, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 2, 3, 6, and 73. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a PNPLA3 gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in any one of Tables 2, 3, 6, and 7, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 2, 3, 6, and 7.

In certain embodiments, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In other embodiments, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

In some embodiments, the sense or antisense strand is selected from the sense or antisense strand of any one of duplexes AD-1526902.2, AD-1526891.3, AD-1526820.3, AD-1526960.2, and AD-1526996.2.

In some embodiments, the sense or antisense strand is selected from the sense or antisense strand of any one of duplexes AD-1526902.2, AD-1526891.3, AD-1526820.3, and AD-1526960.2.

In some embodiments, the sense or antisense strand is selected from the sense or antisense strand of duplex AD-1526902.

In some embodiments, the sense or antisense strand is selected from the sense or antisense strand of duplex AD-1526891.

In some embodiments, the sense or antisense strand is selected from the sense or antisense strand of duplex AD-1526820.

In some embodiments, the sense or antisense strand is selected from the sense or antisense strand of duplex AD-1526960.

It will be understood that, although the sequences in, for example, Table 3, are not described as modified or conjugated sequences, the RNA of the iRNA of the invention *e.g.*, a dsRNA of the invention, may comprise any one of the sequences set forth in any one of Tables 2, 3, 6, and 7 that is

un-modified, un-conjugated, or modified or conjugated differently than described therein. In other words, the invention encompasses dsRNA of Tables 2, 3, 6, and 7 which are un-modified, un-conjugated, modified, or conjugated, as described herein.

The skilled person is well aware that dsRNAs having a duplex structure of about 20 to 23 base pairs, *e.g.,* 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, *EMBO* 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in any one of Tables 2, 3, 6, and 7. dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having any one of the sequences in any one of Tables 2, 3, 6, and 7 minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 19, 20, or more contiguous nucleotides derived from any one of the sequences of any one of Tables 2, 3, 6, and 7, and differing in their ability to inhibit the expression of a PNPLA3 gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs provided in Tables 2, 3, 6, and 7 identify a site(s) in a PNPLA3 transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within one of these sites. As used herein, an iRNA is said to target within a particular site of an RNA transcript if the iRNA promotes cleavage of the transcript anywhere within that particular site. Such an iRNA will generally include at least about 19 contiguous nucleotides from any one of the sequences provided in any one of Tables 2, 3, 6, and 7 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a PNPLA3 gene.

III. Modified iRNAs of the Invention

In certain embodiments, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is un-modified, and does not comprise, *e.g.,* chemical modifications or conjugations known in the art and described herein. In other embodiments, the RNA of an iRNA of the invention, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the invention, substantially all of the nucleotides of an iRNA of the invention are modified. In other embodiments of the invention, all of the nucleotides of an iRNA or substantially all of the nucleotides of an iRNA are modified, *i.e.*, not more than 5, 4, 3, 2, or 1unmodified nucleotides are present in a strand of the iRNA.

The nucleic acids featured in the invention can be synthesized or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Modifications include, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation,

DNA nucleotides, inverted linkages, *etc.*); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (*e.g.*, at the 2'-position or 4'-position) or replacement of the sugar; or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA 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. In some embodiments of the invention, the dsRNA agents of the invention are in a free acid form. In other embodiments of the invention, the dsRNA agents of the invention are in a salt form. In one embodiment, the dsRNA agents of the invention are in a sodium salt form. In certain embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for substantially all of the phosphodiester and/or phosphorothiotate groups present in the agent. Agents in which substantially all of the phosphodiester and/or phosphorothioate linkages have a sodium counterion include not more than 5, 4, 3, 2, or 1 phosphodiester and/or phosphorothioate linkages without a sodium counterion. In some embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for all of the phosphodiester and/or phosphorothiotate groups present in the agent.

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

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. Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

Suitable RNA mimetics are contemplated for use in iRNAs provided herein, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound in which 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 US patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the iRNAs of the invention are described in, for example, in Nielsen *et al.*, *Science*, 1991, 254, 1497-1500.

Some embodiments featured in the invention 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. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506.

Modified RNAs can 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 can 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, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH_2--O--CH_2--$N(CH_2)_2$. Further exemplary modifications include : 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications can 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 can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative US patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Patent Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application,. The entire contents of each of the foregoing are hereby incorporated herein by reference.

An iRNA can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxy-thymine (dT), 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 invention. 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. Patent Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

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

In some embodiments, the RNA of an iRNA can also be modified to include one or more bicyclic sugar moieties. A “bicyclic sugar” is a furanosyl ring modified by the bridging of two atoms. A “bicyclic nucleoside” (“BNA”) is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4′-carbon and the 2′-carbon of the sugar ring. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH_2-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, 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 invention 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 invention 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′-(CH_2)—O-2′ (LNA); 4′-(CH_2)—S-2′; 4′-(CH_2)$_2$—O-2′ (ENA); 4′-CH(CH_3)—O-2′ (also referred to as "constrained ethyl" or "cEt") and 4′-CH(CH_2OCH_3)—O-2′ (and analogs thereof; see, *e.g.*, U.S. Patent No. 7,399,845); 4′-C(CH_3)(CH_3)—O-2′ (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,283); 4′-CH_2—N(OCH_3)-2′ (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,425); 4′-CH_2—O—N(CH_3)-2′ (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4′-CH_2—N(R)—O-2′, wherein R is H, C1-C12 alkyl, or a protecting group (see, *e.g.*, U.S. Patent No. 7,427,672); 4′-CH_2—C(H)(CH_3)-2′ (see, *e.g.*, Chattopadhyaya *et al., J. Org. Chem.*, 2009, 74, 118-134); and 4′-CH_2—C(=CH_2)-2′ (and analogs thereof; see, *e.g.*, U.S. Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

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

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).

The RNA of an iRNA 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(CH_3)-O-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

An iRNA of the invention 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, U.S. Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

In some embodiments, an iRNA of the invention comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'-C4' have been removed (*i.e.* the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (*i.e.* the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed (see

Nuc. Acids Symp. Series, 52, 133-134 (2008) and Fluiter *et al.*, *Mol. Biosyst.,* 2009, 10, 1039 hereby incorporated by reference).

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

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'-0-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 the nucleotides of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, *e.g.,* a 5'-terminal phosphate or phosphate mimic on the antisense strand of an iRNA. Suitable phosphate mimics are disclosed in, for example U.S. Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

A. Modified iRNAs Comprising Motifs of the Invention

In certain aspects of the invention, the double stranded RNA agents of the invention include agents with chemical modifications as disclosed, for example, in WO2013/075035, the entire contents of each of which are incorporated herein by reference. WO2013/075035 provides motifs of three identical modifications on three consecutive nucleotides into a sense strand or antisense strand of a dsRNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the dsRNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The dsRNAi agent may be optionally conjugated with a GalNAc derivative ligand, for instance on the sense strand.

More specifically, when the sense strand and antisense strand of the double stranded RNA agent are completely modified to have one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of at least one strand of a dsRNAi agent, the gene silencing activity of the dsRNAi agent was observed.

Accordingly, the invention provides double stranded RNA agents capable of inhibiting the expression of a target gene (*i.e.*, PNPLA3 gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may be, for example, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length.

The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as "dsRNAi agent." The duplex region of a dsRNAi agent may be,

for example, the duplex region can be 27-30 nucleotide pairs in length, 19-25 nucleotide pairs in length, 19-23 nucleotide pairs in length, 19- 21 nucleotide pairs in length, 21-25 nucleotide pairs in length, or 21-23 nucleotide pairs in length. In another example, the duplex region is selected from 19, 20, 21, 22, 23, 24, 25, 26, and 27 nucleotides in length.

In certain embodiments, the dsRNAi agent may contain one or more overhang regions or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be, independently, 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5 nucleotides in length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. In certain embodiments, the overhang regions can include extended overhang regions as provided above. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence. The first and second strands can also be joined, *e.g.*, by additional bases to form a hairpin, or by other non-base linkers.

In certain embodiments, the nucleotides in the overhang region of the dsRNAi agent can each independently be a modified or unmodified nucleotide including, but no limited to 2'-sugar modified, such as, 2'-F, 2'-O-methyl, thymidine (T), 2`-O-methoxyethyl-5-methyluridine (Teo), 2`-O-methoxyethyladenosine (Aeo), 2`-O-methoxyethyl-5-methylcytidine (m5Ceo), and any combinations thereof.

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

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

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

In certain embodiments, the dsRNAi agent is a double ended bluntmer of 19 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three

consecutive nucleotides at positions 7, 8, 9 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

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

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

In certain embodiments, the dsRNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang. Preferably, the 2 nucleotide overhang is at the 3'-end of the antisense strand.

When the 2 nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In certain embodiments, every nucleotide in the sense strand and the antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In certain embodiments each residue is independently modified with a 2'-O-methyl or 3'-fluoro, *e.g.,* in an alternating motif. Optionally, the dsRNAi agent further comprises a ligand (preferably $GalNAc_3$).

In certain embodiments, the dsRNAi agent comprises a sense and an antisense strand, wherein the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1) positions 1 to 23 of the first strand comprise at least 8 ribonucleotides; the antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, comprises at least 8 ribonucleotides in the positions paired with positions 1- 23 of sense strand to form a duplex; wherein at least the 3 ' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30

nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the double stranded nucleic acid is introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

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

In certain embodiments, the sense strand of the dsRNAi agent contains at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at the cleavage site in the sense strand.

In certain embodiments, the antisense strand of the dsRNAi agent can also contain at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at or near the cleavage site in the antisense strand.

For a dsRNAi agent having a duplex region of 19-23 nucleotides in length, the cleavage site of the antisense strand is typically around the 10, 11, and 12 positions from the 5'-end. Thus the motifs of three identical modifications may occur at the 9, 10, 11 positions; the 10, 11, 12 positions; the 11, 12, 13 positions; the 12, 13, 14 positions; or the 13, 14, 15 positions of the antisense strand, the count starting from the first nucleotide from the 5'-end of the antisense strand, or, the count starting from the first paired nucleotide within the duplex region from the 5'- end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the dsRNAi agent from the 5'-end.

The sense strand of the dsRNAi agent may contain at least one motif of three identical modifications on three consecutive nucleotides at the cleavage site of the strand; and the antisense strand may have at least one motif of three identical modifications on three consecutive nucleotides at or near the cleavage site of the strand. When the sense strand and the antisense strand form a dsRNA

duplex, the sense strand and the antisense strand can be so aligned that one motif of the three nucleotides on the sense strand and one motif of the three nucleotides on the antisense strand have at least one nucleotide overlap, *i.e.*, at least one of the three nucleotides of the motif in the sense strand forms a base pair with at least one of the three nucleotides of the motif in the antisense strand. Alternatively, at least two nucleotides may overlap, or all three nucleotides may overlap.

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

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

In some embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end, or both ends of the strand.

In other embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi agent typically does not include the first one or two paired nucleotides within the duplex region at the 3'-end, 5'-end, or both ends of the strand.

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

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

In some embodiments, every nucleotide in the sense strand and antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs, 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 a RNA. For example, 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. For example, 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, CRN, cET, UNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-deoxy, 2'-hydroxyl, or 2'-fluoro. The strands can contain more than one modification. In one embodiment, each residue of the sense strand and antisense strand is independently modified with 2'- O-methyl or 2'-fluoro.

At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'- O-methyl or 2'-fluoro modifications, or others.

In certain embodiments, the N_a or N_b comprise modifications of an alternating pattern. The term "alternating motif" 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 dsRNAi agent of the invention 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'to 3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 5' to 3' of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5' to 3' of the strand and the alternating motif in the antisense strand may start with "BBAABBAA" from 5' to 3' 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.

In some embodiments, the dsRNAi agent comprises the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the sense strand initially has a shift relative to the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the antisense strand initially, *i.e.*, the 2'-O-methyl modified nucleotide on the sense strand base pairs with a 2'-F modified nucleotide on the antisense strand and vice versa. The 1 position of the sense strand may start with the 2'-F modification, and the 1 position of the antisense strand may start with the 2'- O-methyl modification.

The introduction of one or more motifs of three identical modifications on three consecutive nucleotides to the sense strand or antisense strand interrupts the initial modification pattern present in the sense strand or antisense strand. This interruption of the modification pattern of the sense or antisense strand by introducing one or more motifs of three identical modifications on three consecutive nucleotides to the sense or antisense strand may enhance the gene silencing activity against the target gene.

In some embodiments, when the motif of three identical modifications on three consecutive nucleotides is introduced to any of the strands, the modification of the nucleotide next to the motif is a different modification than the modification of the motif. For example, the portion of the sequence containing the motif is "…N_aYYYN_b…," where "Y" represents the modification of the motif of three identical modifications on three consecutive nucleotide, and "N_a" and "N_b" represent a modification to the nucleotide next to the motif "YYY" that is different than the modification of Y, and where N_a and N_b can be the same or different modifications. Alternatively, N_a or N_b may be present or absent when there is a wing modification present.

The iRNA 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, antisense strand, or both strands 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 may contain 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 one embodiment, a double-stranded RNAi agent comprises 6-8 phosphorothioate internucleotide linkages. In some embodiments, the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-end and two phosphorothioate internucleotide linkages at the 3'-end, and the sense strand comprises at least two phosphorothioate internucleotide linkages at either the 5'-end or the 3'-end.

In some embodiments, the dsRNAi agent comprises a phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region may contain 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 the 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. These terminal three nucleotides may be at the 3'-end of the antisense strand, the 3'-end of the sense strand, the 5'-end of the antisense strand, or the 5'end of the antisense strand.

In some embodiments, the 2-nucleotide overhang is at the 3'-end of the antisense strand, and there are two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. Optionally, the dsRNAi agent may additionally have two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand.

In one embodiment, the dsRNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch may occur in the overhang region or the duplex region. The base pair may be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest

approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

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

In certain embodiments, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from 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.

In other embodiments, the nucleotide at the 3'-end of the sense strand is deoxy-thymine (dT) or the nucleotide at the 3'-end of the antisense strand is deoxy-thymine (dT). For example, there is a short sequence of deoxy-thymine nucleotides, for example, two dT nucleotides on the 3'-end of the sense, antisense strand, or both strands.

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

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

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. Preferably YYY is all 2'-F modified nucleotides.

In some embodiments, the N_a or N_b comprises 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 dsRNAi 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 first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end.

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

5' n_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, 0-10, 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. Preferably, 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 one embodiment, the antisense strand sequence of the RNAi may be represented by formula (II):

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' (II)

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 motif of three identical modifications on three consecutive nucleotides.

In some embodiments, the N_a' or N_b' comprises modifications of alternating pattern.

The Y'Y'Y' motif occurs at or near the cleavage site of the antisense strand. For example, when the dsRNAi 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 first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end. Preferably, the Y′Y′Y′ motif occurs at positions 11, 12, 13.

In certain embodiments, Y′Y′Y′ motif is all 2'-OMe modified nucleotides.

In certain embodiments, k is 1 and l is 0, or k is 0 and l is 1, or both 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' (IIb);

5' $n_{q'}$-N_a′-Y′Y′Y′-N_b′-X′X′X′-$n_{p'}$ 3' (IIc); or

5' $n_{q'}$-N_a′- Z′Z′Z′-N_b′-Y′Y′Y′-N_b′- X′X′X′-N_a′-$n_{p'}$ 3' (IId).

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

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

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

When the antisense strand is represented as formula (IIa), each N_a' 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, CRN, UNA, cEt, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X, Y, Z, X′, Y′, and Z′, in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

In some embodiments, the sense strand of the dsRNAi 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 first nucleotide from the 5'-end, or optionally, the count starting at the first 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 contain Y′Y′Y′ motif occurring at positions 11, 12, 13 of the strand, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first 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 (IIa), (IIb), (IIc), and (IId), respectively.

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

sense: 5' n_p -N_a-(X X X)$_i$ -N_b- Y Y Y -N_b -(Z Z Z)$_j$-N_a-n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y′Y′Y′-N_b'-(Z′Z′Z′)$_l$-N_a'-n_q' 5'

(III)

wherein:

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

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

each N_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 modifications on three consecutive nucleotides.

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

Exemplary combinations of the sense strand and antisense strand forming an iRNA 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'

(IIIa)

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′-N_a'n_q' 5'

(IIIb)

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′-N_a'-n_q' 5'

(IIIc)

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′-N_a-n_q' 5'

(IIId)

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

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

When the dsRNAi agent is represented as formula (IIId), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_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 (III), (IIIa), (IIIb), (IIIc), and (IIId) may be the same or different from each other.

When the dsRNAi agent is represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), 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 dsRNAi agent is represented by formula (IIIb) or (IIId), 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 dsRNAi agent is represented as formula (IIIc) or (IIId), 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 certain 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, or the modification on the X nucleotide is different than the modification on the X' nucleotide.

In certain embodiments, when the dsRNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications. In other embodiments, when the RNAi agent is represented by formula (IIId), 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 yet other embodiments, when the RNAi agent is represented by formula (IIId), 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 GalNAc derivatives attached through a bivalent or trivalent branched linker (described below). In other embodiments, when the RNAi agent is represented by formula (IIId), 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 GalNAc derivatives attached through a bivalent or trivalent branched linker.

In some embodiments, when the dsRNAi 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 GalNAc derivatives attached through a bivalent or trivalent branched linker.

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

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

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

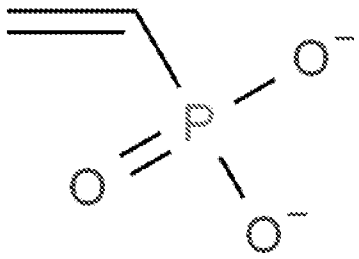
In certain embodiments, an RNAi agent of the invention may contain a low number of nucleotides containing a 2'-fluoro modification, *e.g.*, 10 or fewer nucleotides with 2'-fluoro modification. For example, the RNAi agent may contain 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotides with a 2'-fluoro modification. In a specific embodiment, the RNAi agent of the invention contains 10 nucleotides with a 2'-fluoro modification, *e.g.*, 4 nucleotides with a 2'-fluoro modification in the sense strand and 6 nucleotides with a 2'-fluoro modification in the antisense strand. In another specific embodiment, the RNAi agent of the invention contains 6 nucleotides with a 2'-fluoro

modification, *e.g.*, 4 nucleotides with a 2'-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

In other embodiments, an RNAi agent of the invention may contain an ultra low number of nucleotides containing a 2'-fluoro modification, *e.g.*, 2 or fewer nucleotides containing a 2'-fluoro modification. For example, the RNAi agent may contain 2, 1 of 0 nucleotides with a 2'-fluoro modification. In a specific embodiment, the RNAi agent may contain 2 nucleotides with a 2'-fluoro modification, *e.g.*, 0 nucleotides with a 2-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

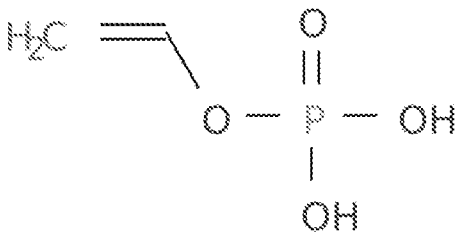
Various publications describe multimeric iRNAs that can be used in the methods of the invention. Such publications include WO2007/091269, U.S. Patent No. 7,858,769, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520 the entire contents of each of which are hereby incorporated herein by reference.

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.

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



As described in more detail below, the iRNA that contains conjugations of one or more carbohydrate moieties to an iRNA can optimize one or more properties of the iRNA. In many cases, the carbohydrate moiety will be attached to a modified subunit of the iRNA. For example, the ribose

sugar of one or more ribonucleotide subunits of a iRNA 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, or 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.

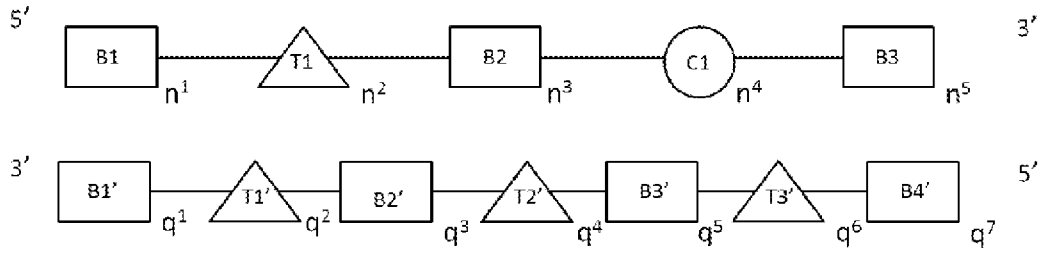
The iRNA may be conjugated to a ligand *via* a carrier, wherein the carrier can be cyclic group or acyclic group; preferably, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl,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

i. 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 or at positions 2-8 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, 2-8, 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.

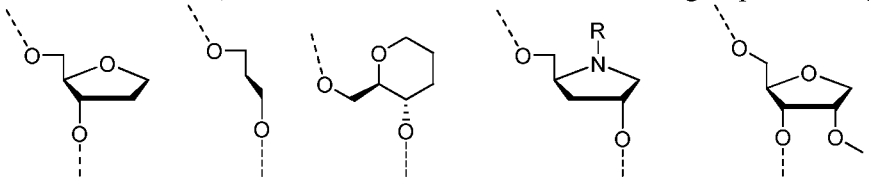
An iRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides. The RNAi agent may be represented by formula (L):



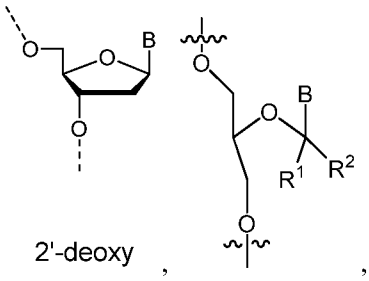
(L),

In formula (L), B1, B2, B3, B1', B2', B3', and B4' each are independently a nucleotide containing a modification selected from the group consisting of 2'-O-alkyl, 2'-substituted alkoxy, 2'-substituted alkyl, 2'-halo, ENA, and BNA/LNA. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe modifications. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe or 2'-F modifications. In one embodiment, at least one of B1, B2, B3, B1', B2', B3', and B4' contain 2'-O-N-methylacetamido (2'-O-NMA) modification.

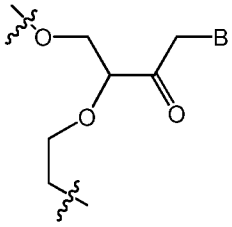
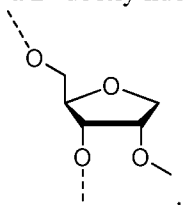
C1 is a thermally destabilizing nucleotide placed at a site opposite to the seed region of the antisense strand (*i.e.*, at positions 2-8 of the 5'-end of the antisense strand or at positions 2-9 of the 5'-end of the antisense strand). For example, C1 is at a position of the sense strand that pairs with a nucleotide at positions 2-8 of the 5'-end of the antisense strand. In one example, C1 is at position 15 from the 5'-end of the sense strand. C1 nucleotide bears the thermally destabilizing modification which can include abasic modification; mismatch with the opposing nucleotide in the duplex; and sugar modification such as 2'-deoxy modification or acyclic nucleotide *e.g.*, unlocked nucleic acids (UNA) or glycerol nucleic acid (GNA). In one embodiment, C1 has thermally destabilizing modification selected from the group consisting of: i) mismatch with the opposing nucleotide in the antisense strand; ii) abasic modification selected from the group consisting of:



; and iii) sugar modification selected from the group consisting of:



, and


, 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. In one embodiment, the thermally destabilizing modification in C1 is a mismatch selected from the group consisting of G:G, G:A, G:U, G:T, A:A, A:C, C:C, C:U, C:T, U:U, T:T, and U:T; and optionally, at least one nucleobase in the mismatch pair is a 2'-deoxy nucleobase. In one example, the thermally destabilizing modification in C1 is GNA or  .

T1, T1', T2', and T3' each independently represent a nucleotide comprising a modification providing the nucleotide a steric bulk that is less or equal to the steric bulk of a 2'-OMe modification. A steric bulk refers to the sum of steric effects of a modification. Methods for determining steric effects of a modification of a nucleotide are known to one skilled in the art. The modification can be at the 2' position of a ribose sugar of the nucleotide, or a modification to a non-ribose nucleotide, acyclic nucleotide, or the backbone of the nucleotide that is similar or equivalent to the 2' position of the ribose sugar, and provides the nucleotide a steric bulk that is less than or equal to the steric bulk of a 2'-OMe modification. For example, T1, T1', T2', and T3' are each independently selected from DNA, RNA, LNA, 2'-F, and 2'-F-5'-methyl. In one embodiment, T1 is DNA. In one embodiment, T1' is DNA, RNA or LNA. In one embodiment, T2' is DNA or RNA. In one embodiment, T3' is DNA or RNA.

n^1, n^3, and q^1 are independently 4 to 15 nucleotides in length.

n^5, q^3, and q^7 are independently 1-6 nucleotide(s) in length.

n^4, q^2, and q^6 are independently 1-3 nucleotide(s) in length; alternatively, n^4 is 0.

q^5 is independently 0-10 nucleotide(s) in length.

n^2 and q^4 are independently 0-3 nucleotide(s) in length.

Alternatively, n^4 is 0-3 nucleotide(s) in length.

In one embodiment, n^4 can be 0. In one example, n^4 is 0, and q^2 and q^6 are 1. In another example, n^4 is 0, and q^2 and q^6 are 1, with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand).

In one embodiment, n^4, q^2, and q^6 are each 1.

In one embodiment, n^2, n^4, q^2, q^4, and q^6 are each 1.

In one embodiment, C1 is at position 14-17 of the 5'-end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^4 is 1. In one embodiment, C1 is at position 15 of the 5'-end of the sense strand

In one embodiment, T3' starts at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1.

In one embodiment, T1' starts at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

In an exemplary embodiment, T3' starts from position 2 from the 5' end of the antisense strand and T1' starts from position 14 from the 5' end of the antisense strand. In one example, T3' starts from position 2 from the 5' end of the antisense strand and q^6 is equal to 1 and T1' starts from position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

In one embodiment, T1' and T3' are separated by 11 nucleotides in length (*i.e.* not counting the T1' and T3' nucleotides).

In one embodiment, T1' is at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1, and the modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose.

In one embodiment, T3' is at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1, and the modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

In one embodiment, T1 is at the cleavage site of the sense strand. In one example, T1 is at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1. In an exemplary embodiment, T1 is at the cleavage site of the sense strand at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1,

In one embodiment, T2' starts at position 6 from the 5' end of the antisense strand. In one example, T2' is at positions 6-10 from the 5' end of the antisense strand, and q^4 is 1.

In an exemplary embodiment, T1 is at the cleavage site of the sense strand, for instance, at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1; T1' is at position 14 from the 5' end of the antisense strand, and q^2 is equal to 1, and the modification to T1' is at the 2' position of a ribose sugar or at positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose; T2' is at positions 6-10 from the 5' end of the antisense strand, and q^4 is 1; and T3' is at position 2 from the 5' end of the antisense strand, and

q^6 is equal to 1, and the modification to T3' is at the 2' position or at positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

In one embodiment, T2' starts at position 8 from the 5' end of the antisense strand. In one example, T2' starts at position 8 from the 5' end of the antisense strand, and q^4 is 2.

In one embodiment, T2' starts at position 9 from the 5' end of the antisense strand. In one example, T2' is at position 9 from the 5' end of the antisense strand, and q^4 is 1.

In one embodiment, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand).

In one embodiment, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 6, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 7, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 6, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 7, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand

(counting from the 5'-end of the sense strand), 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 of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 5, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand.

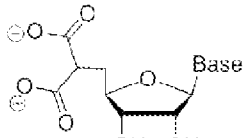
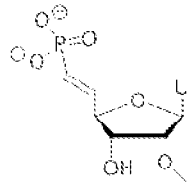
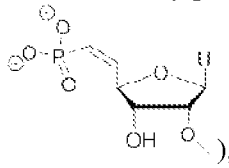
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 5, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 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 one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1.
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1.
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand).

The RNAi 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'-PS_2), 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*-vinylphosphate, ), 5'-*Z*-VP isomer (*i.e.*, *cis*-vinylphosphate, ), or mixtures thereof.

In one embodiment, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the sense strand. In one embodiment, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-P. In one embodiment, the RNAi agent comprises a 5'-P in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS. In one embodiment, the RNAi agent comprises a 5'-PS in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-VP. In one embodiment, the RNAi agent comprises a 5'-VP in the antisense strand. In one embodiment, the RNAi agent comprises a 5'-*E*-VP in the antisense strand. In one embodiment, the RNAi agent comprises a 5'-*Z*-VP in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS_2. In one embodiment, the RNAi agent comprises a 5'-PS_2 in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS_2. In one embodiment, the RNAi agent comprises a 5'-deoxy-5'-*C*-malonyl in the antisense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The dsRNA agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof. In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 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). The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 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). The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 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). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 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). The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 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). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The dsRNAi RNA agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1;

with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two

phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-*E*-VP, 5'-*Z*-VP, or combination thereof), and a targeting ligand.

In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- PS_2 and a targeting ligand. In one embodiment, the 5'- PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 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). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 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). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 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). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-*E*-VP, 5'-*Z*-VP, or combination thereof) and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 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). The RNAi agent also comprises a 5'-PS_2 and a targeting ligand. In one embodiment, the 5'-PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 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). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1;

with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-*E*-VP, 5'-*Z*-VP, or combination thereof) and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-PS_2 and a targeting ligand. In one embodiment, the 5'-PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- VP (*e.g.*, a 5'-*E*-VP, 5'-*Z*-VP, or combination thereof) and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'- PS_2 and a targeting ligand. In one embodiment, the 5'-PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), 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 of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In a particular embodiment, an RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker; and

(iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14 to 16, 18, and 20 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 9, 11 to 13, 15, 17, 19, 21, and 23, and 2'F modifications at positions 2, 4, 6 to 8, 10, 14, 16, 18, 20, and 22 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the dsRNA agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, an RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 15, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14, 16, 18, and 20 (counting from the 5' end); and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, 10, and 12 to 21, 2'-F modifications at positions 7, and 9, and a deoxy-nucleotide (*e.g.* dT) at position 11 (counting from the 5' end); and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 7, 9, 11, 13, 15, 17, and 19 to 23, and 2'-F modifications at positions 2, 4 to 6, 8, 10, 12, 14, 16, and 18 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, 10, 12, 14, and 16 to 21, and 2'-F modifications at positions 7, 9, 11, 13, and 15; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 5, 7, 9, 11, 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2 to 4, 6, 8, 10, 12, 14, 16, 18, and 20 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 9, and 12 to 21, and 2'-F modifications at positions 10, and 11; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, and 13, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, and 14 to 21; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5 to 7, 9, 11 to 13, 15, 17 to 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 8, 10, 14, 16, and 20 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1, 2, 4, 6, 8, 12, 14, 15, 17, and 19 to 21, and 2'-F modifications at positions 3, 5, 7, 9 to 11, 13, 16, and 18; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 25 nucleotides;

(ii) 2'-OMe modifications at positions 1, 4, 6, 7, 9, 11 to 13, 15, 17, and 19 to 23, 2'-F modifications at positions 2, 3, 5, 8, 10, 14, 16, and 18, and desoxy-nucleotides (*e.g.* dT) at positions 24 and 25 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a four nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 8, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 9, 14, and 16 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 19 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 4, 6, and 10 to 19, and 2'-F modifications at positions 5, and 7 to 9; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 21, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 19 and 20, and between nucleotide positions 20 and 21 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In certain embodiments, the iRNA for use in the methods of the invention is an agent selected from agents listed in any one of Tables 2, 3, 6, and 7. These agents may further comprise a ligand.

III. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the iRNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the iRNA *e.g.*, into a cell. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, *Proc. Natl. Acid. Sci. USA*, 1989, 86: 6553-6556). In other embodiments, the ligand is 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 certain embodiments, a ligand alters the distribution, targeting, or lifetime of an iRNA agent into which it is incorporated. In preferred 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. Preferred ligands do 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, N-acetylglucosamine, N-acetylgalactosamine, or hyaluronic acid); or a lipid. The ligand can 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. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha 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-glucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic. In certain embodiments, the ligand is a multivalent galactose, *e.g.*, an N-acetyl-galactosamine.

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 a hepatic cell. Ligands can 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, or intermediate filaments. The drug can be, for example, taxol, 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. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases, or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

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

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems® (Foster City, Calif.). Any other methods 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 iRNAs and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then

reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A. Lipid Conjugates

In certain embodiments, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule preferably binds a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

A lipid based ligand can be used to inhibit, *e.g.*, control 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 certain embodiments, the lipid based ligand binds HSA. Preferably, it binds HSA with a sufficient affinity such that the conjugate will be preferably distributed to a non-kidney tissue. However, it is preferred that the affinity not be so strong that the HSA-ligand binding cannot be reversed.

In other embodiments, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be preferably distributed to the kidney. 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 target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

B. Cell Permeation Agents

In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-permeation agent. Preferably, 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 preferably an alpha-helical agent, which preferably has 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: 14). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO:15) 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:16) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO:17) 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). Examples of a peptide or peptidomimetic tethered to a dsRNA agent *via* an incorporated monomer unit for cell targeting purposes is 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 invention may be linear or cyclic, and may be modified, *e.g.,* glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidiomimemtics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Preferred conjugates of this ligand target PECAM-1 or VEGF.

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).

C. Carbohydrate Conjugates

In some embodiments of the compositions and methods of the invention, an iRNA further comprises a carbohydrate. The carbohydrate conjugated iRNA is 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, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide.

In certain embodiments, the monosaccharide is an N-acetylgalactosamine (GalNAc). GalNAc conjugates, which comprise one or more N-acetylgalactosamine (GalNAc) derivatives, are described, for example, in US 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 conjugated to the 5' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 5' end of the sense strand) *via* a linker, *e.g.*, a linker as described herein.

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

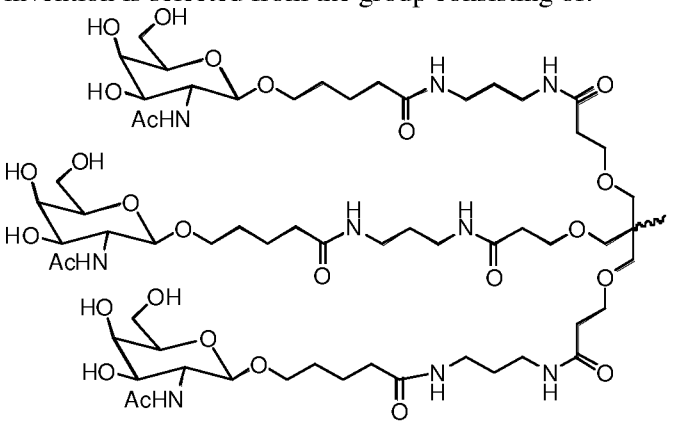
In certain embodiments, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc

derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

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

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

In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



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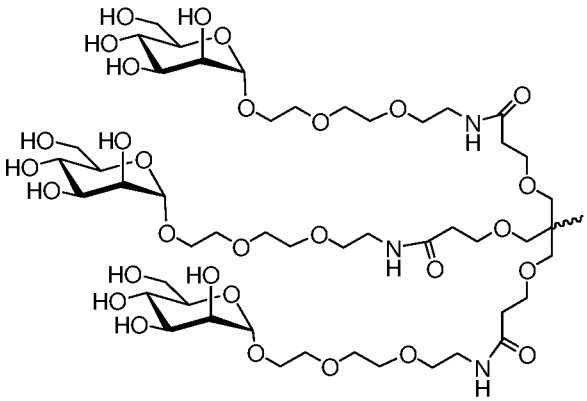

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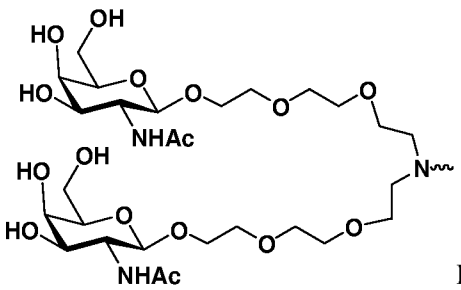

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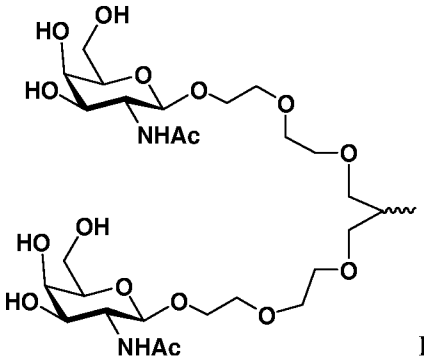

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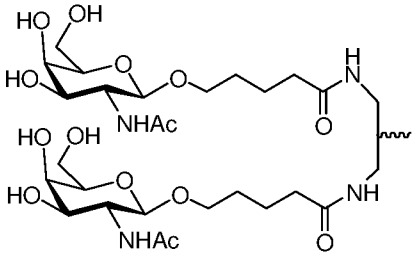

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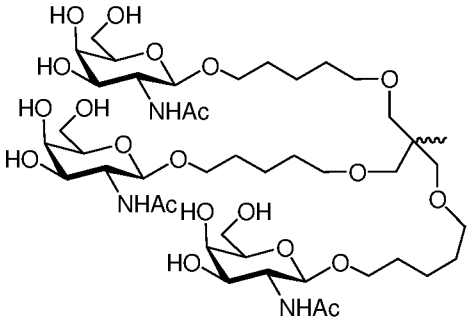

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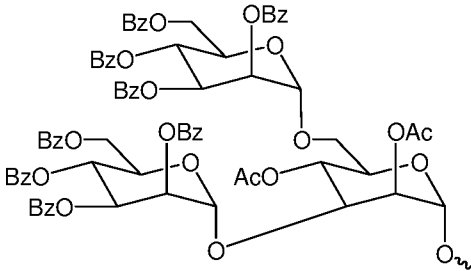

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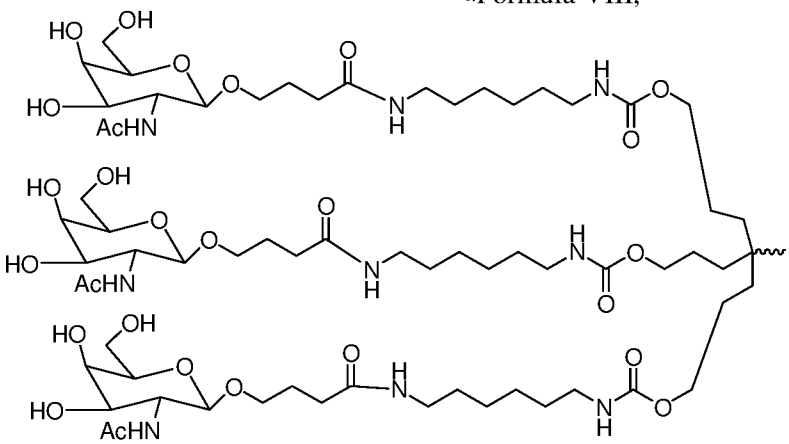

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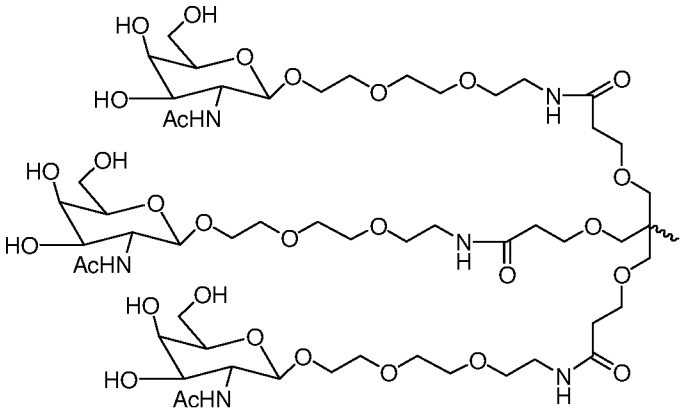

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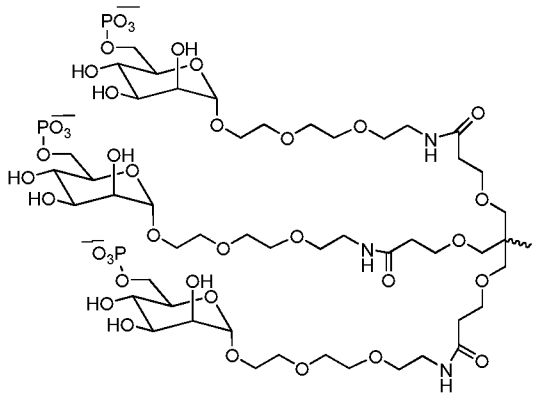

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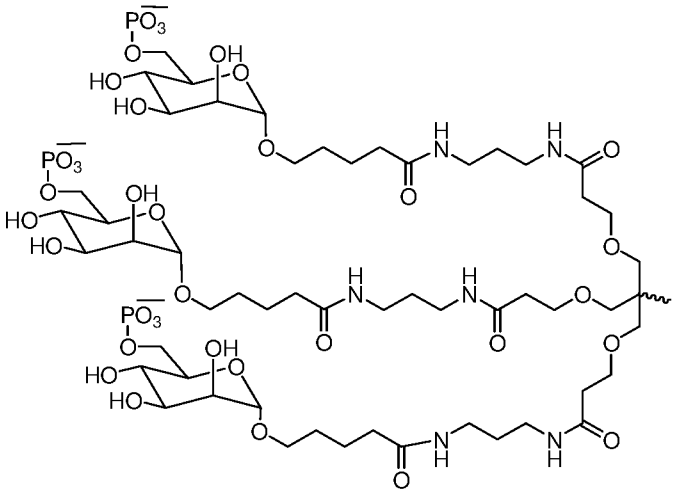

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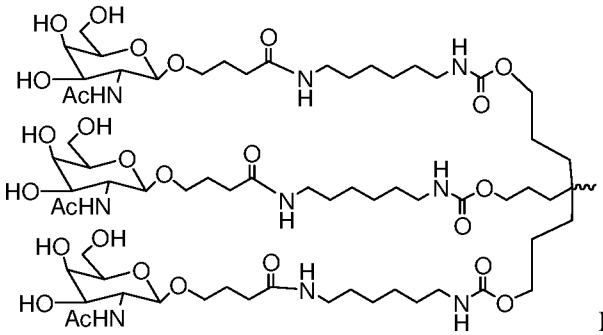

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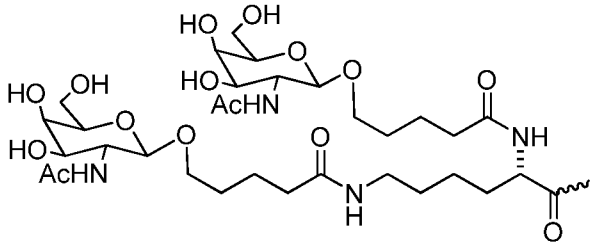

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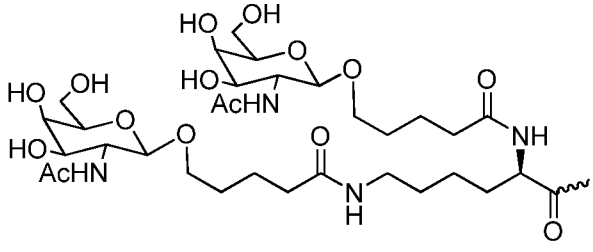

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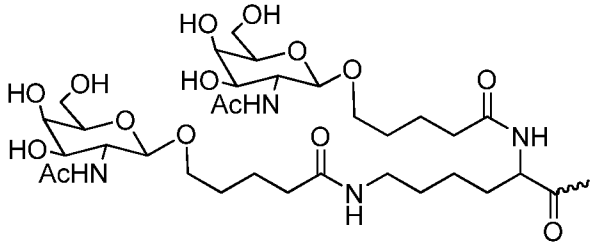

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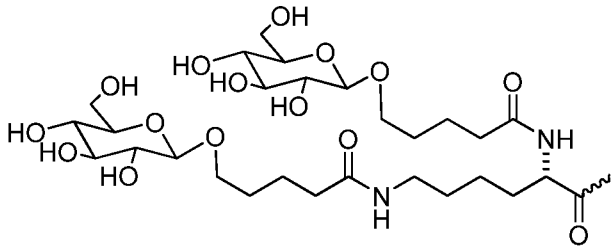

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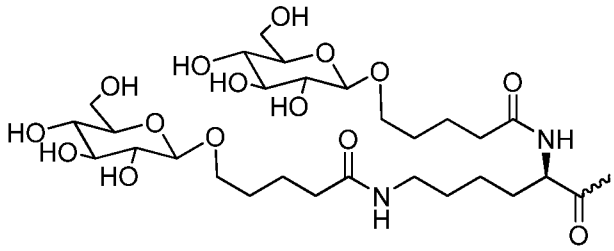

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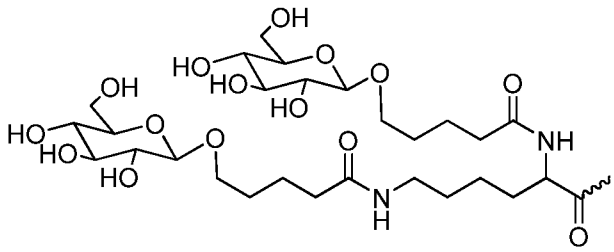

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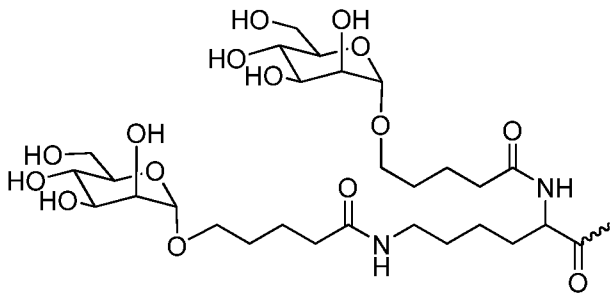

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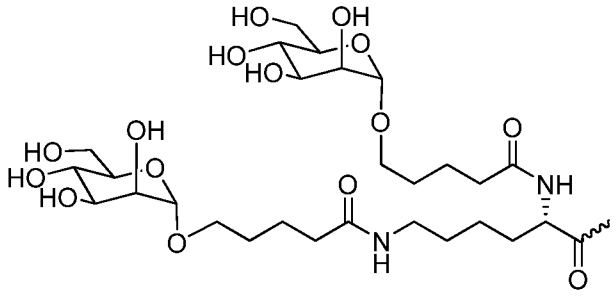

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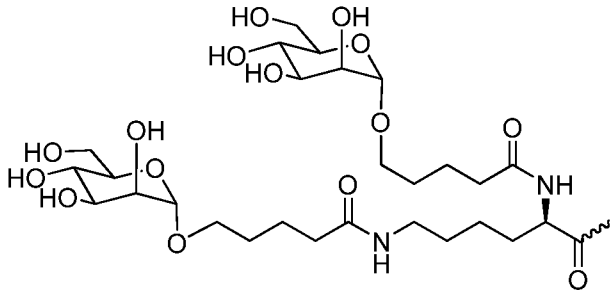

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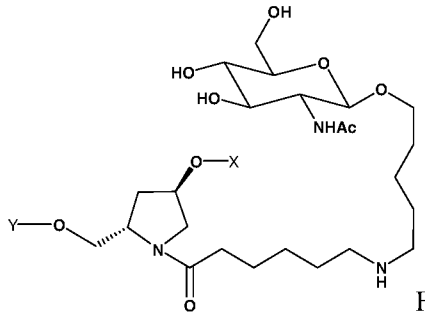

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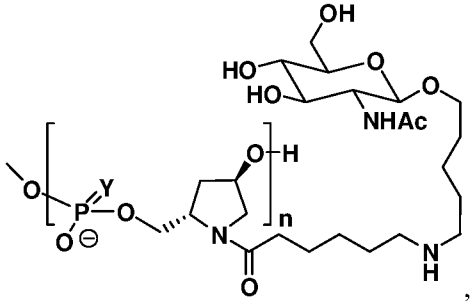
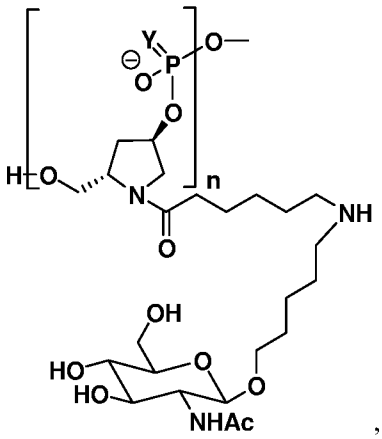
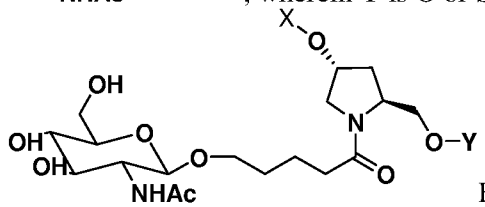


, wherein Y is O or S and n is 3 -6 (Formula XXIV);



, wherein Y is O or S and n is 3-6 (Formula XXV);



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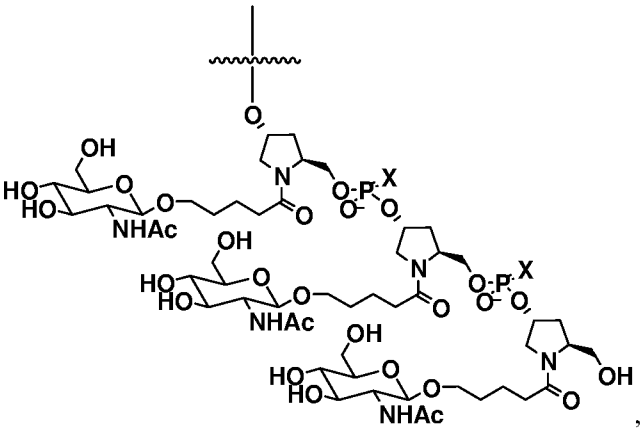
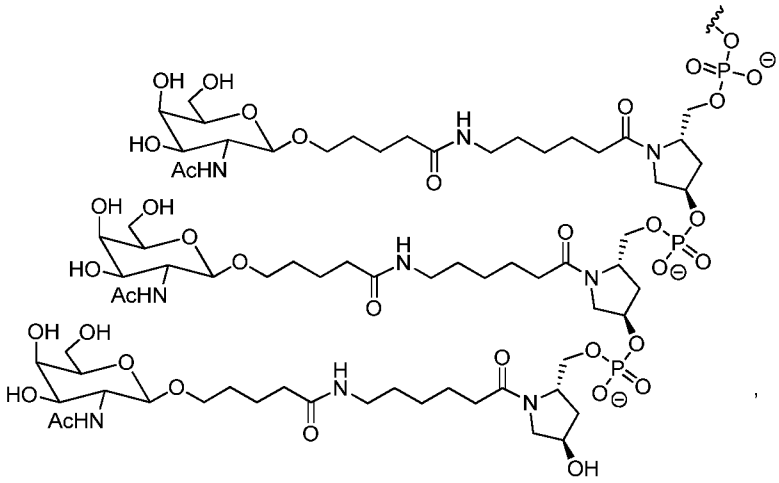


, wherein X is O or S (Formula XXVII);



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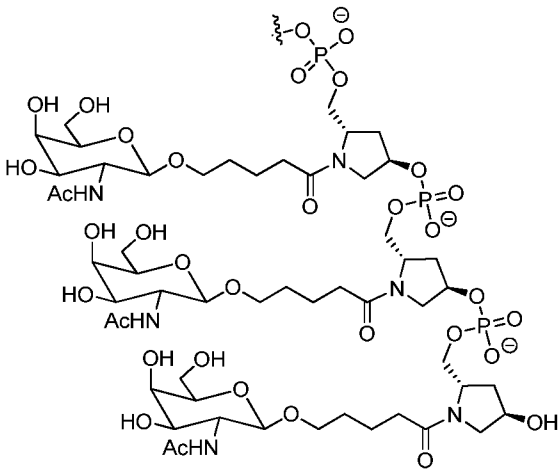

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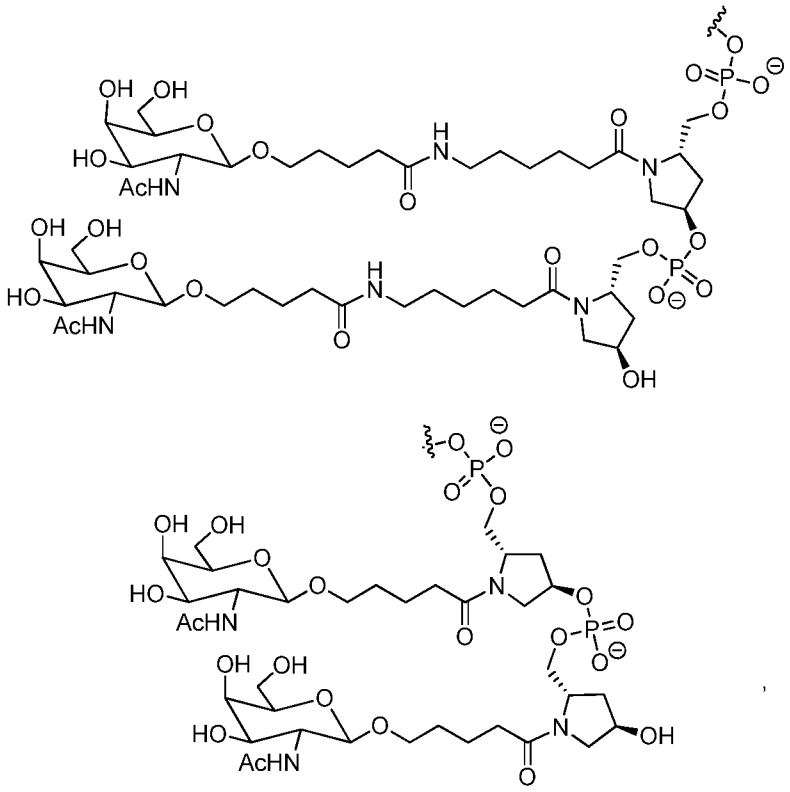
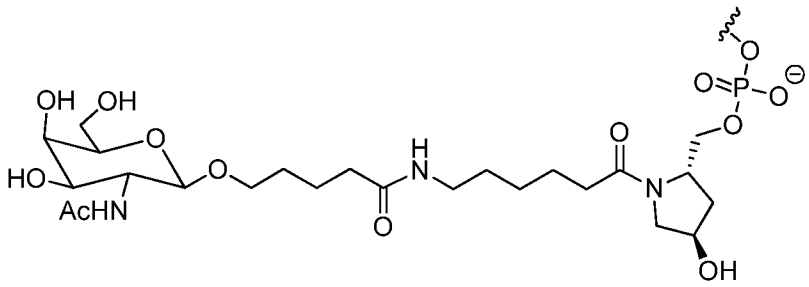
Formula XXVII; Formula XXIX;

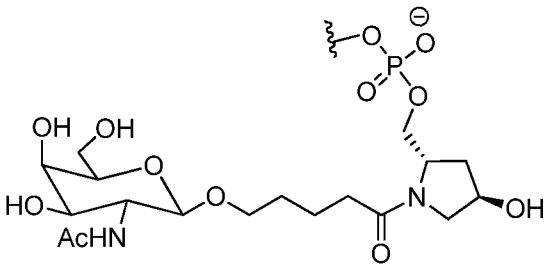


Formula XXX;

Formula XXXI;

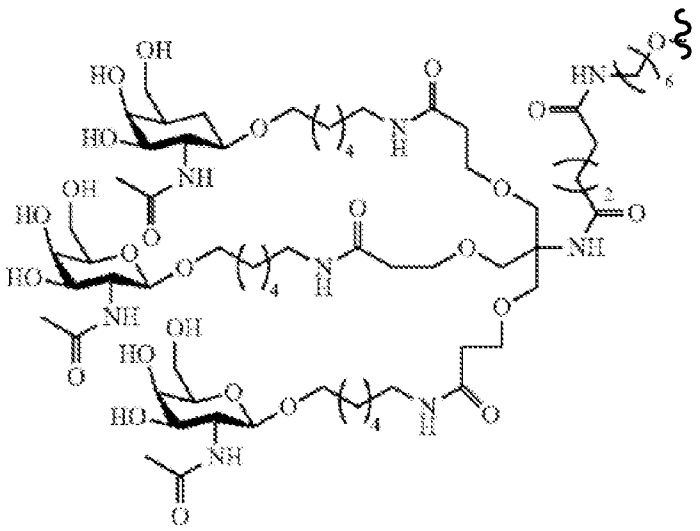


, and



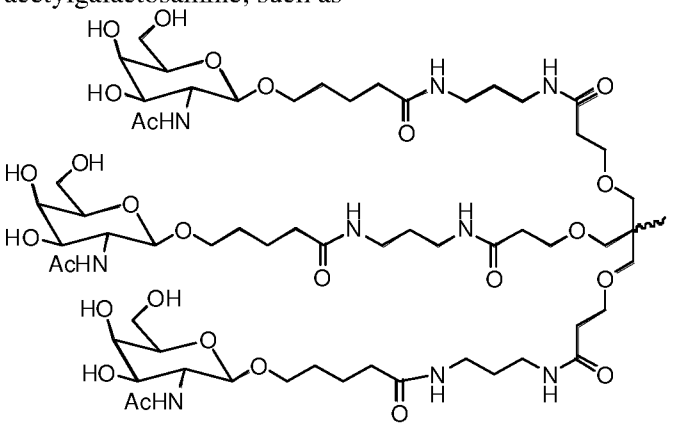
Formula XXXII;

Formula XXXIII.



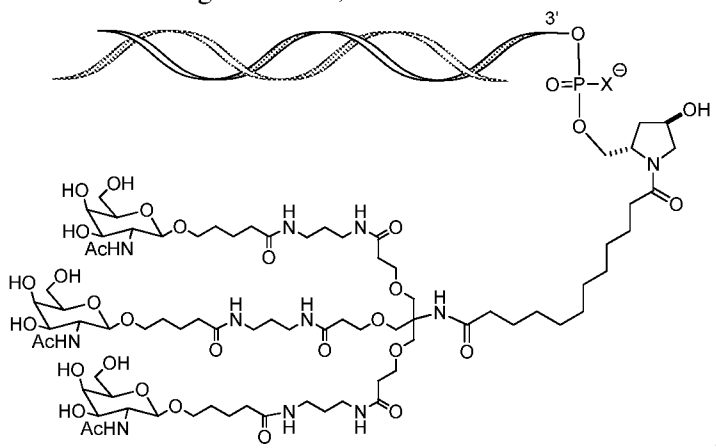
Formula XXXIV.

In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In one embodiment, the monosaccharide is an N-acetylgalactosamine, such as

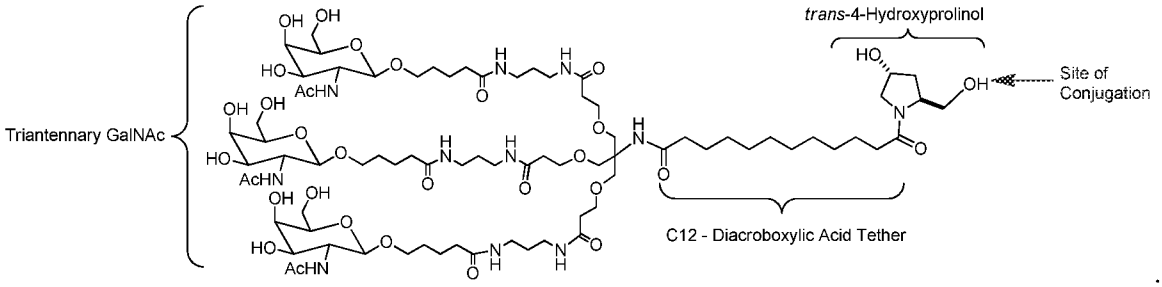


Formula II.

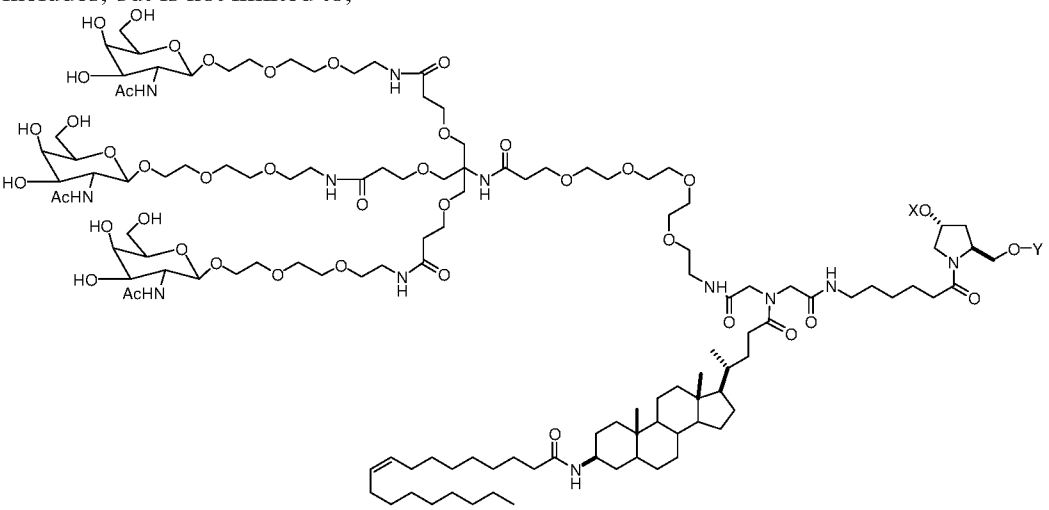
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate *via* a linker as shown in the following schematic, wherein X is O or S



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

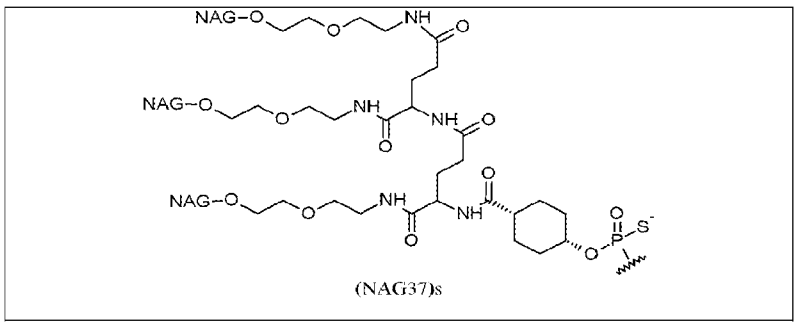


Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



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

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



In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc

derivative is attached to an iRNA agent of the invention *via* a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a trivalent linker.

In one embodiment, the double stranded RNAi agents of the invention comprise one or more GalNAc or GalNAc derivative attached to the iRNA agent. The GalNAc may be attached to any nucleotide *via* a linker on the sense strand or antsisense strand. The GalNac may be attached to the 5'-end of the sense strand, the 3' end of the sense strand, the 5'-end of the antisense strand, or the 3' – end of the antisense strand. In one embodiment, the GalNAc is attached to the 3' end of the sense strand, *e.g., via* a trivalent linker.

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

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

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

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

D. 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.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.,* covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO_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, or substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic, or substituted aliphatic. In one embodiment, the linker is about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18, 7-17, 8-17, 6-16, 7-17, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, or more, or at least 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 preferred 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. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

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

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the

blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 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 certain embodiments, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents 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 other embodiments, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S-. Preferred embodiments are -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -S-P(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O, -S-P(S)(H)-O-, -S-P(O)(H)-S-, and -O-P(S)(H)-S-. A

preferred embodiment is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

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

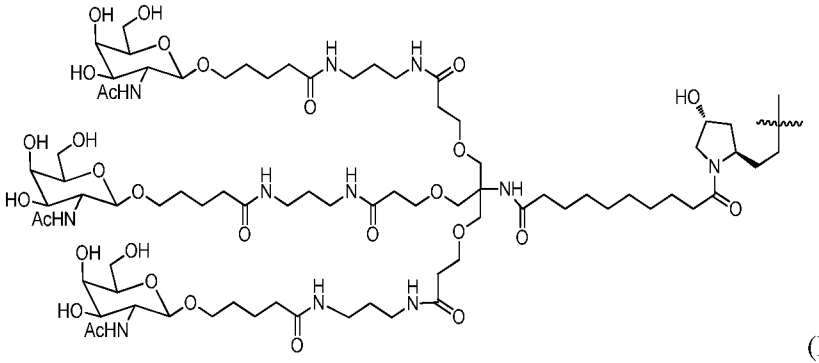
iv. Ester-based linking groups

In other 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.

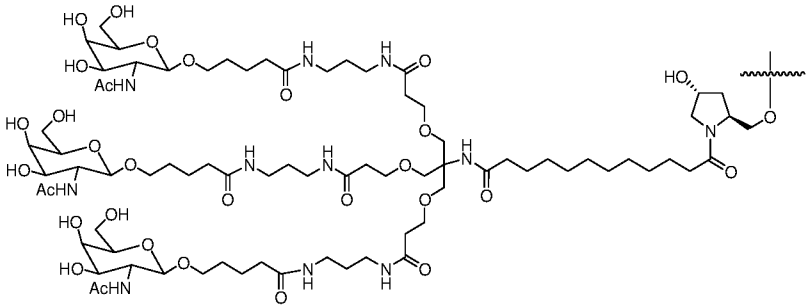
v. Peptide-based cleaving groups

In yet other 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.

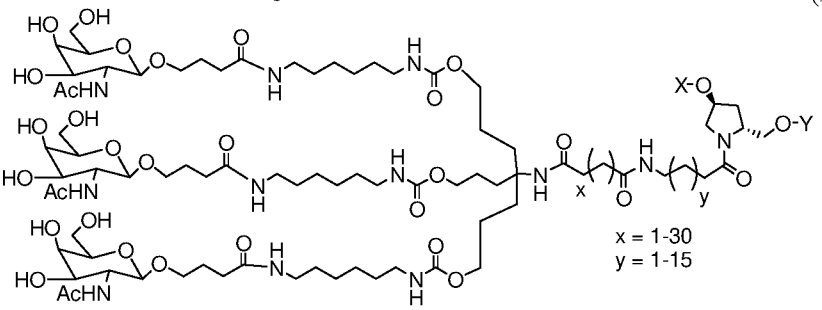
In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



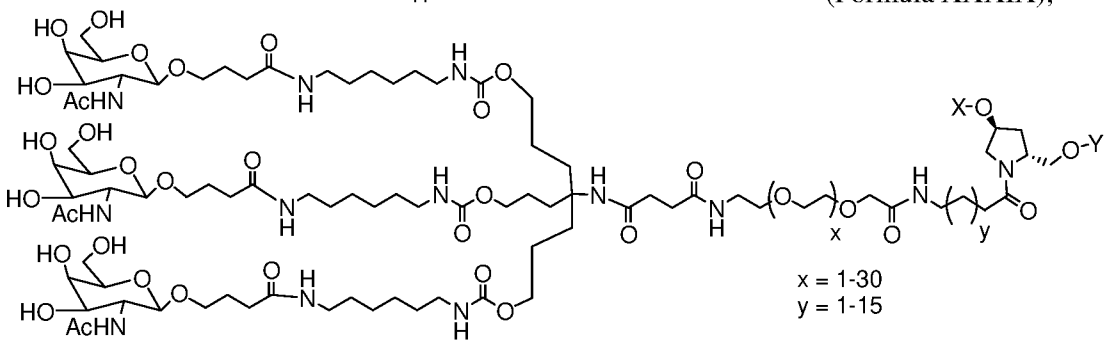
(Formula XXXVII),



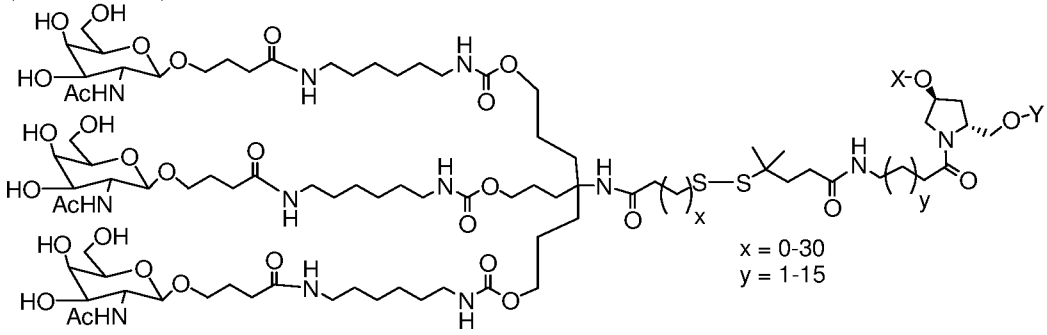
(Formula XXXVIII),



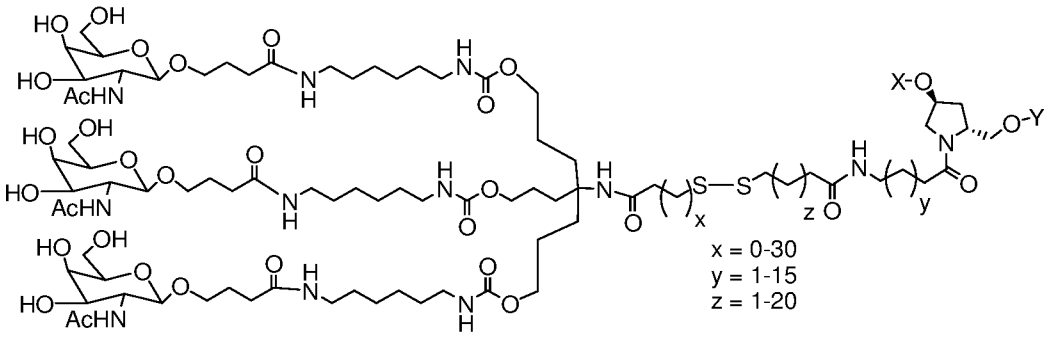
(Formula XXXIX),



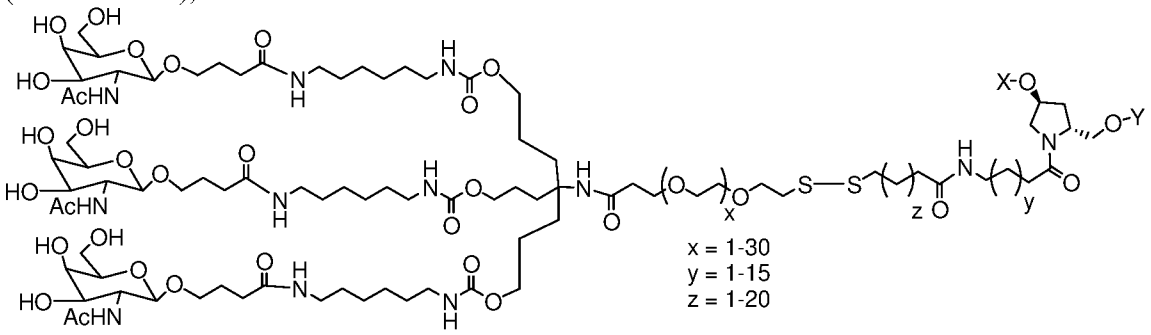
(Formula XL),



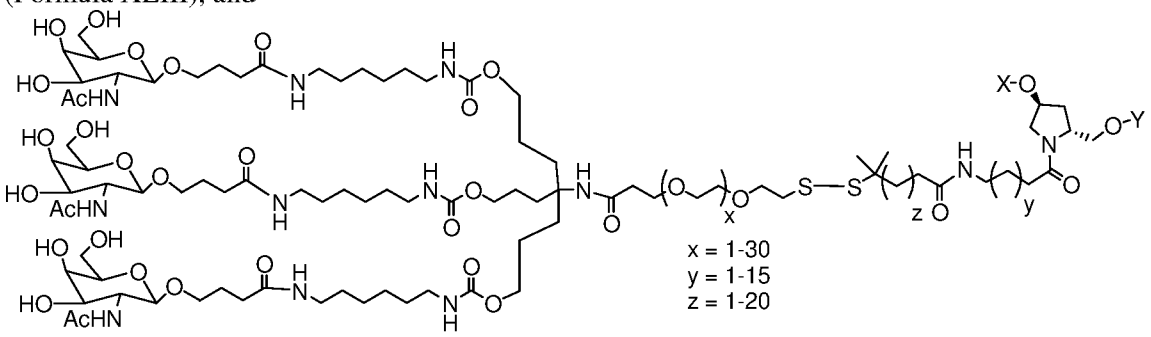
(Formula XLI),



(Formula XLII),



(Formula XLIII), and

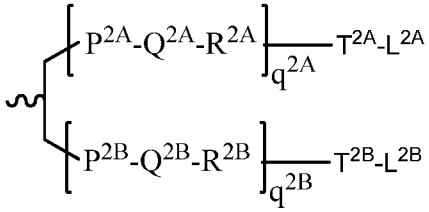


(Formula XLIV), when one of X or Y is an oligonucleotide, the other is a hydrogen.

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

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

Formula XXXXV



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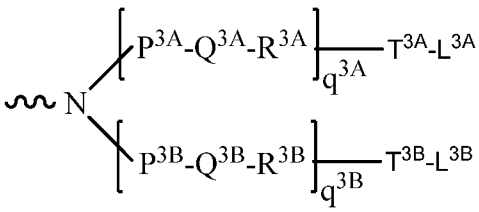
Formula XLVI



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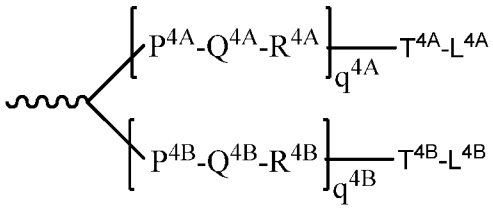
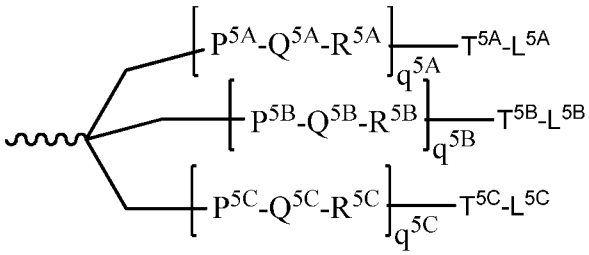


Formula (VI)

,



Formula (VII)

;

Formula XLVII

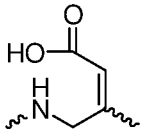
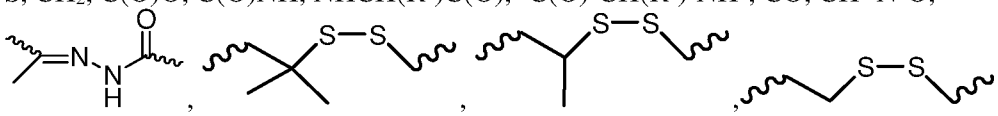
Formula XLVIII

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;

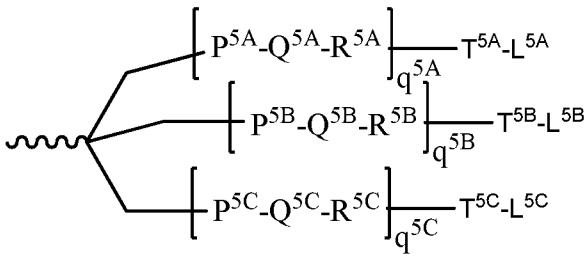
P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or CH_2O;

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,  ,  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 (XLIX):

Formula XLIX



Formula (VII),

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.

Representative U.S. Patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Patent Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928;5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; and 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

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

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

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 RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

IV. Delivery of an iRNA of the Invention

The delivery of an iRNA of the invention to a cell *e.g.,* a cell within a subject, such as a human subject (*e.g.,* a subject in need thereof, such as a subject susceptible to or diagnosed with a PNPLA3-associated disorder, *e.g.*, NAFLD) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an iRNA of the invention either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an iRNA, *e.g.,* a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the iRNA. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an iRNA of the invention (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). For *in vivo* delivery, factors to consider in order to deliver an iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. 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). Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA to the target tissue and avoid undesirable off-target effects. iRNA molecules can 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).

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) J*ournal 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*), "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.

A. Vector encoded iRNAs of the Invention

iRNA targeting the PNPLA3 gene 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. Patent 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).

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, *etc.*; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of 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 known in the art.

V. Pharmaceutical Compositions of the Invention

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for preventing or treating a PNPLA3-associated disorder, *e.g.*, hypertriglyceridemia. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration *via* parenteral delivery, *e.g.*, by subcutaneous (SC), intramuscular (IM), or intravenous (IV) delivery. The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a PNPLA3 gene.

In some embodiments, the pharmaceutical compositions of the invention are sterile. In another embodiment, the pharmaceutical compositions of the invention are pyrogen free.

The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a PNPLA3 gene. In general, a suitable dose of an iRNA of the invention will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. Typically, a suitable dose of an iRNA of the invention will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, preferably about 0.3 mg/kg and about 3.0 mg/kg. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as every month, once every 3-6 months, or once a year. In certain embodiments, the iRNA is administered about once per month to about once per six months.

After an initial treatment regimen, the treatments can be administered on a less frequent basis. Duration of treatment can be determined based on the severity of disease.

In other embodiments, a single dose of the pharmaceutical compositions can be long lasting, such that doses are administered at not more than 1, 2, 3, or 4 month intervals. In some embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered about once per month. In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered quarterly (*i.e.*, about every three months). In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered twice per year (*i.e.*, about once every six months).

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

The iRNA can be delivered in a manner to target a particular tissue (*e.g.*, hepatocytes).

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

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

A. Additional Formulations

i. Emulsions

The compositions of the present invention can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm 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 can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution either in the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

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. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich 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 can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic, and amphoteric (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich 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).

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).

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).

ii. Microemulsions

In one embodiment of the present invention, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil, and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich 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, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215).

iii. Microparticles

An iRNA of the invention may be incorporated into a particle, *e.g.*, a microparticle. Microparticles can be produced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

iv. Penetration Enhancers

In one embodiment, the present invention 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 can cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to

aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

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

v. Excipients

In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent, or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Such agent are well known in the art.

vi. Other Components

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

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

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA and (b) one or more agents which function by a non-iRNA mechanism and which are useful in treating a PNPLA33-associated disorder, *e.g.*, NAFLD.

Toxicity and prophylactic 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 prophylactically effective in 50% of

the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED50, preferably an ED80 or ED90, with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the prophylactically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) or higher levels of inhibition as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents used for the prevention or treatment of aPNPLA3-associated disorder, *e.g.*, NAFLD. 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.

VI. Methods For Inhibiting PNPLA3 Expression

The present invention also provides methods of inhibiting expression of a PNPLA3 gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, double stranded RNA agent, in an amount effective to inhibit expression of PNPLA3 in the cell, thereby inhibiting expression of PNPLA3 in the cell.

Contacting of a cell with an iRNA, *e.g.*, a double stranded RNA agent, may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the iRNA includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the iRNA. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible. Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished *via* a targeting ligand, including any ligand described herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a $GalNAc_3$ ligand, or any other ligand that directs the RNAi agent to a site of interest.

The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating", "suppressing", and other similar terms, and includes any level of inhibition.

The phrase "inhibiting expression of a PNPLA3" is intended to refer to inhibition of expression of any PNPLA3 gene (such as, *e.g.*, a mouse PNPLA3 3 gene, a rat PNPLA3 gene, a monkey PNPLA3 gene, or a human PNPLA3 gene) as well as variants or mutants of a PNPLA3 gene.

Thus, the PNPLA3 gene may be a wild-type PNPLA3 gene, a mutant PNPLA3 gene, or a transgenic PNPLA3 gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of a PNPLA3 gene" includes any level of inhibition of aPNPLA3 gene, *e.g.*, at least partial suppression of the expression of a PNPLA3 gene. The expression of the PNPLA3 gene may be assessed based on the level, or the change in the level, of any variable associated with PNPLA3 gene expression, *e.g.*, PNPLA3 mRNA level or PNPLA3 protein level. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject. It is understood that PNPLA3 is expressed predominantly in the liver, but also in the brain, gall bladder, heart, and kidney, and is present in circulation.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with PNPLA3 expression compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

In some embodiments of the methods of the invention, expression of a PNPLA3 gene is inhibited by at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In preferred embodiments, expression of a PNPLA3 gene is inhibited by at least 70%. It is further understood that inhibition of PNPLA3 expression in certain tissues, *e.g.*, in liver, without a significant inhibition of expression in other tissues, *e.g.*, brain, may be desirable. In preferred embodiments, expression level is determined using the assay method provided in Example 2 with a 10 nM siRNA concentration in the appropriate species matched cell line.

In certain embodiments, inhibition of expression *in vivo* is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.*, an AAV-infected mouse expressing the human target gene (*i.e.*,PNPLA3), *e.g.*, when administered as a single dose, *e.g.,* at 3 mg/kg at the nadir of RNA expression. Knockdown of expression of an endogenous gene in a model animal system can also be determined, *e.g.*, after administration of a single dose at, *e.g.,* 3 mg/kg at the nadir of RNA expression. Such systems are useful when the nucleic acid sequence of the human gene and the model animal gene are sufficiently close such that the human iRNA provides effective knockdown of the model animal gene. RNA expression in liver is determined using the PCR methods provided in Example 2.

Inhibition of the expression of a PNPLA3 gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which a PNPLA3 gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with an iRNA of the invention, or by administering an iRNA of the invention to a subject in which the cells are or were present) such that the expression of a PNPLA3 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s) not treated with an iRNA or not treated with an iRNA targeted to the gene of interest). In preferred embodiments, the inhibition is assessed by the method provided in Example 2 using a 10nM siRNA

concentration in the species matched cell line and expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

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

In other embodiments, inhibition of the expression of a PNPLA3 gene may be assessed in terms of a reduction of a parameter that is functionally linked to PNPLA3 gene expression, *e.g.*, PNPLA3 protein level in blood or serum from a subject. PNPLA3 gene silencing may be determined in any cell expressing PNPLA3, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of a PNPLA3 protein may be manifested by a reduction in the level of the PNPLA3 protein that is expressed by a cell or group of cells or in a subject sample (*e.g.*, the level of protein in a blood sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibition of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells, or the change in the level of protein in a subject sample, *e.g.*, blood or serum derived therefrom.

A control cell, a group of cells, or subject sample that may be used to assess the inhibition of the expression of a PNPLA3 gene includes a cell, group of cells, or subject sample that has not yet been contacted with an RNAi agent of the invention. For example, the control cell, group of cells, or subject sample may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent or an appropriately matched population control.

The level of PNPLA3 mRNA that is expressed by a cell or group of cells may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of PNPLA3 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.*, mRNA of the PNPLA3 gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy™ RNA preparation kits (Qiagen®) or PAXgene™ (PreAnalytix™, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays, northern blotting, *in situ* hybridization, and microarray analysis.

In some embodiments, the level of expression of PNPLA3 is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific PNPLA3. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to PNPLA3 mRNA. In one embodiment, the mRNA

is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix® gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of PNPLA3 mRNA.

An alternative method for determining the level of expression of PNPLA3 in a sample involves the process of nucleic acid amplification or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi *et al.*, U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of PNPLA3 is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System). In preferred embodiments, expression level is determined by the method provided in Example 2 using, *e.g.*, a 10nM siRNA concentration, in the species matched cell line.

The expression levels of PNPLA3 mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of PNPLA3 expression level may also comprise using nucleic acid probes in solution.

In preferred embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of these methods is described and exemplified in the Examples presented herein. In preferred embodiments, expression level is determined by the method provided in Example 2 using a 10nM siRNA concentration in the species matched cell line.

The level of PNPLA3 protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

In some embodiments, the efficacy of the methods of the invention are assessed by a decrease in PNPLA3 mRNA or protein level (*e.g.*, in a liver biopsy).

In some embodiments of the methods of the invention, the iRNA is administered to a subject such that the iRNA is delivered to a specific site within the subject. The inhibition of expression of PNPLA3 may be assessed using measurements of the level or change in the level of PNPLA3 mRNA or PNPLA3 protein in a sample derived from fluid or tissue from the specific site within the subject (*e.g.*, liver or blood).

As used herein, the terms detecting or determining a level of an analyte are understood to mean performing the steps to determine if a material, *e.g.*, protein, RNA, is present. As used herein, methods of detecting or determining include detection or determination of an analyte level that is below the level of detection for the method used.

VII. Prophylactic and Treatment Methods of the Invention

The present invention also provides methods of using an iRNA of the invention or a composition containing an iRNA of the invention to inhibit expression of PNPLA3, thereby preventing or treating an PNPLA3-associated disorder, *e.g.*, fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD). In the methods of the invention the cell may be contacted with the siRNA *in vitro* or *in vivo, i.e.,* the cell may be within a subject.

A cell suitable for treatment using the methods of the invention may be any cell that expresses an PNPLA3 gene, *e.g.*, a liver cell, a brain cell, a gall bladder cell, a heart cell, or a kidney cell, but preferably a liver cell. A cell suitable for use in the methods of the invention may be a mammalian cell, *e.g.,* a primate cell (such as a human cell, including human cell in a chimeric non-human animal, or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), or a non-primate cell. In certain embodiments, the cell is a human cell, *e.g.,* a human liver cell. In the methods of the invention, PNPLA3 expression is inhibited in the cell by at least 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95, or to a level below the level of detection of the assay.

The *in vivo* methods of the invention may include administering to a subject 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 the PNPLA3 gene of the mammal to which the RNAi agent is to be administered. The composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal, and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection. In certain embodiments, the compositions are administered by intramuscular injection.

In one aspect, the present invention also provides methods for inhibiting the expression of an PNPLA3 gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets a PNPLA3 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the PNPLA3 gene, thereby inhibiting expression of the PNPLA3 gene in the cell. Reduction in gene expression can be assessed by any methods known in the art and by methods, *e.g.* qRT-PCR, described herein, *e.g.*, in Example 2. Reduction in protein production can be assessed by any methods known it the art, *e.g.* ELISA. In certain embodiments, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in the PNPLA3 gene or protein expression. In other embodiments, a blood sample serves as the subject sample for monitoring the reduction in thePNPLA3 protein expression.

The present invention further provides methods of treatment in a subject in need thereof, *e.g.*, a subject diagnosed with a PNPLA3-associated disorder, such as, fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD).

The present invention further provides methods of prophylaxis in a subject in need thereof. The treatment methods of the invention include administering an iRNA of the invention to a subject, *e.g.*, a subject that would benefit from a reduction of PNPLA3 expression, in a prophylactically effective amount of an iRNA targeting a PNPLA3 gene or a pharmaceutical composition comprising an iRNA targeting a PNPLA3 gene.

In one aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in PNPLA3 expression, *e.g.,* a PNPLA3-associated disease, such as a chronic fibro-inflammatory liver disease (*e.g.,* cancer, *e.g.,* hepatocellular carcinoma, nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, and nonalcoholic fatty liver disease (NAFLD). In one embodiment, the chronic fibro-inflammatory liver disease is NASH.

In one embodiment, a PNPLA3-associated disease is selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD).

As used herein, "nonalcoholic fatty liver disease," used interchangeably with the term "NAFLD," refers to a disease defined by the presence of macrovascular steatosis in the presence of less than 20 gm of alcohol ingestion per day. NAFLD is the most common liver disease in the United States, and is commonly associated with insulin resistance/type 2 diabetes mellitus and obesity. NAFLD is manifested by steatosis, steatohepatitis, cirrhosis, and sometimes hepatocellaular carcinoma. For a review of NAFLD, see Tolman and Dalpiaz (2007) Ther. Clin. Risk. Manag., 3(6):1153-1163 the entire contents of which are incorporated herein by reference.

As used herein, the terms "steatosis," "hepatic steatosis," and "fatty liver disease" refer to the accumulation of triglycerides and other fats in the liver cells.

As used herein, ther term "Nonalcoholic steatohepatitis" or "NASH" refers to liver inflammation and damage caused by a buildup of fat in the liver. NASH is part of a group of conditions called nonalcoholic fatty liver disease (NAFLD). NASH resembles alcoholic liver disease, but occurs in people who drink little or no alcohol. The major feature in NASH is fat in the liver, along with inflammation and damage. Most people with NASH feel well and are not aware that they have a liver problem. Nevertheless, NASH can be severe and can lead to cirrhosis, in which the liver is permanently damaged and scarred and no longer able to work properly. NASH is usually first suspected in a person who is found to have elevations in liver tests that are included in routine blood test panels, such as alanine aminotransferase (ALT) or aspartate aminotransferase (AST). When further evaluation shows no apparent reason for liver disease (such as medications, viral hepatitis, or excessive use of alcohol) and when x rays or imaging studies of the liver show fat, NASH is suspected. The only means of proving a diagnosis of NASH and separating it from simple fatty liver is a liver biopsy.

As used herein, the term "cirrhosis," defined histologically, is a diffuse hepatic process characterized by fibrosis and conversion of the normal liver architecture into structurally abnormal nodules.

An iRNA of the invention may be administered as a "free iRNA." A free iRNA is administered in the absence of a pharmaceutical composition. The naked iRNA may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the iRNA can be adjusted such that it is suitable for administering to a subject.

Alternatively, an iRNA of the invention may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

Subjects that would benefit from an inhibition of PNPLA3 gene expression are subjects susceptible to or diagnosed with an PNPLA3-associated disorder, such as fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD).

In an embodiment, the method includes administering a composition featured herein such that expression of the target a PNPLA3 gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 1-6, 1-3, or 3-6 months per dose. In certain embodiments, the composition is administered once every 3-6 months.

Preferably, the iRNAs useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target a PNPLA3 gene. Compositions and methods for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

Administration of the iRNA according to the methods of the invention may result prevention or treatment of a PNPLA3-associated disorder, *e.g.*, fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD).

Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 mg/kg.

The iRNA is preferably administered subcutaneously, *i.e.*, by subcutaneous injection. One or more injections may be used to deliver the desired dose of iRNA to a subject. The injections may be repeated over a period of time.

The administration may be repeated on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as once per month to once a year. In certain embodiments, the iRNA is administered about once per month to about once every three months, or about once every three months to about once every six months.

The invention further provides methods and uses of an iRNA agent or a pharmaceutical composition thereof for treating a subject that would benefit from reduction and/or inhibition of PNPLA3 gene expression, *e.g.*, a subject having an PNPLA3-associated disease, in combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders.

Accordingly, in some aspects of the invention, the methods which include either a single iRNA agent of the invention, further include administering to the subject one or more additional therapeutic agents.

The iRNA agent and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

Examples of additional therapeutic agents include those known to treat hypertriglyceridemia and other diseases that are caused by, associated with, or are a consequence of, hypertriglyceridemia. For example, an iRNA featured in the invention can be administered with any such additional theraprutic agents. Examples of such agents include, but are not lmited to an HMG-CoA reductase inhibitor (*e.g.*, a statin), a fibrate, a bile acid sequestrant, niacin, an antiplatelet agent, an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist (e.g., losartan potassium, such as Merck & Co. 's Cozaar®), an acylCoA cholesterol acetyltransferase (ACAT) inhibitor, a cholesterol absorption inhibitor, a cholesterol ester transfer protein (CETP) inhibitor, a microsomal triglyceride transfer protein (MTTP) inhibitor, a cholesterol modulator, a bile acid modulator, a peroxisome proliferation activated receptor (PPAR) agonist, a gene-based therapy, a composite vascular protectant (*e.g.*, AGI-1067, from Atherogenics), a glycoprotein Ilb/IIIa inhibitor, aspirin or an aspirin- like compound, an IBAT inhibitor (*e.g.*, S-8921 , from Shionogi), a squalene synthase inhibitor, a monocyte chemoattractant protein (MCP)-I inhibitor, or fish oil. Exemplary HMG-CoA reductase inhibitors include atorvastatin (Pfizer's Lipitor®/Tahor/Sortis/Torvast/Cardyl), pravastatin (Bristol-Myers Squibb 's Pravachol, Sankyo's Mevalotin/Sanaprav), simvastatin (Merck's Zocor®/Sinvacor, Boehringer Ingelheim's Denan, Banyu's Lipovas), lovastatin (Merck's Mevacor/Mevinacor, Bexal's

Lovastatina, Cepa; Schwarz Pharma's Liposcler), fluvastatin (Novartis' Lescol®/Locol/Lochol, Fujisawa's Cranoc, Solvay's Digaril), cerivastatin (Bayer's Lipobay/GlaxoSmithKline's Baycol), rosuvastatin (AstraZeneca' s Crestor®), and pitivastatin (itavastatin/risivastatin) (Nissan Chemical, Kowa Kogyo, Sankyo, and Novartis). Exemplary fibrates include, e.g., bezafibrate (e.g., Roche's Befizal®/Cedur®/Bezalip®, Kissei's Bezatol), clofibrate (e.g., Wyeth's Atromid-S®), fenofibrate (e.g., Fournier's Lipidil/Lipantil, Abbott's Tricor®, Takeda's Lipantil, generics), gemfibrozil (e.g., Pfizer' s Lopid/Lipur) and ciprofibrate (Sanofi-Synthelabo's Modalim®). Exemplary bile acid sequestrants include, *e.g.*, cholestyramine (Bristol-Myers Squibb's Questran® and Questran Light™), colestipol (*e.g.*, Pharmacia's Colestid), and colesevelam (Genzyme/Sankyo's WelChol™). Exemplary niacin therapies include, e.g., immediate release formulations, such as Aventis' Nicobid, Upsher-Smith's Niacor, Aventis' Nicolar, and Sanwakagaku's Perycit. Niacin extended release formulations include, e.g., Kos Pharmaceuticals' Niaspan and Upsher-Smith's SIo- Niacin. Exemplary antiplatelet agents include, *e.g.*, aspirin (*e.g.*, Bayer's aspirin), clopidogrel (Sanofi-Synthelabo/Bristol-Myers Squibb's Plavix), and ticlopidine (e.g., Sanofi-Synthelabo's Ticlid and Daiichi's Panaldine). Other aspirin-like compounds useful in combination with a dsRNA targeting PNPLA3 include, *e.g.*, Asacard (slow-release aspirin, by Pharmacia) and Pamicogrel (Kanebo/Angelini Ricerche/CEPA). Exemplary angiotensin-converting enzyme inhibitors include, *e.g.*, ramipril (*e.g.*, Aventis' Altace) and enalapril (*e.g.*, Merck & Co.'s Vasotec). Exemplary acyl CoA cholesterol acetyltransferase (AC AT) inhibitors include, *e.g.*, avasimibe (Pfizer), eflucimibe (BioMsrieux Pierre Fabre/Eli Lilly), CS-505 (Sankyo and Kyoto), and SMP-797 (Sumito). Exemplary cholesterol absorption inhibitors include, *e.g.*, ezetimibe (Merck/Schering-Plough Pharmaceuticals Zetia®) and Pamaqueside (Pfizer). Exemplary CETP inhibitors include, *e.g.*, Torcetrapib (also called CP-529414, Pfizer), JTT-705 (Japan Tobacco), and CETi-I (Avant Immunotherapeutics). Exemplary microsomal triglyceride transfer protein (MTTP) inhibitors include, *e.g.*, implitapide (Bayer), R-103757 (Janssen), and CP-346086 (Pfizer). Other exemplary cholesterol modulators include, *e.g.*, NO- 1886 (Otsuka/TAP Pharmaceutical), CI- 1027 (Pfizer), and WAY- 135433 (Wyeth-Ayerst).

Exemplary bile acid modulators include, *e.g.*, HBS-107 (Hisamitsu/Banyu), Btg-511 (British Technology Group), BARI-1453 (Aventis), S-8921 (Shionogi), SD-5613 (Pfizer), and AZD- 7806 (AstraZeneca). Exemplary peroxisome proliferation activated receptor (PPAR) agonists include, *e.g.*, tesaglitazar (AZ-242) (AstraZeneca), Netoglitazone (MCC-555) (Mitsubishi/ Johnson & Johnson), GW-409544 (Ligand Pharniaceuticals/GlaxoSmithKline), GW-501516 (Ligand Pharmaceuticals/GlaxoSmithKline), LY-929 (Ligand Pharmaceuticals and Eli Lilly), LY-465608 (Ligand Pharmaceuticals and Eli Lilly), LY-518674 (Ligand Pharmaceuticals and Eli Lilly), and MK-767 (Merck and Kyorin). Exemplary gene-based therapies include, e.g. , AdGWEGF 121.10 (GenVec), ApoAl (UCB Pharma/Groupe Fournier), EG-004 (Trinam) (Ark Therapeutics), and ATP -binding cassette transporter-Al (ABCA1) (CV Therapeutics/Incyte, Aventis, Xenon). Exemplary Glycoprotein Ilb/IIIa inhibitors include, *e.g.*, roxifiban (also called DMP754, Bristol-Myers Squibb), Gantofiban (Merck KGaA/Yamanouchi), and Cromafiban (Millennium Pharmaceuticals). Exemplary squalene synthase inhibitors include, *e.g.*, BMS- 1884941 (Bristol-Myers Squibb), CP-210172 (Pfizer), CP-295697 (Pfizer),

CP-294838 (Pfizer), and TAK-475 (Takeda). An exemplary MCP-I inhibitor is, *e.g.*, RS-504393 (Roche Bioscience). The anti-atherosclerotic agent BO- 653 (Chugai Pharmaceuticals), and the nicotinic acid derivative Nyclin (Yamanouchi Pharmacuticals) are also appropriate for administering in combination with a dsRNA featured in the invention. Exemplary combination therapies suitable for administration with a dsRNA targeting PNPLA3 include, *e.g.*, advicor (Niacin/lovastatin from Kos Pharmaceuticals), amlodipine/atorvastatin (Pfizer), and ezetimibe/simvastatin (*e.g.*, Vytorin® 10/10, 10/20, 10/40, and 10/80 tablets by Merck/Schering-Plough Pharmaceuticals). Agents for treating hypertriglyceridemia, and suitable for administration in combination with a dsRNA targeting PNPLA3 include, *e.g.*, lovastatin, niacin Altoprev® Extended-Release Tablets (Andrx Labs), lovastatin Caduet® Tablets (Pfizer), amlodipine besylate, atorvastatin calcium Crestor® Tablets (AstraZeneca), rosuvastatin calcium Lescol® Capsules (Novartis), fluvastatin sodium Lescol® (Reliant, Novartis), fluvastatin sodium Lipitor® Tablets (Parke-Davis), atorvastatin calcium Lofibra® Capsules (Gate), Niaspan Extended-Release Tablets (Kos), niacin Pravachol Tablets (Bristol-Myers Squibb), pravastatin sodium TriCor® Tablets (Abbott), fenofibrate Vytorin® 10/10 Tablets (Merck/Schering-Plough Pharmaceuticals), ezetimibe, simvastatin WelChol™ Tablets (Sankyo), colesevelam hydrochloride Zetia® Tablets (Schering), ezetimibe Zetia® Tablets (Merck/Schering-Plough Pharmaceuticals), and ezetimibe Zocor® Tablets (Merck).

In some embodiments, an iRNA featured in the invention can be administered with, *e.g.*, pyridoxine, an ACE inhibitor (angiotensin converting enzyme inhibitors), *e.g.*, benazepril (Lotensin); an angiotensin II receptor antagonist (ARB) (*e.g.*, losartan potassium, such as Merck & Co. 's Cozaar®), *e.g.*, Candesartan (Atacand); an HMG-CoA reductase inhibitor (*e.g.*, a statin); calcium binding agents, *e.g.*, Sodium cellulose phosphate (Calcibind); diuretics, *e.g.*, thiazide diuretics, such as hydrochlorothiazide (Microzide); an insulin sensitizer, such as the PPARγ agonist pioglitazone, a glp-1r agonist, such as liraglutatide, vitamin E, an SGLT2 inhibitor, a DPPIV inhibitor, and kidney/liver transplant; or a combination of any of the foregoing.

In one embodiment, an iRNA agent is administered in combination with an ezetimibe/simvastatin combination (*e.g.*, Vytorin® (Merck/Schering-Plough Pharmaceuticals)). In one embodiment, the iRNA agent is administered to the patient, and then the additional therapeutic agent is administered to the patient (or vice versa). In another embodiment, the iRNA agent and the additional therapeutic agent are administered at the same time.

The iRNA agent and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

In one embodiment, an iRNA agent is administered in combination with an ezetimibe/simvastatin combination (*e.g.*, Vytorin® (Merck/Schering-Plough Pharmaceuticals)). In one embodiment, the iRNA agent is administered to the patient, and then the additional therapeutic agent is administered to the patient (or vice versa). In another embodiment, the iRNA agent and the additional therapeutic agent are administered at the same time.

The iRNA agent and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

VIII. Kits

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

Such kits include one or more dsRNA agent(s) and instructions for use, e.g., instructions for administering a prophylactically or therapeutically effective amount of a dsRNA agent(s). The dsRNA agent may be in a vial or a pre-filled syringe. The kits may optionally further comprise means for administering the dsRNA agent (*e.g.*, an injection device, such as a pre-filled syringe), or means for measuring the inhibition of PNPLA3 (*e.g.,* means for measuring the inhibition of PNPLA3 mRNA, PNPLA3 protein, and/or PNPLA3 activity). Such means for measuring the inhibition of PNPLA3 may comprise a means for obtaining a sample from a subject, such as, *e.g.*, a plasma sample. The kits of the invention may optionally further comprise means for determining the therapeutically effective or prophylactically effective amount.

In certain embodiments the individual components of the pharmaceutical formulation may be provided in one container, *e.g.,* a vial or a pre-filled syringe. Alternatively, it may be desirable to provide the components of the pharmaceutical formulation separately in two or more containers, *e.g.*, one container for a siRNA compound preparation, and at least another for a carrier compound. The kit may be packaged in a number of different configurations such as one or more containers in a single box. The different components can be combined, *e.g.*, according to instructions provided with the kit. The components can be combined according to a method described herein, *e.g.*, to prepare and administer a pharmaceutical composition. The kit can also include a delivery device.

This invention is further illustrated by the following examples which should not be construed as limiting. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the informal Sequence Listing and Figures, are hereby incorporated herein by reference.

EXAMPLES

Example 1. iRNA Synthesis

Source of reagents

Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

siRNA Design

siRNAs targeting the human Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene (human: NCBI refseqID NM_025225.2; NCBI GeneID: 80339) were designed using custom R and Python scripts. The human NM_025225.2 REFSEQ mRNA, has a length of 2805 bases.

Detailed lists of the unmodified PNPLA3 sense and antisense strand nucleotide sequences are shown in Table 2. Detailed lists of the modified PNPLA3 sense and antisense strand nucleotide sequences are shown in Table 3.

It is to be understood that, throughout the application, a duplex name without a decimal is equivalent to a duplex name with a decimal which merely references the batch number of the duplex. For example, AD-959917 is equivalent to AD-959917.1.

siRNA Synthesis

siRNAs were designed, synthesized, and prepared using methods known in the art.

Briefly, siRNA sequences were synthesized on a 1 μmol scale using a Mermade 192 synthesizer (BioAutomation) with phosphoramidite chemistry on solid supports. The solid support was controlled pore glass (500-1000 Å) loaded with a custom GalNAc ligand (3'-GalNAc conjugates), universal solid support (AM Chemicals), or the first nucleotide of interest. Ancillary synthesis reagents and standard 2-cyanoethyl phosphoramidite monomers (2'-deoxy-2'-fluoro, 2'-O-methyl, RNA, DNA) were obtained from Thermo-Fisher (Milwaukee, WI), Hongene (China), or Chemgenes (Wilmington, MA, USA). Additional phosphoramidite monomers were procured from commercial suppliers, prepared in-house, or procured using custom synthesis from various CMOs. Phosphoramidites were prepared at a concentration of 100 mM in either acetonitrile or 9:1 acetonitrile:DMF and were coupled using 5-Ethylthio-1H-tetrazole (ETT, 0.25 M in acetonitrile) with a reaction time of 400 s. Phosphorothioate linkages were generated using a 100 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (9:1 v/v). Oxidation time was 5 minutes. All sequences were synthesized with final removal of the DMT group ("DMT-Off").

Upon completion of the solid phase synthesis, solid-supported oligoribonucleotides were treated with 300 μL of Methylamine (40% aqueous) at room temperature in 96 well plates for approximately 2 hours to afford cleavage from the solid support and subsequent removal of all additional base-labile protecting groups. For sequences containing any natural ribonucleotide linkages

(2'-OH) protected with a tert-butyl dimethyl silyl (TBDMS) group, a second deprotection step was performed using TEA.3HF (triethylamine trihydrofluoride). To each oligonucleotide solution in aqueous methylamine was added 200 µL of dimethyl sulfoxide (DMSO) and 300 µL TEA.3HF and the solution was incubated for approximately 30 mins at 60 °C. After incubation, the plate was allowed to come to room temperature and crude oligonucleotides were precipitated by the addition of 1 mL of 9:1 acetontrile:ethanol or 1:1 ethanol:isopropanol. The plates were then centrifuged at 4 °C for 45 mins and the supernatant carefully decanted with the aid of a multichannel pipette. The oligonucleotide pellet was resuspended in 20 mM NaOAc and subsequently desalted using a HiTrap size exclusion column (5 mL, GE Healthcare) on an Agilent LC system equipped with an autosampler, UV detector, conductivity meter, and fraction collector. Desalted samples were collected in 96 well plates and then analyzed by LC-MS and UV spectrometry to confirm identity and quantify the amount of material, respectively.

Duplexing of single strands was performed on a Tecan liquid handling robot. Sense and antisense single strands were combined in an equimolar ratio to a final concentration of 10 µM in 1x PBS in 96 well plates, the plate sealed, incubated at 100 °C for 10 minutes, and subsequently allowed to return slowly to room temperature over a period of 2-3 hours. The concentration and identity of each duplex was confirmed and then subsequently utilized for in vitro screening assays.

Example 2. *In vitro* screening methods

Hep3B Cell culture and 384-well transfections

Hep3b and HepG2 cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 14.8 µl of Opti-MEM plus 0.2 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 µl of each siRNA duplex to an individual well in a 384-well plate. The mixture was then incubated at room temperature for 15 minutes. Eighty µl of complete growth media without antibiotic containing ~2 x10^4 Hep3B or HepG2 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 50 nM, 10 nM and 1 nM final duplex concentration.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen™, part #: 610-12)

Cells were lysed in 75µl of Lysis/Binding Buffer containing 3 µL of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 90µL) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 10µL RT mixture was added to each well, as described below.

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

A master mix of 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H_2O per reaction were added per well. Plates were sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37 degrees C for 2 hours. Following this, the plates were agitated at 80 degrees C for 8 minutes.

Real time PCR

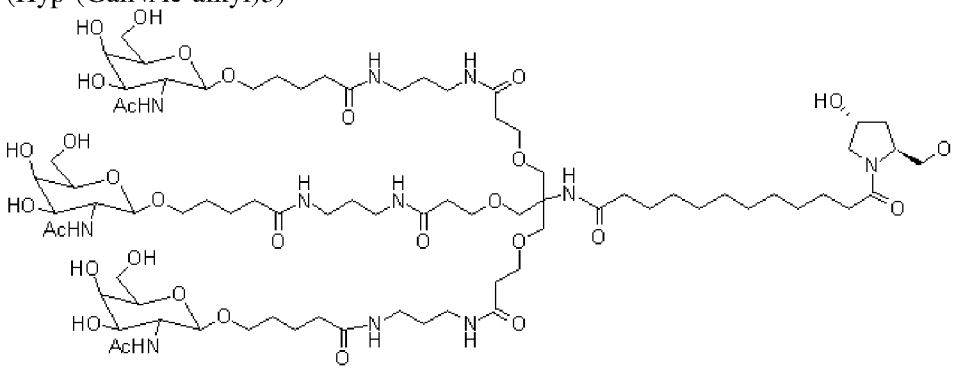
Two microlitre (µl) of cDNA were added to a master mix containing 0.5µl of human GAPDH TaqMan Probe (4326317E), 0.5µl human PNPLA3, 2µl nuclease-free water and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR is done in a LightCycler480 Real Time PCR system (Roche).

To calculate relative fold change, data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC_{50}s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 or mock-transfected. The sense and antisense sequences of AD-1955 are: sense: cuuAcGcuGAGuAcuucGAdTsdT (SEQ ID NO: 18) and antisense UCGAAGuACUcAGCGuAAGdTsdT (SEQ ID NO: 19).

The results of the screening of the dsRNA agents listed in Tables 1 and 2 in Hep3b cells are shown in Table 4. The results of the screening of the dsRNA agents listed in Tables 1 and 2 in HepG2 cells are shown in Table 5.

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; and it is understood that when the nucleotide contains a 2'-fluoro modification, then the fluoro replaces the hydroxy at that position in the parent nucleotide (i.e., it is a 2'-deoxy-2'-fluoronucleotide).

Abbre*via*tion	**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
As	adenosine-3'-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
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gb	beta-L-guanosine-3`-phosphate

Abbreviation	Nucleotide(s)
Gbs	beta-L-guanosine-3`-phosphorothioate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine -3'-phosphorothioate
Us	uridine -3'-phosphorothioate
N	any nucleotide, modified or unmodified
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'- phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'- phosphorothioate
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'- phosphorothioate
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage
L10	N-(cholesterylcarboxamidocaproyl)-4-hydroxyprolinol (Hyp-C6-Chol)
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol (Hyp-(GalNAc-alkyl)3) 
Y34	2-hydroxymethyl-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose)
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofurane-5-phosphate)
(Agn)	Adenosine-glycol nucleic acid (GNA)
(Cgn)	Cytidine-glycol nucleic acid (GNA)
(Ggn)	Guanosine-glycol nucleic acid (GNA)
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
P	Phosphate
VP	Vinyl-phosphonate
dA	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

Abbre*via*tion	**Nucleotide(s)**
dT	2`-deoxythymidine-3`-phosphate
dTs	2`-deoxythymidine-3`-phosphorothioate
dU	2`-deoxyuridine
dUs	2`-deoxyuridine-3`-phosphorothioate
(C2p)	cytidine-2`-phosphate
(G2p)	guanosine-2`-phosphate
(U2p)	uridine-2`-phosphate
(A2p)	adenosine-2`-phosphate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Ahd)	2'-O-hexadecyl-adenosine-3'-phosphate
(Ghd)	2'-O-hexadecyl-guanosine-3'-phosphate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate

Table 2. Unmodifed Sense and Antisense Strand Sequences of PNPLA3 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1526763	CGCGGCUGGAGCUUGUCCUUU	20	189-209	AAAGGACAAGCUCCAGCCGCGCU	301	187-209
AD-1526764	GCGGCUUCCUGGGCUUCUACU	21	217-237	AGUAGAAGCCCAGGAAGCCGCAG	302	215-237
AD-1526765	CGGCUUCCUGGGCUUCUACCU	22	218-238	AGGUAGAAGCCCAGGAAGCCGCA	303	216-238
AD-1526766	CGGCUUCCUGGGCUUCUACCU	22	218-238	AGGUAGAAGCCCAGGAAGCCGCA	303	216-238
AD-1526767	CUUCCUGGGCUUCUACCACGU	23	221-241	ACGUGGTAGAAGCCCAGGAAGCC	304	219-241
AD-1526768	CCUGGGCUUCUACCACGUCGU	24	224-244	ACGACGUGGUAGAAGCCCAGGAA	305	222-244
AD-1526769	CUGGGCUUCUACCACGUCGGU	25	225-245	ACCGACGUGGUAGAAGCCCAGGA	306	223-245
AD-1526770	CGCGACGCGCGCAUGUUGUUU	26	285-305	AAACAACAUGCGCGCGUCGCGGA	307	283-305
AD-1526771	CCGCUGGAGCAGACUCUGCAU	27	354-374	AUGCAGAGUCUGCUCCAGCGGGA	308	352-374
AD-1526772	GGAGCAGACUCUGCAGGUCCU	28	359-379	AGGACCTGCAGAGUCUGCUCCAG	309	357-379
AD-1526773	GACUCUGCAGGUCCUCUCAGU	29	365-385	ACUGAGAGGACCUGCAGAGUCUG	310	363-385
AD-1527072	CUCUGCAGGUCCUCUCAGAUU	30	367-387	AAUCTGAGAGGACCUGCAGAGUC	311	365-387
AD-1527073	CUGCAGGUCCUCUCAGAUCUU	31	369-389	AAGATCTGAGAGGACCUGCAGAG	312	367-389
AD-1526776	UGCAGGUCCUCUCAGAUCUUU	32	370-390	AAAGAUCUGAGAGGACCUGCAGA	313	368-390
AD-1526777	GCAGGUCCUCUCAGAUCUUGU	33	371-391	ACAAGAUCUGAGAGGACCUGCAG	314	369-391
AD-1526778	AGGCCAGGAGUCGGAACAUUU	34	397-417	AAAUGUTCCGACUCCUGGCCUUC	315	395-417
AD-1527074	GGCCAGGAGUCGGAACAUUGU	35	398-418	ACAATGTUCCGACUCCUGGCCUU	316	396-418
AD-1526780	GCCAGGAGUCGGAACAUUGGU	36	399-419	ACCAAUGUUCCGACUCCUGGCCU	317	397-419
AD-1526781	GCAUCUUCCAUCCAUCCUUCU	37	418-438	AGAAGGAUGGAUGGAAGAUGCCA	318	416-438
AD-1526782	UGCCUCCCGGCCAAUGUCCAU	38	474-494	AUGGACAUUGGCCGGGAGGCAUU	319	472-494
AD-1527075	CAAUGUCCACCAGCUCAUCUU	39	485-505	AAGATGAGCUGGUGGACAUUGGC	320	483-505
AD-1526784	AAUGUCCACCAGCUCAUCUCU	40	486-506	AGAGAUGAGCUGGUGGACAUUGG	321	484-506
AD-1526785	GUCCAAAGACGAAGUCGUGGU	41	572-592	ACCACGACUUCGUCUUUGGACCG	322	570-592
AD-1526786	UCCAAAGACGAAGUCGUGGAU	42	573-593	AUCCACGACUUCGUCUUUGGACC	323	571-593
AD-1526787	CCAAAGACGAAGUCGUGGAUU	43	574-594	AAUCCACGACUUCGUCUUUGGAC	324	572-594

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1526788	CAAAGACGAAGUCGUGGAUGU	44	575-595	ACAUCCACGACUUCGUCUUUGGA	325	573-595
AD-1526789	AGACGAAGUCGUGGAUGCCUU	45	578-598	AAGGCATCCACGACUUCGUCUUU	326	576-598
AD-1526790	CUUCUACAGUGGCCUUAUCCU	46	620-640	AGGAUAAGGCCACUGUAGAAGGG	327	618-640
AD-1526791	UUCUACAGUGGCCUUAUCCCU	47	621-641	AGGGAUAAGGCCACUGUAGAAGG	328	619-641
AD-1526792	UCUACAGUGGCCUUAUCCCUU	48	622-642	AAGGGAUAAGGCCACUGUAGAAG	329	620-642
AD-1526793	CUACAGUGGCCUUAUCCCUCU	49	623-643	AGAGGGAUAAGGCCACUGUAGAA	330	621-643
AD-1526794	AGUGGCCUUAUCCCUCCUUCU	50	627-647	AGAAGGAGGGAUAAGGCCACUGU	331	625-647
AD-1526795	UGGCCUUAUCCCUCCUUCCUU	51	629-649	AAGGAAGGAGGGAUAAGGCCACU	332	627-649
AD-1526796	CUUAUCCCUCCUUCCUUCAGU	52	633-653	ACUGAAGGAAGGAGGGAUAAGGC	333	631-653
AD-1526797	UUAUCCCUCCUUCCUUCAGAU	53	634-654	AUCUGAAGGAAGGAGGGAUAAGG	334	632-654
AD-1526798	CCCUCCUUCCUUCAGAGGCGU	54	638-658	ACGCCUCUGAAGGAAGGAGGGAU	335	636-658
AD-1526799	CCUCCUUCCUUCAGAGGCGUU	55	639-659	AACGCCUCUGAAGGAAGGAGGGA	336	637-659
AD-1526800	CCUCCUUCCUUCAGAGGCGUU	55	639-659	AACGCCTCUGAAGGAAGGAGGGA	337	637-659
AD-1526801	CCUUCCUUCAGAGGCGUGCGU	56	642-662	ACGCACGCCUCUGAAGGAAGGAG	338	640-662
AD-1526802	UCCUUCAGAGGCGUGCGAUAU	57	645-665	AUAUCGCACGCCUCUGAAGGAAG	339	643-665
AD-1526803	CUUCAGAGGCGUGCGAUAUGU	58	647-667	ACAUAUCGCACGCCUCUGAAGGA	340	645-667
AD-1526804	UUCAGAGGCGUGCGAUAUGUU	59	648-668	AACAUAUCGCACGCCUCUGAAGG	341	646-668
AD-1526805	UCAGAGGCGUGCGAUAUGUGU	60	649-669	ACACAUAUCGCACGCCUCUGAAG	342	647-669
AD-1526806	CAGAGGCGUGCGAUAUGUGGU	61	650-670	ACCACAUAUCGCACGCCUCUGAA	343	648-670
AD-1526807	AGAGGCGUGCGAUAUGUGGAU	62	651-671	AUCCACAUAUCGCACGCCUCUGA	344	649-671
AD-1526808	AGGCGUGCGAUAUGUGGAUGU	63	653-673	ACAUCCACACAUAUCGCACGCCUCU	345	651-673
AD-1526809	GGCGUGCGAUAUGUGGAUGGU	64	654-674	ACCAUCCACACAUAUCGCACGCCUC	346	652-674
AD-1526810	CGUGCGAUAUGUGGAUGGAGU	65	656-676	ACUCCAUCCACACAUAUCGCACGCC	347	654-676
AD-1526811	GUGCGAUAUGUGGAUGGAGGU	66	657-677	ACCUCCAUCCACACAUAUCGCACGC	348	655-677
AD-1526812	GUGAGUGACACAACGUACCCUUU	67	678-698	AAAGGGTACGUUGUCACACUCACUC	349	676-698
AD-1526813	AGUGACAACGUACCCUUCAUU	68	681-701	AAUGAAGGGUACGUUGUCACACUCA	350	679-701

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1526814	GUGACAACGUACCCUUCAUUU	69	682-702	AAAUGAAGGGUACGUUGUCACUC	351	680-702
AD-1526815	UGACAACGUACCCUUCAUUGU	70	683-703	ACAAUGAAGGGUACGUUGUCACU	352	681-703
AD-1526816	GACAACGUACCCUUCAUUGAU	71	684-704	AUCAAUGAAGGGUACGUUGUCAC	353	682-704
AD-1526817	ACAACGUACCCUUCAUUGAUU	72	685-705	AAUCAAUGAAGGGUACGUUGUCA	354	683-705
AD-1526818	CAACGUACCCUUCAUUGAUGU	73	686-706	ACAUCAAUGAAGGGUACGUUGUC	355	684-706
AD-1526819	AACGUACCCUUCAUUGAUGCU	74	687-707	AGCAUCAAUGAAGGGUACGUUGU	356	685-707
AD-1526820	CGUACCCUUCAUUGAUGCCAU	75	689-709	AUGGCATCAAUGAAGGGUACGUU	357	687-709
AD-1526821	GUACCCUUCAUUGAUGCCAAU	76	690-710	AUUGGCAUCAAUGAAGGGUACGU	358	688-710
AD-1526822	UACGACAUCUGCCCUAAAGUU	77	744-764	AACUUUAGGGCAGAUGUCGUACU	359	742-764
AD-1526823	CGACAUCUGCCCUAAAGUCAU	78	746-766	AUGACUUUAGGGCAGAUGUCGUA	360	744-766
AD-1526824	GACAUCUGCCCUAAAGUCAAU	79	747-767	AUUGACTUUAGGGCAGAUGUCGU	361	745-767
AD-1526825	ACAUCUGCCCUAAAGUCAAGU	80	748-768	ACUUGACUUUAGGGCAGAUGUCG	362	746-768
AD-1526826	UCUGCCCUAAAGUCAAGUCCU	81	751-771	AGGACUUGACUUUAGGGCAGAUG	363	749-771
AD-1526827	UCUGCCCUAAAGUCAAGUCCU	81	751-771	AGGACUTGACUUUAGGGCAGAUG	364	749-771
AD-1526828	CUGCCCUAAAGUCAAGUCCAU	82	752-772	AUGGACTUGACUUUAGGGCAGAU	365	750-772
AD-1526829	AUGUGGACAUCACCAAGCUCU	83	784-804	AGAGCUUGGUGAUGUCCACAUGA	366	782-804
AD-1526830	AUGUGGACAUCACCAAGCUCU	83	784-804	AGAGCUTGGUGAUGUCCACAUGA	367	782-804
AD-1526831	UGUGGACAUCACCAAGCUCAU	84	785-805	AUGAGCTUGGUGAUGUCCACAUG	368	783-805
AD-1527076	GUCUACGCCUCUGCACAGGGU	85	805-825	ACCCTGTGCAGAGGCGUAGACUG	369	803-825
AD-1526833	CCUCUGCACAGGGAACCUCUU	86	812-832	AAGAGGTUCCCUGUGCAGAGGCG	370	810-832
AD-1526834	UGCACAGGGAACCUCUACCUU	87	816-836	AAGGUAGAGGUUCCCUGUGCAGA	371	814-836
AD-1526835	GCACAGGGAACCUCUACCUUU	88	817-837	AAAGGUAGAGGUUCCCUGUGCAG	372	815-837
AD-1526836	CACAGGGAACCUCUACCUUCU	89	818-838	AGAAGGTAGAGGUUCCCUGUGCA	373	816-838
AD-1526837	CAGGGAACCUCUACCUUCUCU	90	820-840	AGAGAAGGUAGAGGUUCCCUGUG	374	818-840
AD-1526838	AGGGAACCUCUACCUUCUCUU	91	821-841	AAGAGAAGGUAGAGGUUCCCUGU	375	819-841
AD-1526839	GGGAACCUCUACCUUCUCUCU	92	822-842	AGAGAGAAGGUAGAGGUUCCCUG	376	820-842

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1527077	CAAGGUGCUGGGAGAGAUAUU	93	866-886	AAUATCTCUCCCAGCACCUUGAG	377	864-886
AD-1526841	AAGGUGCUGGGAGAGAUAUGU	94	867-887	ACAUAUCUCUCCCAGCACCUUGA	378	865-887
AD-1526842	AGGUGCUGGGAGAGAUAUGCU	95	868-888	AGCAUAUCUCUCCCAGCACCUUG	379	866-888
AD-1526843	GGUGCUGGGAGAGAUAUGCCU	96	869-889	AGGCAUAUCUCUCCCAGCACCUU	380	867-889
AD-1526844	GUGCUGGGAGAGAUAUGCCUU	97	870-890	AAGGCATAUCUCUCCCAGCACCU	381	868-890
AD-1526845	UGCUGGGAGAGAUAUGCCUUU	98	871-891	AAGGCAUAUCUCUCCCAGCACC	382	869-891
AD-1526846	UGGGAGAGAUAUGCCUUCGAU	99	874-894	AUCGAAGGCAUAUCUCUCCCAGC	383	872-894
AD-1526847	GGGAGAGAUAUGCCUUCGAGU	100	875-895	ACUCGAAGGCAUAUCUCUCCCAG	384	873-895
AD-1526848	GGAGAGAUAUGCCUUCGAGGU	101	876-896	ACCUCGAAGGCAUAUCUCUCCCA	385	874-896
AD-1526849	GAGAGAUAUGCCUUCGAGGAU	102	877-897	AUCCUCGAAGGCAUAUCUCUCCC	386	875-897
AD-1526850	GAGAUAUGCCUUCGAGGAUAU	103	879-899	AUAUCCTCGAAGGCAUAUCUCUC	387	877-899
AD-1526851	GAUAUGCCUUCGAGGAUAUUU	104	881-901	AAAUAUCCUCGAAGGCAUAUCUC	388	879-901
AD-1526852	AUAUGCCUUCGAGGAUAUUUU	105	882-902	AAAAUAUCCUCGAAGGCAUAUCU	389	880-902
AD-1526853	UAUGCCUUCGAGGAUAUUUGU	106	883-903	ACAAAUAUCCUCGAAGGCAUAUC	390	881-903
AD-1526854	AUGCCUUCGAGGAUAUUUGGU	107	884-904	ACCAAAUAUCCUCGAAGGCAUAU	391	882-904
AD-1526855	UGCCUUCGAGGAUAUUUGGAU	108	885-905	AUCCAAAUAUCCUCGAAGGCAUA	392	883-905
AD-1526856	CAUUCAGGUUCUUGGAAGAGU	109	907-927	ACUCUUCCAAGAACCUGAAUGCA	393	905-927
AD-1526857	AGGUUCUUGGAAGAGAAGGGU	110	912-932	ACCCUUCUCUUCCAAGAACCUGA	394	910-932
AD-1526858	GGUUCUUGGAAGAGAAGGGCU	111	913-933	AGCCCUUCUCUUCCAAGAACCUG	395	911-933
AD-1526859	GGUUCUUGGAAGAGAAGGGCU	111	913-933	AGCCCUTCUCUUCCAAGAACCUG	396	911-933
AD-1526860	GUUCUUGGAAGAGAAGGGCAU	112	914-934	AUGCCCTUCUCUUCCAAGAACCU	397	912-934
AD-1526861	AAGAGAAGGGCAUCUGCAACU	113	922-942	AGUUGCAGAUGCCCUUCUCUUCC	398	920-942
AD-1527078	GCCUGAAGUCAUCCUCAGAAU	114	955-975	AUUCTGAGGAUGACUUCAGGCCU	399	953-975
AD-1526863	CUGAAGUCAUCCUCAGAAGGU	115	957-977	ACCUUCUGAGGAUGACUUCAGGC	400	955-977
AD-1526864	UGAAGUCAUCCUCAGAAGGGU	116	958-978	ACCCUUCUGAGGAUGACUUCAGG	401	956-978
AD-1526865	GAAGUCAUCCUCAGAAGGGAU	117	959-979	AUCCCUUCUGAGGAUGACUUCAG	402	957-979

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1526866	AAGUCAUCCUCAGAAGGGAUU	118	960-980	AAUCCCUUCUGAGGAUGACUUCA	403	958-980
AD-1526867	AAGUCAUCCUCAGAAGGGAUU	118	960-980	AAUCCCTUCUGAGGAUGACUUCA	404	958-980
AD-1526868	AGUCAUCCUCAGAAGGGAUGU	119	961-981	ACAUCCCUUCUGAGGAUGACUUC	405	959-981
AD-1526869	UCAUCCUCAGAAGGGAUGGAU	120	963-983	AUCCAUCCCUUCUGAGGAUGACU	406	961-983
AD-1526870	GGGAGAUGAGCUGCUAGACCU	121	1064-1084	AGGUCUAGCAGCUCAUCUCCCUC	407	1062-1084
AD-1527079	GGAGAUGAGCUGCUAGACCAU	122	1065-1085	AUGGTCTAGCAGCUCAUCUCCCU	408	1063-1085
AD-1526872	AGAUGAGCUGCUAGACCACCU	123	1067-1087	AGGUGGTCUAGCAGCUCAUCUCC	409	1065-1087
AD-1526873	CUGCUAGACCACCUGCGUCUU	124	1074-1094	AAGACGCAGGUGGUCUAGCAGCU	410	1072-1094
AD-1526874	CUAGACCACCUGCGUCUCAGU	125	1077-1097	ACUGAGACGCAGGUGGUCUAGCA	411	1075-1097
AD-1526875	CUAGACCACCUGCGUCUCAGU	125	1077-1097	ACUGAGACGCAGGUGGUCUAGCA	411	1075-1097
AD-1526876	ACCACCUGCGUCUCAGCAUCU	126	1081-1101	AGAUGCTGAGACGCAGGUGGUCU	412	1079-1101
AD-1526877	GCGUCUCAGCAUCCUGCCCUU	127	1088-1108	AAGGGCAGGAUGCUGAGACGCAG	413	1086-1108
AD-1526878	GCAUCCUGCCCUGGGAUGAGU	128	1096-1116	ACUCAUCCCAGGGCAGGAUGCUG	414	1094-1116
AD-1526879	CAUCCUGCCCUGGGAUGAGAU	129	1097-1117	AUCUCATCCCAGGGCAGGAUGCU	415	1095-1117
AD-1527080	AUCCUGCCCUGGGAUGAGAGU	130	1098-1118	ACUCTCAUCCCCAGGGCAGGAUGC	416	1096-1118
AD-1526881	UGCCCUGGGAUGAGAGCAUCU	131	1102-1122	AGAUGCTCUCAUCCCAGGGCAGG	417	1100-1122
AD-1526882	CCUGGGAUGAGAGCAUCCUGU	132	1105-1125	ACAGGAUGCUCUCAUCCCAGGGC	418	1103-1125
AD-1526883	AAUGAAAGACAAAGGUGGAUU	133	1166-1186	AAUCCACCUUUGUCUUUCAUUUC	419	1164-1186
AD-1526884	AUGAAAGACAAAGGUGGAUAU	134	1167-1187	AUAUCCACCUUUGUCUUUCAUUU	420	1165-1187
AD-1527081	AGACAAAGGUGGAUACAUGAU	135	1172-1192	AUCATGTAUCCACCUUUGUCUUU	421	1170-1192
AD-1526886	ACAAAGGUGGAUACAUGAGCU	136	1174-1194	AGCUCAUGUAUCCACCUUUGUCU	422	1172-1194
AD-1527082	CAAAGGUGGAUACAUGAGCAU	137	1175-1195	AUGCTCAUGUAUCCACCUUUGUC	423	1173-1195
AD-1526888	AAGGUGGAUACAUGAGCAAGU	138	1177-1197	ACUUGCTCAUGUAUCCACCUUUG	424	1175-1197
AD-1526889	GGAUACAUGAGCAAGAUUUGU	139	1182-1202	ACAAAUCUUGCUCAUGUAUCCAC	425	1180-1202
AD-1526890	GAUACAUGAGCAAGAUUUGCU	140	1183-1203	AGCAAAUCUUGCUCAUGUAUCCA	426	1181-1203
AD-1526891	AUACAUGAGCAAGAUUUGCAU	141	1184-1204	AUGCAAAUCUUGCUCAUGUAUCC	427	1182-1204

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1526892	UACAUGAGCAAGAUUUGCAAU	142	1185-1205	AUUGCAAAUCUUGCUCAUGUAUC	428	1183-1205
AD-1526893	ACAUGAGCAAGAUUUGCAACU	143	1186-1206	AGUUGCAAAUCUUGCUCAUGUAU	429	1184-1206
AD-1526894	UGAGCAAGAUUUGCAACUUGU	144	1189-1209	ACAAGUUGCAAAUCUUGCUCAUG	430	1187-1209
AD-1526895	GAGCAAGAUUUGCAACUUGCU	145	1190-1210	AGCAAGTUGCAAAUCUUGCUCAU	431	1188-1210
AD-1526896	AGCAAGAUUUGCAACUUGCUU	146	1191-1211	AAGCAAGUUGCAAAUCUUGCUCA	432	1189-1211
AD-1526897	GCAAGAUUUGCAACUUGCUAU	147	1192-1212	AUAGCAAGUUGCAAAUCUUGCUC	433	1190-1212
AD-1526898	GCAAGAUUUGCAACUUGCUAU	147	1192-1212	AUAGCAAGUUGCAAAUCUUGCUC	433	1190-1212
AD-1526899	UGCAACUUGCUACCCAUUAGU	148	1200-1220	ACUAAUGGGUAGCAAGUUGCAAA	434	1198-1220
AD-1526900	GCAACUUGCUACCCAUUAGGU	149	1201-1221	ACCUAAUGGGUAGCAAGUUGCAA	435	1199-1221
AD-1526901	CAACUUGCUACCCAUUAGGAU	150	1202-1222	AUCCUAAUGGGUAGCAAGUUGCA	436	1200-1222
AD-1526902	AACUUGCUACCCAUUAGGAUU	151	1203-1223	AAUCCUAAUGGGUAGCAAGUUGC	437	1201-1223
AD-1526903	ACUUGCUACCCAUUAGGAUAU	152	1204-1224	AUAUCCTAAUGGGUAGCAAGUUG	438	1202-1224
AD-1526904	GCUGCCCUGUACCCUGCCUGU	153	1238-1258	ACAGGCAGGGUACAGGGCAGCAU	439	1236-1258
AD-1526905	CCCUGUACCCUGCCUGUGGAU	154	1242-1262	AUCCACAGGCAGGGUACAGGGCA	440	1240-1262
AD-1526906	AAUCUGCCAUUGCGAUUGUCU	155	1261-1281	AGACAAUCGCAAUGGCAGAUUCC	441	1259-1281
AD-1526907	CUGCCAUUGCGAUUGUCCAGU	156	1264-1284	ACUGGACAAUCGCAAUGGCAGAU	442	1262-1284
AD-1526908	UGCCAUUGCGAUUGUCCAGAU	157	1265-1285	AUCUGGACAAUCGCAAUGGCAGA	443	1263-1285
AD-1527083	CAUUGCGAUUGUCCAGAGACU	158	1268-1288	AGUCTCTGGACAAUCGCAAUGGC	444	1266-1288
AD-1526910	UUGCGAUUGUCCAGAGACUGU	159	1270-1290	ACAGUCUCUGGACAAUCGCAAUG	445	1268-1290
AD-1527084	UUGCGAUUGUCCAGAGACUGU	159	1270-1290	ACAGTCTCUGGACAAUCGCAAUG	446	1268-1290
AD-1526912	UGCGAUUGUCCAGAGACUGGU	160	1271-1291	ACCAGUCUCUGGACAAUCGCAAU	447	1269-1291
AD-1526913	GCGAUUGUCCAGAGACUGGUU	161	1272-1292	AACCAGTCUCUGGACAAUCGCAA	448	1270-1292
AD-1526914	CGAUUGUCCAGAGACUGGUGU	162	1273-1293	ACACCAGUCUCUGGACAAUCGCA	449	1271-1293
AD-1526915	GAUUGUCCAGAGACUGGUGAU	163	1274-1294	AUCACCAGUCUCUGGACAAUCGC	450	1272-1294
AD-1527085	UGUCCAGAGACUGGUGACAUU	164	1277-1297	AAUGTCACCAGUCUCUGGACAAU	451	1275-1297
AD-1526917	CAGAGACUGGUGACAUGGCUU	165	1281-1301	AAGCCAUGUCACCAGUCUCUGGA	452	1279-1301

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1526918	AGAGACUGGUGACAUGGCUUU	166	1282-1302	AAAGCCAUGUCACCAGUCUCUGG	453	1280-1302
AD-1526919	AGAGACUGGUGACAUGGCUUU	166	1282-1302	AAAGCCAUGUCACCAGUCUCUGG	453	1280-1302
AD-1526920	ACUGGUGACAUGGCUUCCAGU	167	1286-1306	ACUGGAAGCCAUGUCACCAGUCU	454	1284-1306
AD-1526921	CUGGUGACAUGGCUUCCAGAU	168	1287-1307	AUCUGGAAGCCAUGUCACCAGUC	455	1285-1307
AD-1527086	GUGACAUGGCUUCCAGAUAUU	169	1290-1310	AAUATCTGGAAGCCAUGUCACCA	456	1288-1310
AD-1526923	UGACAUGGCUUCCAGAUAUGU	170	1291-1311	ACAUAUCUGGAAGCCAUGUCACC	457	1289-1311
AD-1526924	GACAUGGCUUCCAGAUAUGCU	171	1292-1312	AGCAUAUCUGGAAGCCAUGUCAC	458	1290-1312
AD-1526925	ACAUGGCUUCCAGAUAUGCCU	172	1293-1313	AGGCAUAUCUGGAAGCCAUGUCA	459	1291-1313
AD-1526926	CAUGGCUUCCAGAUAUGCCCU	173	1294-1314	AGGGCATAUCUGGAAGCCAUGUC	460	1292-1314
AD-1526927	AUGGCUUCCAGAUAUGCCCGU	174	1295-1315	ACGGGCAUAUCUGGAAGCCAUGU	461	1293-1315
AD-1526928	AUGGCUUCCAGAUAUGCCCGU	174	1295-1315	ACGGGCAUAUCUGGAAGCCAUGU	461	1293-1315
AD-1526929	GCUUCCAGAUAUGCCCGACGU	175	1298-1318	ACGUCGGGCAUAUCUGGAAGCCA	462	1296-1318
AD-1526930	GCAGUGGGUGACCUCACAGGU	176	1331-1351	ACCUGUGAGGUCACCCACUGCAA	463	1329-1351
AD-1526931	CCUCCAGGUCCCAAAUGCCAU	177	1384-1404	AUGGCATUUGGGACCUGGAGGCG	464	1382-1404
AD-1526932	CUCCAGGUCCCAAAUGCCAGU	178	1385-1405	ACUGGCAUUUGGGACCUGGAGGC	465	1383-1405
AD-1526933	CUCCAGGUCCCAAAUGCCAGU	178	1385-1405	ACUGGCAUUUGGGACCUGGAGGC	465	1383-1405
AD-1526934	AGGUCCCAAAUGCCAGUGAGU	179	1389-1409	ACUCACUGGCAUUUGGGACCUGG	466	1387-1409
AD-1527087	GUCCCAAAUGCCAGUGAGCAU	180	1391-1411	AUGCTCACUGGCAUUUGGGACCU	467	1389-1411
AD-1527088	CCUCAGGUCCAGCCUGAACUU	181	1520-1540	AAGUTCAGGCUGGACCUGAGGAU	468	1518-1540
AD-1526937	UCAGGUCCAGCCUGAACUUCU	182	1522-1542	AGAAGUUCAGGCUGGACCUGAGG	469	1520-1542
AD-1526938	UCAGGUCCAGCCUGAACUUCU	182	1522-1542	AGAAGUTCAGGCUGGACCUGAGG	470	1520-1542
AD-1526939	CAGGUCCAGCCUGAACUUCUU	183	1523-1543	AAGAAGTUCAGGCUGGACCUGAG	471	1521-1543
AD-1526940	AGGUCCAGCCUGAACUUCUUU	184	1524-1544	AAAGAAGUUCAGGCUGGACCUGA	472	1522-1544
AD-1526941	GGUCCAGCCUGAACUUCUUCU	185	1525-1545	AGAAGAAGUUCAGGCUGGACCUG	473	1523-1545
AD-1526942	UUGGGCAAUAAAGUACCUGCU	186	1545-1565	AGCAGGTACUUUAUUGCCCAAGA	474	1543-1565
AD-1526943	GGCAAUAAAGUACCUGCUGGU	187	1548-1568	ACCAGCAGGUACUUUAUUGCCCA	475	1546-1568

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1526944	AAUAAAGUACCUGCUGGUGCU	188	1551-1571	AGCACCAGCAGGUACUUUAUUGC	476	1549-1571
AD-1526945	AAUAAAGUACCUGCUGGUGCU	188	1551-1571	AGCACCAGCAGGUACUUUAUUGC	476	1549-1571
AD-1526946	AAAGUACCUGCUGGUGCUGAU	189	1554-1574	AUCAGCACCAGCAGGUACUUUAU	477	1552-1574
AD-1526947	ACUUGAGGAGGCGAGUCUAGU	190	1623-1643	ACUAGACUCGCCUCCUCAAGUGA	478	1621-1643
AD-1526948	GUUUCCCAUCUUUGUGCAGCU	191	1666-1686	AGCUGCACAAAGAUGGGAAACUU	479	1664-1686
AD-1526949	UCCCAUCUUUGUGCAGCUACU	192	1669-1689	AGUAGCUGCACAAAGAUGGGAAA	480	1667-1689
AD-1526950	CAUCUUUGUGCAGCUACCUCU	193	1672-1692	AGAGGUAGCUGCACAAAGAUGGG	481	1670-1692
AD-1526951	CAUCUUUGUGCAGCUACCUCU	193	1672-1692	AGAGGUAGCUGCACAAAGAUGGG	481	1670-1692
AD-1526952	UGUGCAGCUACCUCCGCAUUU	194	1678-1698	AAAUGCGGAGGUAGCUGCACAAA	482	1676-1698
AD-1526953	UGCAGCUACCUCCGCAUUGCU	195	1680-1700	AGCAAUGCGGAGGUAGCUGCACA	483	1678-1700
AD-1526954	UGCCUGUGACGUGGAGGAUCU	196	1714-1734	AGAUCCTCCACGUCACAGGCAGG	484	1712-1734
AD-1526955	CAGCCUCUGAGCUGAGUUGGU	197	1735-1755	ACCAACUCAGCUCAGAGGCUGGG	485	1733-1755
AD-1526956	AGCCUCUGAGCUGAGUUGGUU	198	1736-1756	AACCAACUCAGCUCAGAGGCUGG	486	1734-1756
AD-1526957	GCCUCUGAGCUGAGUUGGUUU	199	1737-1757	AAACCAACUCAGCUCAGAGGCUG	487	1735-1757
AD-1526958	CCUCUGAGCUGAGUUGGUUUU	200	1738-1758	AAAACCAACUCAGCUCAGAGGCU	488	1736-1758
AD-1526959	CUCUGAGCUGAGUUGGUUUUU	201	1739-1759	AAAAACCAACUCAGCUCAGAGGC	489	1737-1759
AD-1526960	UCUGAGCUGAGUUGGUUUUAU	202	1740-1760	AUAAAACCAACUCAGCUCAGAGG	490	1738-1760
AD-1526961	CUGAGCUGAGUUGGUUUUAUU	203	1741-1761	AAUAAAACCAACUCAGCUCAGAG	491	1739-1761
AD-1526962	UGAGCUGAGUUGGUUUUAUGU	204	1742-1762	ACAUAAAACCAACUCAGCUCAGA	492	1740-1762
AD-1526963	GAGCUGAGUUGGUUUUAUGAU	205	1743-1763	AUCAUAAAACCAACUCAGCUCAG	493	1741-1763
AD-1526964	AGCUGAGUUGGUUUUAUGAAU	206	1744-1764	AUUCAUAAAACCAACUCAGCUCA	494	1742-1764
AD-1526965	GCUGAGUUGGUUUUAUGAAAU	207	1745-1765	AUUUCATAAAACCAACUCAGCUC	495	1743-1765
AD-1193373	CUGAGUUGGUUUUAUGAAAAU	208	1746-1766	AUUUTCAUAAAACCAACUCAGCU	496	1744-1766
AD-1526967	GAGUUGGUUUUAUGAAAAGCU	209	1748-1768	AGCUUUUCAUAAAACCAACUCAG	497	1746-1768
AD-1526968	AGUUGGUUUUAUGAAAAGCUU	210	1749-1769	AAGCUUUUCAUAAAACCAACUCA	498	1747-1769
AD-1526969	GUUGGUUUUAUGAAAAGCUAU	211	1750-1770	AUAGCUTUUCAUAAAACCAACUC	499	1748-1770

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1526970	UUGGUUUUAUGAAAAGCUAGU	212	1751-1771	ACUAGCUUUUCAUAAAACCAACU	500	1749-1771
AD-1526971	UUGGUUUUAUGAAAAGCUAGU	212	1751-1771	ACUAGCTUUUCAUAAAACCAACU	501	1749-1771
AD-1526972	UGGUUUUAUGAAAAGCUAGGU	213	1752-1772	ACCUAGCUUUUCAUAAAACCAAC	502	1750-1772
AD-1526973	GUUUUAUGAAAAGCUAGGAAU	214	1754-1774	AUUCCUAGCUUUUCAUAAAACCA	503	1752-1774
AD-1526974	GUUUUAUGAAAAGCUAGGAAU	214	1754-1774	AUUCCUAGCUUUUCAUAAAACCA	503	1752-1774
AD-1526975	UUUUAUGAAAAGCUAGGAAGU	215	1755-1775	ACUUCCUAGCUUUUCAUAAAACC	504	1753-1775
AD-1526976	UUUUAUGAAAAGCUAGGAAGU	215	1755-1775	ACUUCCTAGCUUUUCAUAAAACC	505	1753-1775
AD-1526977	UAUGAAAAGCUAGGAAGCAAU	216	1758-1778	AUUGCUTCCUAGCUUUUCAUAAA	506	1756-1778
AD-1526978	AUUCAGCUGGUUGGGAAAUGU	217	1832-1852	ACAUUUCCCAACCAGCUGAAUUA	507	1830-1852
AD-1526979	CAGCUGGUUGGGAAAUGACAU	218	1835-1855	AUGUCATUUCCCAACCAGCUGAA	508	1833-1855
AD-1527089	AGCUGGUUGGGAAAUGACACU	219	1836-1856	AGUGTCAUUUCCCAACCAGCUGA	509	1834-1856
AD-1526981	GUGCAGAGGGUCCCUUACUGU	220	1867-1887	ACAGUAAGGGACCCUCUGCACUG	510	1865-1887
AD-1526982	UGCAGAGGGUCCCUUACUGAU	221	1868-1888	AUCAGUAAGGGACCCUCUGCACU	511	1866-1888
AD-1526983	GCAGAGGGUCCCUUACUGACU	222	1869-1889	AGUCAGTAAGGGACCCUCUGCAC	512	1867-1889
AD-1526984	UUAAUGGUCAGACUGUUCCAU	223	1904-1924	AUGGAACAGUCUGACCAUUAAUA	513	1902-1924
AD-1526985	UAAUGGUCAGACUGUUCCAGU	224	1905-1925	ACUGGAACAGUCUGACCAUUAAU	514	1903-1925
AD-1526986	ACGACACUGCCUGUCAGGUGU	225	2078-2098	ACACCUGACAGGCAGUGUCGUUC	515	2076-2098
AD-1526987	ACACCUUUUUCACCUAACUAU	226	2178-2198	AUAGUUAGGUGAAAAAGGUGUUC	516	2176-2198
AD-1526988	CACCUUUUUCACCUAACUAAU	227	2179-2199	AUUAGUTAGGUGAAAAAGGUGUU	517	2177-2199
AD-1526989	ACCUUUUUCACCUAACUAAAU	228	2180-2200	AUUUAGTUAGGUGAAAAAGGUGU	518	2178-2200
AD-1526990	CUUUUUCACCUAACUAAAAUU	229	2182-2202	AAUUUUAGUUAGGUGAAAAAGGU	519	2180-2202
AD-1526991	UUUUUCACCUAACUAAAAUAU	230	2183-2203	AUAUUUUAGUUAGGUGAAAAAGG	520	2181-2203
AD-1526992	UUUUCACCUAACUAAAAUAAU	231	2184-2204	AUUAUUUUAGUUAGGUGAAAAAG	521	2182-2204
AD-1526993	UUUCACCUAACUAAAAUAAUU	232	2185-2205	AAUUAUUUUAGUUAGGUGAAAAA	522	2183-2205
AD-1526994	UUCACCUAACUAAAAUAAUGU	233	2186-2206	ACAUUAUUUUAGUUAGGUGAAAA	523	2184-2206
AD-1526995	UCACCUAACUAAAAUAAUGUU	234	2187-2207	AACAUUAUUUUAGUUAGGUGAAA	524	2185-2207

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1526996	CACCUAACUAAAAAUAAUGUUU	235	2188-2208	AAACAUUAUUUUAGUUAGGUGAA	525	2186-2208
AD-1526997	ACCUAACUAAAAAUAAUGUUUU	236	2123-2143	AAAACAUUAUUUUAGUUAGGUGA	526	2121-2143
AD-1526998	CCUAACUAAAAAUAAUGUUUAU	237	2124-2144	AUAAACAUUAUUUUAGUUAGGUG	527	2122-2144
AD-1526999	CCUAACUAAAAAUAAUGUUUAU	237	2124-2144	AUAAACAUUAUUUUAGUUAGGUG	527	2122-2144
AD-1527000	CUAACUAAAAAUAAUGUUUAAU	238	2125-2145	AUUAAACAUUAUUUUAGUUAGGU	528	2123-2145
AD-1527001	UAACUAAAAAUAAUGUUUAAAU	239	2126-2146	AUUUAAACAUUAUUUUAGUUAGG	529	2124-2146
AD-1527002	AACUAAAAAUAAUGUUUAAAGU	240	2127-2147	ACUUUAAACAUUAUUUUAGUUAG	530	2125-2147
AD-1527003	ACUAAAAAUAAUGUUUAAAGAU	241	2128-2148	AUCUUUAAACAUUAUUUUAGUUA	531	2126-2148
AD-1527004	ACCUGUUGAAUUUUUGUAUUAU	242	2245-2265	AUAAUACAAAAAUUCAACAGGUAA	532	2243-2265
AD-1527005	CCUGUUGAAUUUUUGUAUUAUU	243	2180-2200	AAUAAUACAAAAAUUCAACAGGUA	533	2178-2200
AD-1527006	UGCGGCUUCCUGGGCUUCUAU	244	216-236	AUAGAAGCCCAGGAAGCCGCAGC	534	214-236
AD-1527090	UUCCUGGGCUUCUACCACGUU	245	222-242	AACGTGGUAGAAGCCCAGGAAGC	535	220-242
AD-1527008	CCCGCUGGAGCAGACUCUGCU	246	353-373	AGCAGAGUCUGCUCCAGCGGGAU	536	351-373
AD-1527009	CAGACUCUGCAGGUCCUCUCU	247	363-383	AGAGAGGACCUGCAGAGUCUGCU	537	361-383
AD-1527010	ACUCUGCAGGUCCUCUCAGAU	248	366-386	AUCUGAGAGGACCUGCAGAGUCU	538	364-386
AD-1527011	UCUGCAGGUCCUCUCAGAUCU	249	368-388	AGAUCUGAGAGGACCUGCAGAGU	539	366-388
AD-1527012	UGCAGGUCCUCUCAGAUCUUU	32	370-390	AAAGAUCUGAGAGGACCUGCAGA	313	368-390
AD-1527013	CAUCUUCCAUCCAUCCUUCAU	250	419-439	AUGAAGGAUGGAUGGAAGAUGCC	540	417-439
AD-1527014	AAUGUCCACCAGCUCAUCUCU	40	486-506	AGAGAUGAGCUGGUGGACAUUGG	321	484-506
AD-1527015	UACAGUGGCCUUAUCCCUCCU	251	624-644	AGGAGGGAUAAGGCCACUGUAGA	541	622-644
AD-1527016	CAGUGGCCUUAUCCCUCCUUU	252	626-646	AAAGGAGGGAUAAGGCCACUGUA	542	624-646
AD-1527017	GUGGCCUUAUCCCUCCUUCCU	253	628-648	AGGAAGGAGGGAUAAGGCCACUG	543	626-648
AD-1527018	CCCUCCUUCCUUCAGAGGCGU	54	638-658	ACGCCUCUGAAGGAAGGAGGGAU	335	636-658
AD-1527019	UGAGUGACAACGUACCCUUCU	254	679-699	AGAAGGGUACGUUGUCACUCACU	544	677-699
AD-1527020	GAGUGACAACGUACCCUUCAU	255	680-700	AUGAAGGGUACGUUGUCACUCAC	545	678-700
AD-1527021	ACGUACCCUUCAUUGAUGCCU	256	688-708	AGGCAUCAAUGAAGGGUACGUUG	546	686-708

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1527022	AGUACGACAUCUGCCCUAAAU	257	742-762	AUUUAGGGCAGAUGUCGUACUCC	547	740-762
AD-1527091	AUCUGCCCUAAAGUCAAGUCU	258	750-770	AGACUUGACUUUAGGGCAGAUGU	548	748-770
AD-1527024	CUCUGCACAGGGAACCUCUAU	259	813-833	AUAGAGGUUCCCUGUGCAGAGGC	549	811-833
AD-1527025	UCUGCACAGGGAACCUCUACU	260	814-834	AGUAGAGGUUCCCUGUGCAGAGG	550	812-834
AD-1527026	ACAGGGAACCUCUACCUUCUU	261	819-839	AAGAAGGUAGAGGUUCCCUGUGC	551	817-839
AD-1527027	CUGGGAGAGAUAUGCCUUCGU	262	873-893	ACGAAGGCAUAUCUCUCCCAGCA	552	871-893
AD-1527028	UGGGAGAGAUAUGCCUUCGAU	99	874-894	AUCGAAGGCAUAUCUCUCCCAGC	383	872-894
AD-1527029	AGAGAUAUGCCUUCGAGGAUU	263	878-898	AAUCCUCGAAGGCAUAUCUCUCC	553	876-898
AD-1527092	AGAUAUGCCUUCGAGGAUAUU	264	880-900	AAUATCCUCGAAGGCAUAUCUCU	554	878-900
AD-1527031	GAUAUGCCUUCGAGGAUAUUU	104	881-901	AAAUAUCCUCGAAGGCAUAUCUC	388	879-901
AD-1527032	GAAGAGAAGGGCAUCUGCAAU	265	921-941	AUUGCAGAUGCCCUUCUCUUCCA	555	919-941
AD-1527033	GAGCUGCUAGACCACCUGCGU	266	1071-1091	ACGCAGGUGGUCUAGCAGCUCAU	556	1069-1091
AD-1527034	GACCACCUGCGUCUCAGCAUU	267	1080-1100	AAUGCUGAGACGCAGGUGGUCUA	557	1078-1100
AD-1527035	CACCUGCGUCUCAGCAUCCUU	268	1083-1103	AAGGAUGCUGAGACGCAGGUGGU	558	1081-1103
AD-1527036	CCCUGGGAUGAGAGCAUCCUU	269	1104-1124	AAGGAUGCUCUCUCAUCCCAGGGCA	559	1102-1124
AD-1527093	AGGUGGAUACAUGAGCAAGAU	270	1178-1198	AUCUUGCUCAUGUAUCCACCUUU	560	1176-1198
AD-1527094	GGUGGAUACAUGAGCAAGAUU	271	1179-1199	AAUCUUGCUCAUGUAUCCACCUU	561	1177-1199
AD-1527095	CAUGAGCAAGAUUUGCAACUU	272	1187-1207	AAGUUGCAAAUCUUUGCUCAUGUA	562	1185-1207
AD-1527096	AUGAGCAAGAUUUGCAACUUU	273	1188-1208	AAAGUUGCAAAUCUUUGCUCAUGU	563	1186-1208
AD-1527041	UUUGCAACUUGCUACCCAUUU	274	1198-1218	AAAUGGGUAGCAAGUUUGCAAAUC	564	1196-1218
AD-1527042	AUGCUGCCCUGUACCCUGCCU	275	1236-1256	AGGCAGGGUACAGGGCAGCAUUA	565	1234-1256
AD-1527097	GCCAUUGCGAUUUGUCCAGAGU	276	1266-1286	ACUCUGGACAAUCGCAAUGGCAG	566	1264-1286
AD-1527044	CCAUUGCGAUUUGUCCAGAGAU	277	1267-1287	AUCUCUGGACAAUCGCAAUGGCA	567	1265-1287
AD-1527045	AUUGCGAUUUGUCCAGAGACUU	278	1269-1289	AAGUCUCUGGACAAUCGCAAUGG	568	1267-1289
AD-1527046	CCAGAGACUGGUGACAUGGCU	279	1280-1300	AGCCAUGUCACCAGUCUCUGGAC	569	1278-1300
AD-1527047	GACUGGUGACAUGGCUUCCAU	280	1285-1305	AUGGAAGCCAUGUCACCAGUCUC	570	1283-1305

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2
AD-1527098	UGGUGACAUGGCUUCCAGAUU	281	1288-1308	AAUCUGGAAGCCAUGUCACCAGU	571	1286-1308
AD-1527049	GGUGACAUGGCUUCCAGAUAU	282	1289-1309	AUAUCUGGAAGCCAUGUCACCAG	572	1287-1309
AD-1527050	UGGCUUCCAGAUAUGCCCGAU	283	1296-1316	AUCGGGCAUAUCUGGAAGCCAUG	573	1294-1316
AD-1527051	GGCUUCCAGAUAUGCCCGACU	284	1297-1317	AGUCGGGCAUAUCUGGAAGCCAU	574	1295-1317
AD-1527052	GUUGCAGUGGGUGACCUCACU	285	1328-1348	AGUGAGGUCACCCACUGCAACCA	575	1326-1348
AD-1527099	CCAGGUCCCAAAUGCCAGUGU	286	1387-1407	ACACTGGCAUUUGGGACCUGGAG	576	1385-1407
AD-1527054	AGGUCCAGCCUGAACUUCUUU	184	1524-1544	AAAGAAGUUCAGGCUGGACCUGA	472	1522-1544
AD-1527100	GCUCUCCACCUUUCCCAGUUU	287	1577-1597	AAACTGGGAAAGGUGGAGAGCCC	577	1575-1597
AD-1527056	AUUCUUUCAGAGGUGCUAAAU	288	1646-1666	AUUUAGCACCUCUGAAAGAAUCU	578	1644-1666
AD-1527057	UUCCCAUCUUUGUGCAGCUAU	289	1668-1688	AUAGCUGCACACAAAGAUGGGAAAC	579	1666-1688
AD-1527058	UGUGCAGCUACCUCCGCAUUU	194	1678-1698	AAAUGCGGAGGUAGCUGCACAAA	482	1676-1698
AD-1527059	GACGUGGAGGAUCCCAGCCUU	290	1721-1741	AAGGCUGGGAUCCUCCACGUCAC	580	1719-1741
AD-1527060	UGGAGGAUCCCAGCCUCUGAU	291	1725-1745	AUCAGAGGCUGGGAUCCUCCACG	581	1723-1745
AD-1527061	CUCUGAGCUGAGUUGGUUUUU	201	1739-1759	AAAAACCAACUCAGCUCAGAGGC	489	1737-1759
AD-1527101	UGAGUUGGUUUUUAUGAAAAGU	292	1747-1767	ACUUUUCAUAAAAACCAACUCAGC	582	1745-1767
AD-1527102	GGUUUUAUGAAAAGCUAGGAU	293	1753-1773	AUCCUAGCUUUUUCAUAAAACCAA	583	1751-1773
AD-1527103	UUUAUGAAAAGCUAGGAAGCU	294	1756-1776	AGCUUCCUAGCUUUUUCAUAAAAC	584	1754-1776
AD-1527104	UUAUGAAAAAGCUAGGAAGCAU	295	1757-1777	AUGCUUCCUAGCUUUUUCAUAAAA	585	1755-1777
AD-1527105	AAUUCAGCUGGUUGGGAAAUU	296	1831-1851	AAUUUCCCAACCAGCUGAAUUAA	586	1829-1851
AD-1527067	CAGAGGGUCCCUUACUGACUU	297	1870-1890	AAGUCAGUAAGGGACCCUCUGCA	587	1868-1890
AD-1527068	UAUUAAUGGUCAGACUGUUCU	298	1902-1922	AGAACAGUCUGACCAUUAAUAGG	588	1900-1922
AD-1527106	AACACCUUUUUCACCUAACUU	299	2177-2197	AAGUUAGGUGAAAAAGGUGUUCU	589	2175-2197
AD-1527107	CCUUUUUCACCUAACUAAAAU	300	2181-2201	AUUUUAGUUAGGUGAAAAAGGUG	590	2179-2201
AD-1527108	ACCUGUUGAAUUUUGUAUUAU	242	2245-2265	AUAATACAAAAAUUCAACAGGUAA	591	2243-2265

Table 3. Modified Sense and Antisense Strand Sequences of PNPLA3 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1526763	csgscggcUfgGfAfGfcuuguccuuuL96	592	asAfsaggAfcaagcucCfaGfccgcgscsu	873	AGCGCGGCUGGAGCUUGUCCUUC	1182
AD-1526764	gscsggcuUfcCfUfGfggcuucuacuL96	593	asGfsuadGa(Agn)gcccagGfaAfgccgcsasg	874	CUGCGGCUUCCUGGGCUUCUACC	1183
AD-1526765	csgsgcuuCfcUfGfGfgcuucuaccuL96	594	asGfsguaGfaagcccaGfgAfagccgscsa	875	UGCGGCUUCCUGGGCUUCUACCA	1184
AD-1526766	csgsgcuuCfcUfGfGfgcuucuaccuL96	594	asGfsgudAg(Agn)agcccaGfgAfagccgscsa	876	UGCGGCUUCCUGGGCUUCUACCA	1184
AD-1526767	csusuccuGfgGfCfUfucuaccacguL96	595	asCfsgudGg(Tgn)agaagcCfcAfggaagscsc	877	GGCUUCCUGGGCUUCUACCACGU	1185
AD-1526768	cscsugggCfuUfCfUfaccacgucguL96	596	asCfsgacGfugguagaAfgCfccaggsasa	878	UUCCUGGGCUUCUACCACGUCGG	1186
AD-1526769	csusgggcUfuCfUfAfccacgucgguL96	597	asCfscgaCfgugguagAfaGfcccagsgsa	879	UCCUGGGCUUCUACCACGUCGGG	1187
AD-1526770	csgscgacGfcGfCfGfcauguuguuuL96	598	asAfsacaAfcaugcgcGfcGfucgcgsgsa	880	UCCGCGACGCGCGCAUGUUGUUC	1188
AD-1526771	cscsgcugGfaGfCfAfgacucugcauL96	599	asUfsgcdAg(Agn)gucugcUfcCfagcggsgsa	881	UCCCGCUGGAGCAGACUCUGCAG	1189
AD-1526772	gsgsagcaGfaCfUfCfugcagguccuL96	600	asGfsgadCc(Tgn)gcagagUfcUfgcuccsasg	882	CUGGAGCAGACUCUGCAGGUCCU	1190
AD-1526773	gsascucuGfcAfGfGfuccucucaguL96	601	asCfsugaGfaggaccuGfcAfgagucsusg	883	CAGACUCUGCAGGUCCUCUCAGA	1191
AD-1527072	csuscugcAfgGfUfCfcucucagauuL96	602	asAfsucdTg(Agn)gaggacCfuGfcagagsusc	884	GACUCUGCAGGUCCUCUCAGAUC	1192
AD-1527073	csusgcagGfuCfCfUfcucagaucuuL96	603	asAfsgadTc(Tgn)gagaggAfcCfugcagsasg	885	CUCUGCAGGUCCUCUCAGAUCUU	1193
AD-1526776	usgscaggUfcCfUfCfucagaucuuuL96	604	asAfsagaUfcugagagGfaCfcugcasgsa	886	UCUGCAGGUCCUCUCAGAUCUUG	1194
AD-1526777	gscsagguCfcUfCfUfcagaucuuguL96	605	asCfsaagAfucugagaGfgAfccugcsasg	887	CUGCAGGUCCUCUCAGAUCUUGU	1195
AD-1526778	asgsgccaGfgAfGfUfcggaacauuuL96	606	asAfsaudGu(Tgn)ccgacuCfcUfggccususc	888	GAAGGCCAGGAGUCGGAACAUUG	1196
AD-1527074	gsgsccagGfaGfUfCfggaacauuguL96	607	asCfsaadTg(Tgn)uccgacUfcCfuggccsusu	889	AAGGCCAGGAGUCGGAACAUUGG	1197
AD-1526780	gscscaggAfgUfCfGfgaacauugguL96	608	asCfscaaUfguuccgaCfuCfcuggcscsu	890	AGGCCAGGAGUCGGAACAUUGGC	1198
AD-1526781	gscsaucuUfcCfAfUfccauccuucuL96	609	asGfsaadGg(Agn)uggaugGfaAfgaugcscsa	891	UGGCAUCUUCCAUCCAUCCUUCA	1199
AD-1526782	usgsccucCfcGfGfCfcaauguccauL96	610	asUfsggdAc(Agn)uuggccGfgGfaggcasusu	892	AAUGCCUCCCGGCCAAUGUCCAC	1200
AD-1527075	csasauguCfcAfCfCfagcucaucuuL96	611	asAfsgadTg(Agn)gcugguGfgAfcauugsgsc	893	GCCAAUGUCCACCAGCUCAUCUC	1201
AD-1526784	asasugucCfaCfCfAfgcucaucucuL96	612	asGfsagaUfgagcuggUfgGfacauusgsg	894	CCAAUGUCCACCAGCUCAUCUCC	1202
AD-1526785	gsusccaaAfgAfCfGfaagucgugguL96	613	asCfscacGfacuucguCfuUfuggacscsg	895	CGGUCCAAAGACGAAGUCGUGGA	1203
AD-1526786	uscscaaaGfaCfGfAfagucguggauL96	614	asUfsccaCfgacuucgUfcUfuuggascsc	896	GGUCCAAAGACGAAGUCGUGGAU	1204
AD-1526787	cscsaaagAfcGfAfAfgucguggauuL96	615	asAfsuccAfcgacuucGfuCfuuuggsasc	897	GUCCAAAGACGAAGUCGUGGAUG	1205

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1526788	csasaagaCfgAfAfGfucguggaugudL96	616	asCfsaucCfacgacuuCfgUfcuuugsgsa	898	UCCAAAGACGAAGUCGUGGAUGC	1206
AD-1526789	asgsacgaAfgUfCfGfuggaugccuuL96	617	asAfsggdCa(Tgn)ccacgaCfuUfcgucususu	899	AAAGACGAAGUCGUGGAUGCCUU	1207
AD-1526790	csusucuaCfaGfUfGfgccuuauccuL96	618	asGfsgauAfaggccacUfgUfagaagsgsg	900	CCCUUCUACAGUGGCCUUAUCCC	1208
AD-1526791	ususcuacAfgUfGfGfccuuauccccuL96	619	asGfsggaUfaaggccaCfuGfuagaasgsg	901	CCUUCUACAGUGGCCUUAUCCCU	1209
AD-1526792	uscsuacaGfuGfGfGfCfcuuauccccuuL96	620	asAfsgggAfuaaggccAfcUfguagasasg	902	CUUCUACAGUGGCCUUAUCCCUC	1210
AD-1526793	csusacagUfgGfGfCfCfuuauccccucuL96	621	asGfsagdGg(Agn)uaaggcCfaCfuguagsasa	903	UUCUACAGUGGCCUUAUCCCUCC	1211
AD-1526794	asgsuggcCfuUfAfUfcccuccuucuL96	622	asGfsaadGg(Agn)gggauaAfgGfccacusgsu	904	ACAGUGGCCUUAUCCCUCCUUCC	1212
AD-1526795	usgsgccuUfaUfCfCfCfcuccuuccuuL96	623	asAfsggaAfggagggaUfaAfggccascsu	905	AGUGGCCUUAUCCCUCCUUCCUU	1213
AD-1526796	csusuaucCfcUfCfCfCfuuccuucaguL96	624	asCfsugaAfggaaggaGfgGfauaagsgsc	906	GCCUUAUCCCUCCUUCCUUCAGA	1214
AD-1526797	ususauccCfuCfCfUfCfuccuucagauL96	625	asUfscudGa(Agn)ggaaggAfgGfgauaasgsg	907	CCUUAUCCCUCCUUCCUUCAGAG	1215
AD-1526798	cscscuccUfuCfCfUfUfucagaggcguL96	626	asCfsgccUfcugaaggAfaGfgagggsasu	908	AUCCCUCCUUCCUUCAGAGGCGU	1216
AD-1526799	cscsuccuUfcCfUfUfUfcagaggcguuL96	627	asAfscgcCfucugaagGfaAfggagggsgsa	909	UCCCUCCUUCCUUCAGAGGCGUG	1217
AD-1526800	cscsuccuUfcCfUfUfUfcagaggcguuL96	627	asAfscgdCc(Tgn)cugaagGfaAfggagggsgsa	910	UCCCUCCUUCCUUCAGAGGCGUG	1217
AD-1526801	cscsuuccUfuCfAfUfgaggcgugcguL96	628	asCfsgcaCfgccucugAfaGfgaaggsasg	911	CUCCUUCCUUCAGAGGCGUGCGA	1218
AD-1526802	uscscuucAfgAfGfUfGfcgugcgauauL96	629	asUfsaucGfcacgccuCfuGfaaggasasg	912	CUUCCUUCAGAGGCGUGCGAUAU	1219
AD-1526803	csusucagAfgGfCfCfUfgcgauauguL96	630	asCfsauaUfcgcacgcCfuCfugaagsgsa	913	UCCUUCAGAGGCGUGCGAUAUGU	1220
AD-1526804	ususcagaGfgCfCfGfUfgcgauauguuL96	631	asAfscauAfucgcacgCfcUfcugaasgsg	914	CCUUCAGAGGCGUGCGAUAUGUG	1221
AD-1526805	uscsagagGfcGfUfGfCfgauauguguL96	632	asCfsacaUfaucgcacGfcCfucugasasg	915	CUUCAGAGGCGUGCGAUAUGUGG	1222
AD-1526806	csasgaggCfgUfGfCfCfgauauguggguL96	633	asCfscacAfuaucgcaCfgCfcucugsasa	916	UUCAGAGGCGUGCGAUAUGUGGA	1223
AD-1526807	asgsaggcGfuGfCfCfGfauauguggauL96	634	asUfsccaCfauaucgcAfcGfccucusgsa	917	UCAGAGGCGUGCGAUAUGUGGAU	1224
AD-1526808	asgsgcguGfcGfAfUfauguggauguL96	635	asCfsaucCfacauaucGfcAfcgccuscsu	918	AGAGGCGUGCGAUAUGUGGAUGG	1225
AD-1526809	ggsgcgugCfgAfUfAfuguggauggguL96	636	asCfscauCfcacauauCfgCfacgccsusc	919	GAGGCGUGCGAUAUGUGGAUGGA	1226
AD-1526810	csgsugcgAfuAfUfGfuggauggaguL96	637	asCfsuccAfuccacauAfuCfgcacgscsc	920	GGCGUGCGAUAUGUGGAUGGAGG	1227
AD-1526811	gsusgcgaUfaUfGfUfggauggagguL96	638	asCfscucCfauccacaUfaUfcgcacsgsc	921	GCGUGCGAUAUGUGGAUGGAGGA	1228
AD-1526812	gsusgaguGfaCfAfAfcguaccccuuuL96	639	asAfsagdGg(Tgn)acguugUfcAfcucacsusc	922	GAGUGAGUGACAACGUACCCCUUC	1229
AD-1526813	asgsugacAfaCfGfUfaccccuacauuL96	640	asAfsugaAfggguacgUfuGfucacuscsa	923	UGAGUGACAACGUACCCCUUCAUU	1230

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1526814	gsusgacaAfcGfUfAfccuucauuuL96	641	asAfsaugAfagguacGfuUfgucacsusc	924	GAGUGACAACGUACCCUUCAUUG	1231
AD-1526815	usgsacaaCfgUfAfCfccuucauuguL96	642	asCfsaauGfaagguaCfgUfugucascsu	925	AGUGACAACGUACCCUUCAUUGA	1232
AD-1526816	gsascaacGfuAfCfCfcuucauugauL96	643	asUfscaaUfgaaggguAfcGfuugucsasc	926	GUGACAACGUACCCUUCAUUGAU	1233
AD-1526817	ascsaacgUfaCfCfCfuucauugauuL96	644	asAfsucaAfugaaggUfaCfguuguscsa	927	UGACAACGUACCCUUCAUUGAUG	1234
AD-1526818	csasacguAfcCfCfUfucauugauguL96	645	asCfsaucAfaugaaggGfuAfcguugsusc	928	GACAACGUACCCUUCAUUGAUGC	1235
AD-1526819	asascguaCfcCfUfUfcauugaugcuL96	646	asGfscauCfaaugaagGfgUfacguusgsu	929	ACAACGUACCCUUCAUUGAUGCC	1236
AD-1526820	csgsuaccCfuUfCfAfuugaugccauL96	647	asUfsggdCa(Tgn)caaugaAfgGfguacgsusu	930	AACGUACCCUUCAUUGAUGCCAA	1237
AD-1526821	gsusacccUfuCfAfUfugaugccaauL96	648	asUfsugdGc(Agn)ucaaugAfaGfgguacsgsu	931	ACGUACCCUUCAUUGAUGCCAAA	1238
AD-1526822	usascgacAfuCfUfGfcccuaaaguuL96	649	asAfscuuUfagggcagAfuGfucguascsu	932	AGUACGACAUCUGCCCUAAAGUC	1239
AD-1526823	csgsacauCfuGfCfCfcfuaaagucauL96	650	asUfsgacUfuuagggcAfgAfugucgsusa	933	UACGACAUCUGCCCUAAAGUCAA	1240
AD-1526824	gsascaucUfgCfCfCfCfuaaagucaauL96	651	asUfsugdAc(Tgn)uuagggCfaGfaugucsgsu	934	ACGACAUCUGCCCUAAAGUCAAG	1241
AD-1526825	ascsaucuGfcCfCfUfaaagucaaguL96	652	asCfsuugAfcuuuaggGfcAfgauguscsg	935	CGACAUCUGCCCUAAAGUCAAGU	1242
AD-1526826	uscsugccCfuAfAfAfgucaaguccuL96	653	asGfsgacUfugacuuuAfgGfgcagasusg	936	CAUCUGCCCUAAAGUCAAGUCCA	1243
AD-1526827	uscsugccCfuAfAfAfgucaaguccuL96	653	asGfsgadCu(Tgn)gacuuuAfgGfgcagasusg	937	CAUCUGCCCUAAAGUCAAGUCCA	1243
AD-1526828	csusgcccUfaAfAfGfucaaguccauL96	654	asUfsggdAc(Tgn)ugacuuUfaGfggcagsasu	938	AUCUGCCCUAAAGUCAAGUCCAC	1244
AD-1526829	asusguggAfcAfUfCfaccaagcucuL96	655	asGfsagcUfuggugauGfuCfcacausgsa	939	UCAUGUGGACAUCACCAAGCUCA	1245
AD-1526830	asusguggAfcAfUfCfaccaagcucuL96	655	asGfsagdCu(Tgn)gggugauGfuCfcacausgsa	940	UCAUGUGGACAUCACCAAGCUCA	1245
AD-1526831	usgsuggaCfaUfCfAfccaagcucauL96	656	asUfsgadGc(Tgn)uggugaUfgUfccacasusg	941	CAUGUGGACAUCACCAAGCUCAG	1246
AD-1527076	gsuscuacGfcCfUfCfugcacaggguL96	657	asCfsccdTg(Tgn)gcagagGfcGfuagacsusg	942	CAGUCUACGCCUCUGCACAGGGA	1247
AD-1526833	cscsucugCfaCfAfGfggaaccucuuL96	658	asAfsgadGg(Tgn)ucccugUfgCfagaggscsg	943	CGCCUCUGCACAGGGAACCUCUA	1248
AD-1526834	usgscacaGfgGfAfAfccucuaccuuL96	659	asAfsgguAfgagguucCfcUfgugcasgsa	944	UCUGCACAGGGAACCUCUACCUU	1249
AD-1526835	gscsacagGfgAfAfCfcucuaccuuuL96	660	asAfsaggUfagagguuCfcCfugugcsasg	945	CUGCACAGGGAACCUCUACCUUC	1250
AD-1526836	csascaggGfaAfCfCfcucuaccuucuL96	661	asGfsaadGg(Tgn)agagguUfcCfcugugcsca	946	UGCACAGGGAACCUCUACCUUCU	1251
AD-1526837	csasgggaAfcCfUfCfuaccuucucuL96	662	asGfsagaAfgguagagGfuUfcccugsusg	947	CACAGGGAACCUCUACCUUCUCU	1252
AD-1526838	asgsggaaCfcUfCfUfaccuucucuuL96	663	asAfsgagAfagguagaGfgUfucccusgsu	948	ACAGGGAACCUCUACCUUCUCUC	1253
AD-1526839	ggsgaacCfuCfUfAfccuucucucuL96	664	asGfsagaGfaagguagAfgGfuucccsusg	949	CAGGGAACCUCUACCUUCUCUCG	1254

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1527077	csasagguGfcUfGfGfgagagauauuL96	665	asAfsuadTc(Tgn)cuccccaGfcAfccuugsasg	950	CUCAAGGUGCUGGGAGAGAUAUG	1255
AD-1526841	asasggugCfuGfGfGfGfagagauauguL96	666	asCfsauaUfcucucccAfgCfaccuusgsa	951	UCAAGGUGCUGGGAGAGAUAUGC	1256
AD-1526842	asgsgugcUfgGfGfAfgagauaugccuL96	667	asGfscauAfucucucccCfaGfcaccususg	952	CAAGGUGCUGGGAGAGAUAUGCC	1257
AD-1526843	gsgsugcuGfgGfAfGfagauaugccuL96	668	asGfsgcaUfaucucucCfcAfgcaccsusu	953	AAGGUGCUGGGAGAGAUAUGCCU	1258
AD-1526844	gsusgcugGfgAfGfAfgauaugccuuL96	669	asAfsggdCa(Tgn)aucucuCfcCfagcacscsu	954	AGGUGCUGGGAGAGAUAUGCCUU	1259
AD-1526845	usgscuggGfaGfAfGfauaugccuuuL96	670	asAfsagdGc(Agn)uaucucUfcCfcagcascsc	955	GGUGCUGGGAGAGAUAUGCCUUC	1260
AD-1526846	usgsggagAfgAfUfAfugccuucgauL96	671	asUfscgaAfggcauauCfuCfucccasgsc	956	GCUGGGAGAGAUAUGCCUUCGAG	1261
AD-1526847	gsgsgagaGfaUfAfUfgccuucgaguL96	672	asCfsucgAfaggcauaUfcUfcucccsasg	957	CUGGGAGAGAUAUGCCUUCGAGG	1262
AD-1526848	gsgsagagAfuAfUfGfccuucgagguL96	673	asCfscucGfaaggcauAfuCfucucccsa	958	UGGGAGAGAUAUGCCUUCGAGGA	1263
AD-1526849	gsasgagaUfaUfGfCfcuucgaggauL96	674	asUfsccuCfgaaggcaUfaUfcucucscsc	959	GGGAGAGAUAUGCCUUCGAGGAU	1264
AD-1526850	gsasgauaUfgCfCfUfucgaggauauL96	675	asUfsaudCc(Tgn)cgaaggCfaUfaucucsusc	960	GAGAGAUAUGCCUUCGAGGAUAU	1265
AD-1526851	gsasuaugCfcUfUfCfgaggauauuuL96	676	asAfsauaUfccucgaaGfgCfauaucsusc	961	GAGAUAUGCCUUCGAGGAUAUUU	1266
AD-1526852	asusaugcCfuUfCfCfgaggauauuuuL96	677	asAfsaauAfuccucgaAfgGfcauauuscsu	962	AGAUAUGCCUUCGAGGAUAUUUG	1267
AD-1526853	usasugccUfuCfGfAfggauauuuguL96	678	asCfsaaaUfauccucgAfaGfgcauasusc	963	GAUAUGCCUUCGAGGAUAUUUGG	1268
AD-1526854	asusgccuUfcGfAfGfgauauuuggL96	679	asCfscaaAfuauccucGfaAfggcausasu	964	AUAUGCCUUCGAGGAUAUUUGGA	1269
AD-1526855	usgsccuuCfgAfGfGfauauuuggauL96	680	asUfsccaAfauauccuCfgAfaggcasusa	965	UAUGCCUUCGAGGAUAUUUGGAU	1270
AD-1526856	csasuucaGfgUfUfCfuuggaagaguL96	681	asCfsucuUfccaagaaCfcUfgaaugscsa	966	UGCAUUCAGGUUCUUGGAAGAGA	1271
AD-1526857	asgsguucUfuGfGfAfagagaaggguL96	682	asCfsccuUfcucuuccAfaGfaaccusgsa	967	UCAGGUUCUUGGAAGAGAAGGGC	1272
AD-1526858	gsgsuucuUfgGfAfAfgagaagggcuL96	683	asGfscccUfucucuucCfaAfgaaccsusg	968	CAGGUUCUUGGAAGAGAAGGGCA	1273
AD-1526859	gsgsuucuUfgGfAfAfgagaagggcuL96	683	asGfscccUfucucuucCfaAfgaaccsusg	969	CAGGUUCUUGGAAGAGAAGGGCA	1273
AD-1526860	gsusucuuGfgAfAfGfagaagggcauL96	684	asUfsgccUfcucuuCfcAfagaacscsu	970	AGGUUCUUGGAAGAGAAGGGCAU	1274
AD-1526861	asasgagaAfgGfGfCfaucugcaacuL96	685	asGfsuudGc(Agn)gaugccCfuUfcucuuscsc	971	GGAAGAGAAGGGCAUCUGCAACA	1275
AD-1527078	gscscugaAfgUfCfAfuccucagaauL96	686	asUfsucdTg(Agn)ggaugaCfuUfcaggcscsu	972	AGGCCUGAAGUCAUCCUCAGAAG	1276
AD-1526863	csusgaagUfcAfUfCfcucagaagguL96	687	asCfscuuCfugaggauGfaCfuucagsgsc	973	GCCUGAAGUCAUCCUCAGAAGGG	1277
AD-1526864	usgsaaguCfaUfCfCfucagaaggguL96	688	asCfsccuUfcugaggaUfgAfcuucasgsg	974	CCUGAAGUCAUCCUCAGAAGGGA	1278
AD-1526865	gsasagucAfuCfCfUfcagaaggguL96	689	asUfscccUfucugaggAfuGfacuucsasg	975	CUGAAGUCAUCCUCAGAAGGGAU	1279

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1526866	asasgucaUfcCfUfCfagaagggauuL96	690	asAfsuccCfuucugagGfaUfgacuuscsa	976	UGAAGUCAUCCUCAGAAGGGAUG	1280
AD-1526867	asasgucaUfcCfUfCfagaagggauuL96	690	asAfsucdCc(Tgn)ucugagGfaUfgacuuscsa	977	UGAAGUCAUCCUCAGAAGGGAUG	1280
AD-1526868	asgsucauCfcUfCfAfgaagggauguL96	691	asCfsaucCfcuucugaGfgAfugacususc	978	GAAGUCAUCCUCAGAAGGGAUGG	1281
AD-1526869	uscsauccUfcAfGfAfagggauggauL96	692	asUfsccaUfcccuucuGfaGfgaugascsu	979	AGUCAUCCUCAGAAGGGAUGGAU	1282
AD-1526870	gsgsgagaUfgAfGfCfugcuagaccuL96	693	asGfsgudCu(Agn)gcagcuCfaUfcucccsusc	980	GAGGGAGAUGAGCUGCUAGACCA	1283
AD-1527079	gsgsagauGfaGfCfUfgcuagaccauL96	694	asUfsggdTc(Tgn)agcagcUfcAfucucccscsu	981	AGGGAGAUGAGCUGCUAGACCAC	1284
AD-1526872	asgsaugaGfcUfGfCfuagaccaccuL96	695	asGfsgudGg(Tgn)cuagcaGfcUfcaucuscsc	982	GGAGAUGAGCUGCUAGACCACCU	1285
AD-1526873	csusgcuaGfaCfCfAfccugcgucuuL96	696	asAfsgacGfcagguggUfcUfagcagscsu	983	AGCUGCUAGACCACCUGCGUCUC	1286
AD-1526874	csusagacCfaCfCfUfgcgucucaguL96	697	asCfsugaGfacgcaggUfgGfucuagscsa	984	UGCUAGACCACCUGCGUCUCAGC	1287
AD-1526875	csusagacCfaCfCfUfgcgucucaguL96	697	asCfsugdAg(Agn)cgcaggUfgGfucuagscsa	985	UGCUAGACCACCUGCGUCUCAGC	1287
AD-1526876	ascscaccUfgCfGfUfcucagcaucuL96	698	asGfsaudGc(Tgn)gagacgCfaGfgugguscsu	986	AGACCACCUGCGUCUCAGCAUCC	1288
AD-1526877	gscsgucuCfaGfCfAfuccugcccuuL96	699	asAfsggdGc(Agn)ggaugcUfgAfgacgcsasg	987	CUGCGUCUCAGCAUCCUGCCCUG	1289
AD-1526878	gscsauccUfgCfCfCfugggaugaguL96	700	asCfsucaUfcccagggCfaGfgaugcsusg	988	CAGCAUCCUGCCCUGGGAUGAGA	1290
AD-1526879	csasuccuGfcCfCfUfgggaugagauL96	701	asUfscudCa(Tgn)cccaggGfcAfggaugscsu	989	AGCAUCCUGCCCUGGGAUGAGAG	1291
AD-1527080	asusccugCfcCfUfGfggaugagaguL96	702	asCfsucdTc(Agn)ucccagGfgCfaggausgsc	990	GCAUCCUGCCCUGGGAUGAGAGC	1292
AD-1526881	usgscccuGfgGfAfUfgagagcaucuL96	703	asGfsaudGc(Tgn)cucaucCfcAfgggcasgsg	991	CCUGCCCUGGGAUGAGAGCAUCC	1293
AD-1526882	cscsugggAfuGfAfGfagcauccuguL96	704	asCfsaggAfugcucucAfuCfccaggsgsc	992	GCCCUGGGAUGAGAGCAUCCUGG	1294
AD-1526883	asasugaaAfgAfCfAfaagguggauuL96	705	asAfsuccAfccuuuguCfuUfucauususc	993	GAAAUGAAAGACAAAGGUGGAUA	1295
AD-1526884	asusgaaaGfaCfAfAfagguggauauL96	706	asUfsaudCc(Agn)ccuuugUfcUfuucaususu	994	AAAUGAAAGACAAAGGUGGAUAC	1296
AD-1527081	asgsacaaAfgGfUfGfgauacaugauL96	707	asUfscadTg(Tgn)auccacCfuUfugucususu	995	AAAGACAAAGGUGGAUACAUGAG	1297
AD-1526886	ascsaaagGfuGfGfAfuacaugagcuL96	708	asGfscucAfuguauccAfcCfuuuguscsu	996	AGACAAAGGUGGAUACAUGAGCA	1298
AD-1527082	csasaaggUfgGfAfUfacaugagcauL96	709	asUfsgcdTc(Agn)uguaucCfaCfcuuugsusc	997	GACAAAGGUGGAUACAUGAGCAA	1299
AD-1526888	asasggugGfaUfAfCfaugagcaaguL96	710	asCfsuudGc(Tgn)cauguaUfcCfaccuususg	998	CAAAGGUGGAUACAUGAGCAAGA	1300
AD-1526889	gsgsauacAfuGfAfGfcaagauuuguL96	711	asCfsaaaUfcuugcucAfuGfuauccsasc	999	GUGGAUACAUGAGCAAGAUUUGC	1301
AD-1526890	gsasuacaUfgAfGfCfaagauuugcuL96	712	asGfscaaAfucuugcuCfaUfguaucscsa	1000	UGGAUACAUGAGCAAGAUUUGCA	1302
AD-1526891	asusacauGfaGfCfAfagauuugcauL96	713	asUfsgcaAfaucuugcUfcAfuguauscsc	1001	GGAUACAUGAGCAAGAUUUGCAA	1303

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1526892	usascaugAfgCfAfAfgauuugcaauL96	714	asUfsugcAfaaucuugCfuCfauguasusc	1002	GAUACAUGAGCAAGAUUUGCAAC	1304
AD-1526893	ascsaugaGfcAfAfGfauuugcaacuL96	715	asGfsuudGc(Agn)aaucuuGfcUfcaugusasu	1003	AUACAUGAGCAAGAUUUGCAACU	1305
AD-1526894	usgsagcaAfgAfUfUfugcaacuuguL96	716	asCfsaagUfugcaaauCfuUfgcucasusg	1004	CAUGAGCAAGAUUUGCAACUUGC	1306
AD-1526895	gsasgcaaGfaUfUfUfgcaacuugcuL96	717	asGfscadAg(Tgn)ugcaaaUfcUfugcucsasu	1005	AUGAGCAAGAUUUGCAACUUGCU	1307
AD-1526896	asgscaagAfuUfUfGfcaacuugcuuL96	718	asAfsgcaAfguugcaaAfuCfuugcuscsa	1006	UGAGCAAGAUUUGCAACUUGCUA	1308
AD-1526897	gscsaagaUfuUfGfCfaacuugcuauL96	719	asUfsagcAfaguugcaAfaUfcuugcsusc	1007	GAGCAAGAUUUGCAACUUGCUAC	1309
AD-1526898	gscsaagaUfuUfGfCfaacuugcuauL96	719	asUfsagdCa(Agn)guugcaAfaUfcuugcsusc	1008	GAGCAAGAUUUGCAACUUGCUAC	1309
AD-1526899	usgscaacUfuGfCfUfacccauuaguL96	720	asCfsuaaUfggguagcAfaGfuugcasasa	1009	UUUGCAACUUGCUACCCAUUAGG	1310
AD-1526900	gscsaacuUfgCfUfAfcccauuagguL96	721	asCfscuaAfuggguagCfaAfguugcsasa	1010	UUGCAACUUGCUACCCAUUAGGA	1311
AD-1526901	csasacuuGfcUfAfCfccauuaggauL96	722	asUfsccuAfauggguaGfcAfaguugscsa	1011	UGCAACUUGCUACCCAUUAGGAU	1312
AD-1526902	asascuugCfuAfCfCfcauuaggauuL96	723	asAfsuccUfaauggguAfgCfaaguusgsc	1012	GCAACUUGCUACCCAUUAGGAUA	1313
AD-1526903	ascsuugcUfaCfCfCfauuaggauauL96	724	asUfsaudCc(Tgn)aaugggUfaGfcaagususg	1013	CAACUUGCUACCCAUUAGGAUAA	1314
AD-1526904	gscsugccCfuGfUfAfcccugccuguL96	725	asCfsaggCfagguacAfgGfgcagcsasu	1014	AUGCUGCCCUGUACCCUGCCUGU	1315
AD-1526905	cscscuguAfcCfCfUfgccuguggauL96	726	asUfsccdAc(Agn)ggcaggGfuAfcagggscsa	1015	UGCCCUGUACCCUGCCUGUGGAA	1316
AD-1526906	asasucugCfcAfUfUfgcgauugucuL96	727	asGfsacaAfucgcaauGfgCfagauuscsc	1016	GGAAUCUGCCAUUGCGAUUGUCC	1317
AD-1526907	csusgccaUfuGfCfGfauuguccaguL96	728	asCfsuggAfcaaucgcAfaUfggcagsasu	1017	AUCUGCCAUUGCGAUUGUCCAGA	1318
AD-1526908	usgsccauUfgCfGfAfuuguccagauL96	729	asUfscudGg(Agn)caaucgCfaAfuggcasgsa	1018	UCUGCCAUUGCGAUUGUCCAGAG	1319
AD-1527083	csasuugcGfaUfUfGfuccagagacuL96	730	asGfsucdTc(Tgn)ggacaaUfcGfcaaugsgsc	1019	GCCAUUGCGAUUGUCCAGAGACU	1320
AD-1526910	ususgcgaUfuGfUfCfcagagacuguL96	731	asCfsaguCfucuggacAfaUfcgcaasusg	1020	CAUUGCGAUUGUCCAGAGACUGG	1321
AD-1527084	ususgcgaUfuGfUfCfcagagacuguL96	731	asCfsagdTc(Tgn)cuggacAfaUfcgcaasusg	1021	CAUUGCGAUUGUCCAGAGACUGG	1321
AD-1526912	usgscgauUfgUfCfCfcagagacuggL96	732	asCfscagUfcucuggaCfaAfucgcasasu	1022	AUUGCGAUUGUCCAGAGACUGGU	1322
AD-1526913	gscsgauuGfuCfCfAfgagacuggL96	733	asAfsccdAg(Tgn)cucuggAfcAfaucgcsasa	1023	UUGCGAUUGUCCAGAGACUGGUG	1323
AD-1526914	csgsauugUfcCfAfGfagacuggugL96	734	asCfsaccAfgucucugGfaCfaaucgscsa	1024	UGCGAUUGUCCAGAGACUGGUGA	1324
AD-1526915	gsasuuguCfcAfGfAfgacuggugauL96	735	asUfscadCc(Agn)gucucuGfgAfcaaucsgsc	1025	GCGAUUGUCCAGAGACUGGUGAC	1325
AD-1527085	usgsuccaGfaGfAfCfuggugacauuL96	736	asAfsugdTc(Agn)ccagucUfcUfggacasasu	1026	AUUGUCCAGAGACUGGUGACAUG	1326
AD-1526917	csasgagaCfuGfGfUfgacauggcuuL96	737	asAfsgccAfugucaccAfgUfcucugsgsa	1027	UCCAGAGACUGGUGACAUGGCUU	1327

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1526918	asgsagacUfgGfUfGfacauggcuuuL96	738	asAfsagcCfaugucacCfaGfucucusgsg	1028	CCAGAGACUGGUGACAUGGCUUC	1328
AD-1526919	asgsagacUfgGfUfGfacauggcuuuL96	738	asAfsagdCc(Agn)ugucacCfaGfucucusgsg	1029	CCAGAGACUGGUGACAUGGCUUC	1328
AD-1526920	ascsugguGfaCfAfUfggcuuccaguL96	739	asCfsuggAfagccaugUfcAfccaguscsu	1030	AGACUGGUGACAUGGCUUCCAGA	1329
AD-1526921	csusggugAfcAfUfGfgcuuccagauL96	740	asUfscudGg(Agn)agccauGfuCfaccagsusc	1031	GACUGGUGACAUGGCUUCCAGAU	1330
AD-1527086	gsusgacaUfgGfCfUfuccagauauuL96	741	asAfsuadTc(Tgn)ggaagcCfaUfgucacscsa	1032	UGGUGACAUGGCUUCCAGAUAUG	1331
AD-1526923	usgsacauGfgCfUfUfccagauauguL96	742	asCfsauaUfcuggaagCfcAfugucascsc	1033	GGUGACAUGGCUUCCAGAUAUGC	1332
AD-1526924	gsascaugGfcUfUfCfcagauaugcuL96	743	asGfscauAfucuggaaGfcCfaugucsasc	1034	GUGACAUGGCUUCCAGAUAUGCC	1333
AD-1526925	ascsauggCfuUfCfCfagauaugccuL96	744	asGfsgcaUfaucuggaAfgCfcaguscsa	1035	UGACAUGGCUUCCAGAUAUGCCC	1334
AD-1526926	csasuggcUfuCfCfAfgauaugcccuL96	745	asGfsggdCa(Tgn)aucuggAfaGfccaugsusc	1036	GACAUGGCUUCCAGAUAUGCCCG	1335
AD-1526927	asusggcuUfcCfAfGfauaugcccguL96	746	asCfsgggCfauaucugGfaAfgccausgsu	1037	ACAUGGCUUCCAGAUAUGCCCGA	1336
AD-1526928	asusggcuUfcCfAfGfauaugcccguL96	746	asCfsggdGc(Agn)uaucugGfaAfgccausgsu	1038	ACAUGGCUUCCAGAUAUGCCCGA	1336
AD-1526929	gscsuuccAfgAfUfAfugcccgacguL96	747	asCfsgucGfggcauauCfuGfgaagcscsa	1039	UGGCUUCCAGAUAUGCCCGACGA	1337
AD-1526930	gscsagugGfgUfGfAfccucacagguL96	748	asCfscugUfgaggucaCfcCfacugcsasa	1040	UUGCAGUGGGUGACCUCACAGGU	1338
AD-1526931	cscsuccaGfgUfCfCfcaaaugccauL96	749	asUfsggdCa(Tgn)uugggaCfcUfggaggscsg	1041	CGCCUCCAGGUCCCAAAUGCCAG	1339
AD-1526932	csusccagGfuCfCfCfaaaugccaguL96	750	asCfsuggCfauuugggAfcCfuggagsgsc	1042	GCCUCCAGGUCCCAAAUGCCAGU	1340
AD-1526933	csusccagGfuCfCfCfaaaugccaguL96	750	asCfsugdGc(Agn)uuugggAfcCfuggagsgsc	1043	GCCUCCAGGUCCCAAAUGCCAGU	1340
AD-1526934	asgsguccCfaAfAfUfgccagugaguL96	751	asCfsucaCfuggcauuUfgGfgaccusgsg	1044	CCAGGUCCCAAAUGCCAGUGAGC	1341
AD-1527087	gsuscccaAfaUfGfCfcagugagcauL96	752	asUfsgcdTc(Agn)cuggcaUfuUfgggacscsu	1045	AGGUCCCAAAUGCCAGUGAGCAG	1342
AD-1527088	cscsucagGfuCfCfAfgccugaacuuL96	753	asAfsgudTc(Agn)ggcuggAfcCfugaggsasu	1046	AUCCUCAGGUCCAGCCUGAACUU	1343
AD-1526937	uscsagguCfcAfGfCfcugaacuucuL96	754	asGfsaagUfucaggcuGfgAfccugasgsg	1047	CCUCAGGUCCAGCCUGAACUUCU	1344
AD-1526938	uscsagguCfcAfGfCfcugaacuucuL96	754	asGfsaadGu(Tgn)caggcuGfgAfccugasgsg	1048	CCUCAGGUCCAGCCUGAACUUCU	1344
AD-1526939	csasggucCfaGfCfCfcugaacuucuuL96	755	asAfsgadAg(Tgn)ucaggcUfgGfaccugsasg	1049	CUCAGGUCCAGCCUGAACUUCUU	1345
AD-1526940	asgsguccAfgCfCfUfgaacuucuuuL96	756	asAfsagaAfguucaggCfuGfgaccusgsa	1050	UCAGGUCCAGCCUGAACUUCUUC	1346
AD-1526941	gsgsuccaGfcCfUfGfaacuucuucuL96	757	asGfsaagAfaguucagGfcUfggaccsusg	1051	CAGGUCCAGCCUGAACUUCUUCU	1347
AD-1526942	ususgggcAfaUfAfAfaguaccugcuL96	758	asGfscadGg(Tgn)acuuuaUfuGfcccaasgsa	1052	UCUUGGGCAAUAAAGUACCUGCU	1348
AD-1526943	gsgscaauAfaAfGfUfaccugcugguL96	759	asCfscagCfagguacuUfuAfuugccscsa	1053	UGGGCAAUAAAGUACCUGCUGGU	1349

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1526944	asasuaaaGfuAfCfCfugcuggugcuL96	760	asGfscacCfagcagguAfcUfuuauusgsc	1054	GCAAUAAAGUACCUGCUGGUGCU	1350
AD-1526945	asasuaaaGfuAfCfCfugcuggugcuL96	760	asGfscadCc(Agn)gcagguAfcUfuuauusgsc	1055	GCAAUAAAGUACCUGCUGGUGCU	1350
AD-1526946	asasaguaCfcUfGfCfuggugcugauL96	761	asUfscadGc(Agn)ccagcaGfgUfacuuusasu	1056	AUAAAGUACCUGCUGGUGCUGAG	1351
AD-1526947	ascsuugaGfgAfGfGfcgagucuaguL96	762	asCfsuagAfcucgccuCfcUfcaagusgsa	1057	UCACUUGAGGAGGCGAGUCUAGC	1352
AD-1526948	gsusuuccCfaUfCfUfuugugcagcuL96	763	asGfscudGc(Agn)caaagaUfgGfgaaacsusu	1058	AAGUUUCCCAUCUUUGUGCAGCU	1353
AD-1526949	uscsccauCfuUfUfGfugcagcuacuL96	764	asGfsuadGc(Tgn)gcacaaAfgAfugggasasa	1059	UUUCCCAUCUUUGUGCAGCUACC	1354
AD-1526950	csasucuuUfgUfGfCfagcuaccucuL96	765	asGfsaggUfagcugcaCfaAfagaugsgsg	1060	CCCAUCUUUGUGCAGCUACCUCC	1355
AD-1526951	csasucuuUfgUfGfCfagcuaccucuL96	765	asGfsagdGu(Agn)gcugcaCfaAfagaugsgsg	1061	CCCAUCUUUGUGCAGCUACCUCC	1355
AD-1526952	usgsugcaGfcUfAfCfcuccgcauuuL96	766	asAfsaugCfggagguaGfcUfgcacasasa	1062	UUUGUGCAGCUACCUCCGCAUUG	1356
AD-1526953	usgscagcUfaCfCfUfccgcauugcuL96	767	asGfscaaUfgcggaggUfaGfcugcascsa	1063	UGUGCAGCUACCUCCGCAUUGCU	1357
AD-1526954	usgsccugUfgAfCfGfuggaggaucuL96	768	asGfsaudCc(Tgn)ccacguCfaCfaggcasgsg	1064	CCUGCCUGUGACGUGGAGGAUCC	1358
AD-1526955	csasgccuCfuGfAfGfcugaguugguL96	769	asCfscaaCfucagcucAfgAfggcugsgsg	1065	CCCAGCCUCUGAGCUGAGUUGGU	1359
AD-1526956	asgsccucUfgAfGfCfugaguugguuL96	770	asAfsccaAfcucagcuCfaGfaggcusgsg	1066	CCAGCCUCUGAGCUGAGUUGGUU	1360
AD-1526957	gscscucuGfaGfCfUfgaguugguuuL96	771	asAfsaccAfacucagcUfcAfgaggcsusg	1067	CAGCCUCUGAGCUGAGUUGGUUU	1361
AD-1526958	cscsucugAfgCfUfGfaguugguuuuL96	772	asAfsaacCfaacucagCfuCfagaggscsu	1068	AGCCUCUGAGCUGAGUUGGUUUU	1362
AD-1526959	csuscugaGfcUfGfAfguugguuuuuL96	773	asAfsaaaCfcaacucaGfcUfcagagsgsc	1069	GCCUCUGAGCUGAGUUGGUUUUA	1363
AD-1526960	uscsugagCfuGfAfGfuugguuuuauL96	774	asUfsaaaAfccaacucAfgCfucagasgsg	1070	CCUCUGAGCUGAGUUGGUUUUAU	1364
AD-1526961	csusgagcUfgAfGfUfugguuuuauuL96	775	asAfsuaaAfaccaacuCfaGfcucagsasg	1071	CUCUGAGCUGAGUUGGUUUUAUG	1365
AD-1526962	usgsagcuGfaGfUfUfgguuuuauguL96	776	asCfsauaAfaaccaacUfcAfgcucasgsa	1072	UCUGAGCUGAGUUGGUUUUAUGA	1366
AD-1526963	gsasgcugAfgUfUfGfguuuuaugauL96	777	asUfscauAfaaaccaaCfuCfagcucsasg	1073	CUGAGCUGAGUUGGUUUUAUGAA	1367
AD-1526964	asgscugaGfuUfGfGfuuuuaugaauL96	778	asUfsucaUfaaaaccaAfcUfcagcuscsa	1074	UGAGCUGAGUUGGUUUUAUGAAA	1368
AD-1526965	gscsugagUfuGfGfUfuuuaugaaauL96	779	asUfsuudCa(Tgn)aaaaccAfaCfucagcsusc	1075	GAGCUGAGUUGGUUUUAUGAAAA	1369
AD-1193373	csusgaguUfgGfUfUfuuaugaaaauL96	780	asUfsuudTc(Agn)uaaaacCfaAfcucagscsu	1076	AGCUGAGUUGGUUUUAUGAAAAG	1370
AD-1526967	gsasguugGfuUfUfUfaugaaaagcuL96	781	asGfscuuUfucauaaaAfcCfaacucsasg	1077	CUGAGUUGGUUUUAUGAAAAGCU	1371
AD-1526968	asgsuuggUfuUfUfAfugaaaagcuuL96	782	asAfsgcuUfuucauaaAfaCfcaacuscsa	1078	UGAGUUGGUUUUAUGAAAAGCUA	1372
AD-1526969	gsusugguUfuUfAfUfgaaaagcuauL96	783	asUfsagdCu(Tgn)uucauaAfaAfccaacsusc	1079	GAGUUGGUUUUAUGAAAAGCUAG	1373

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1526970	ususgguuUfuAfUfGfaaaagcuaguL96	784	asCfsuagCfuuuucauAfaAfaccaascsu	1080	AGUUGGUUUUAUGAAAAGCUAGG	1374
AD-1526971	ususgguuUfuAfUfGfaaaagcuaguL96	784	asCfsuadGc(Tgn)uuucauAfaAfaccaascsu	1081	AGUUGGUUUUAUGAAAAGCUAGG	1374
AD-1526972	usgsguuuUfuAfUfGfAfaaagcuagguL96	785	asCfscuaGfcuuuucaUfaAfaaccasasc	1082	GUUGGUUUUAUGAAAAGCUAGGA	1375
AD-1526973	gsusuuuaUfgAfAfAfagcuaggaauL96	786	asUfsuccUfagcuuuuCfaUfaaaacscsa	1083	UGGUUUUAUGAAAAGCUAGGAAG	1376
AD-1526974	gsusuuuaUfgAfAfAfagcuaggaauL96	786	asUfsucdCu(Agn)gcuuuuCfaUfaaaacscsa	1084	UGGUUUUAUGAAAAGCUAGGAAG	1376
AD-1526975	ususuuauGfaAfAfAfgcuaggaaguL96	787	asCfsuucCfuagcuuuUfcAfuaaaascsc	1085	GGUUUUAUGAAAAGCUAGGAAGC	1377
AD-1526976	ususuuauGfaAfAfAfgcuaggaaguL96	787	asCfsuudCc(Tgn)agcuuuUfcAfuaaaascsc	1086	GGUUUUAUGAAAAGCUAGGAAGC	1377
AD-1526977	usasugaaAfaGfCfUfaggaagcaauL96	788	asUfsugdCu(Tgn)ccuagcUfuUfucauasasa	1087	UUUAUGAAAAGCUAGGAAGCAAC	1378
AD-1526978	asusucagCfuGfGfUfugggaaauguL96	789	asCfsauuUfcccaaccAfgCfugaaususa	1088	UAAUUCAGCUGGUUGGGAAAUGA	1379
AD-1526979	csasgcugGfuUfGfGfgaaaugacauL96	790	asUfsgudCa(Tgn)uuucccaAfcCfagcugsasa	1089	UUCAGCUGGUUGGGAAAUGACAC	1380
AD-1527089	asgscuggUfuGfGfGfaaaugacacuL96	791	asGfsugdTc(Agn)uuucccAfaCfcagcusgsa	1090	UCAGCUGGUUGGGAAAUGACACC	1381
AD-1526981	gsusgcagAfgGfGfUfcccuuacuguL96	792	asCfsaguAfagggaccCfuCfugcacsusg	1091	CAGUGCAGAGGGUCCCUUACUGA	1382
AD-1526982	usgscagaGfgGfUfCfccuuacugauL96	793	asUfscagUfaagggacCfcUfcugcascsu	1092	AGUGCAGAGGGUCCCUUACUGAC	1383
AD-1526983	gscsagagGfgUfCfCfcuuacugacuL96	794	asGfsucdAg(Tgn)aagggaCfcCfucugcsasc	1093	GUGCAGAGGGUCCCUUACUGACU	1384
AD-1526984	ususaaugGfuCfAfGfacuguuccauL96	795	asUfsggaAfcagucugAfcCfauuaasusa	1094	UAUUAAUGGUCAGACUGUUCCAG	1385
AD-1526985	usasauggUfcAfGfAfcuguuccaguL96	796	asCfsuggAfacagucuGfaCfcauuasasu	1095	AUUAAUGGUCAGACUGUUCCAGC	1386
AD-1526986	ascsgacaCfuGfCfCfugucagguguL96	797	asCfsaccUfgacaggcAfgUfgucgususc	1096	GAACGACACUGCCUGUCAGGUGG	1387
AD-1526987	ascsaccuUfuUfUfCfaccuaacuauL96	798	asUfsaguUfaggugaaAfaAfggugususc	1097	GAACACCUUUUUCACCUAACUAA	1388
AD-1526988	csasccuuUfuUfCfAfccuaacuaauL96	799	asUfsuadGu(Tgn)aggugaAfaAfaggugsusu	1098	AACACCUUUUUCACCUAACUAAA	1389
AD-1526989	ascscuuuUfuCfAfCfcuaacuaaauL96	800	asUfsuudAg(Tgn)uaggugAfaAfaaggusgsu	1099	ACACCUUUUUCACCUAACUAAAA	1390
AD-1526990	csusuuuuCfaCfCfUfaacuaaaauuL96	801	asAfsuuuUfaguuaggUfgAfaaaagsgsu	1100	ACCUUUUUCACCUAACUAAAAUA	1391
AD-1526991	ususuuucAfcCfUfAfacuaaaauauL96	802	asUfsauuUfuaguuagGfuGfaaaaasgsg	1101	CCUUUUUCACCUAACUAAAAUAA	1392
AD-1526992	ususuucaCfcUfAfAfcuaaaauaauL96	803	asUfsuauUfuuaguuaGfgUfgaaaasasg	1102	CUUUUUCACCUAACUAAAAUAAU	1393
AD-1526993	ususucacCfuAfAfCfuaaaauaauuL96	804	asAfsuuaUfuuuaguuAfgGfugaaasasa	1103	UUUUUCACCUAACUAAAAUAAUG	1394
AD-1526994	ususcaccUfaAfCfUfaaaauaauguL96	805	asCfsauuAfuuuuaguUfaGfgugaasasa	1104	UUUUCACCUAACUAAAAUAAUGU	1395
AD-1526995	uscsaccuAfaCfUfAfaaauaauguuL96	806	asAfscauUfauuuuagUfuAfggugasasa	1105	UUUCACCUAACUAAAAUAAUGUU	1396

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1526996	csasccuaAfcUfAfAfaauaauguuuL96	807	asAfsacaUfuauuuuaGfuUfaggugsasa	1106	UUCACCUAACUAAAAUAAUGUUU	1397
AD-1526997	ascscuaaCfuAfAfAfauaauguuuuL96	808	asAfsaacAfuuauuuuAfgUfuaggusgsa	1107	UCACCUAACUAAAAUAAUGUUUA	1398
AD-1526998	cscsuaacUfaAfAfAfuaauguuuauL96	809	asUfsaaaCfauuauuuUfaGfuuaggsusg	1108	CACCUAACUAAAAUAAUGUUUAA	1399
AD-1526999	cscsuaacUfaAfAfAfuaauguuuauL96	809	asUfsaadAc(Agn)uuauuuUfaGfuuaggsusg	1109	CACCUAACUAAAAUAAUGUUUAA	1399
AD-1527000	csusaacuAfaAfAfUfaauguuuaauL96	810	asUfsuaaAfcauuauuUfuAfguuagsgsu	1110	ACCUAACUAAAAUAAUGUUUAAA	1400
AD-1527001	usasacuaAfaAfUfAfauguuuaaauL96	811	asUfsuuaAfacauuauUfuUfaguuasgsg	1111	CCUAACUAAAAUAAUGUUUAAAG	1401
AD-1527002	asascuaaAfaUfAfAfuguuuaaaguL96	812	asCfsuuuAfaacauuaUfuUfuaguusasg	1112	CUAACUAAAAUAAUGUUUAAAGA	1402
AD-1527003	ascsuaaaAfuAfAfUfguuuaaagauL96	813	asUfscuuUfaaacauuAfuUfuuagususa	1113	UAACUAAAAUAAUGUUUAAAGAG	1403
AD-1527004	ascscuguUfgAfAfUfuuuguauuauL96	814	asUfsaauAfcaaaauuCfaAfcaggusasa	1114	UUACCUGUUGAAUUUUGUAUUAU	1404
AD-1527005	cscsuguuGfaAfUfUfuuguauuauuL96	815	asAfsuaaUfacaaaauUfcAfacaggsusa	1115	UACCUGUUGAAUUUUGUAUUAUG	1405
AD-1527006	usgscggcUfuCfCfUfgggcuucuauL96	816	asUfsagdAa(G2p)cccaggAfaGfccgcasgsc	1116	GCUGCGGCUUCCUGGGCUUCUAC	1406
AD-1527090	ususccugGfgCfUfUfcuaccacguuL96	817	asAfscgdTg(G2p)uagaagCfcCfaggaasgsc	1117	GCUUCCUGGGCUUCUACCACGUC	1407
AD-1527008	cscscgcuGfgAfGfCfagacucugcuL96	818	asGfscadGa(G2p)ucugcuCfcAfgcgggsasu	1118	AUCCCGCUGGAGCAGACUCUGCA	1408
AD-1527009	csasgacuCfuGfCfAfgguccucucuL96	819	asGfsagdAg(G2p)accugcAfgAfgucugscsu	1119	AGCAGACUCUGCAGGUCCUCUCA	1409
AD-1527010	ascsucugCfaGfGfUfccucucagauL96	820	asUfscudGa(G2p)aggaccUfgCfagaguscsu	1120	AGACUCUGCAGGUCCUCUCAGAU	1410
AD-1527011	uscsugcaGfgUfCfCfucucagaucuL96	821	asGfsaudCu(G2p)agaggaCfcUfgcagasgsu	1121	ACUCUGCAGGUCCUCUCAGAUCU	1411
AD-1527012	usgscaggUfcCfUfCfucagaucuuuL96	604	asAfsagdAu(C2p)ugagagGfaCfcugcasgsa	1122	UCUGCAGGUCCUCUCAGAUCUUG	1194
AD-1527013	csasucuuCfcAfUfCfcauccuucauL96	822	asUfsgadAg(G2p)auggauGfgAfagaugscsc	1123	GGCAUCUUCCAUCCAUCCUUCAA	1412
AD-1527014	asasugucCfaCfCfAfgcucaucucuL96	612	asGfsagdAu(G2p)agcuggUfgGfacauusgsg	1124	CCAAUGUCCACCAGCUCAUCUCC	1202
AD-1527015	usascaguGfgCfCfUfuaucccuccuL96	823	asGfsgadGg(G2p)auaaggCfcAfcuguasgsa	1125	UCUACAGUGGCCUUAUCCCUCCU	1413
AD-1527016	csasguggCfcUfUfAfucccuccuuuL96	824	asAfsagdGa(G2p)ggauaaGfgCfcacugsusa	1126	UACAGUGGCCUUAUCCCUCCUUC	1414
AD-1527017	gsusggccUfuAfUfCfccuccuuccuL96	825	asGfsgadAg(G2p)agggauAfaGfgccacsusg	1127	CAGUGGCCUUAUCCCUCCUUCCU	1415
AD-1527018	cscscuccUfuCfCfUfucagaggcguL96	626	asCfsgcdCu(C2p)ugaaggAfaGfgagggsasu	1128	AUCCCUCCUUCCUUCAGAGGCGU	1216
AD-1527019	usgsagugAfcAfAfCfguacccuucuL96	826	asGfsaadGg(G2p)uacguuGfuCfacucascsu	1129	AGUGAGUGACAACGUACCCUUCA	1416
AD-1527020	gsasgugaCfaAfCfGfuacccuucauL96	827	asUfsgadAg(G2p)guacguUfgUfcacucsasc	1130	GUGAGUGACAACGUACCCUUCAU	1417
AD-1527021	ascsguacCfcUfUfCfauugaugccuL96	828	asGfsgcdAu(C2p)aaugaaGfgGfuacgususg	1131	CAACGUACCCUUCAUUGAUGCCA	1418

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1527022	asgsuacgAfcAfUfCfugcccuaaauL96	829	asUfsuudAg(G2p)gcagauGfuCfguacuscsc	1132	GGAGUACGACAUCUGCCCUAAAG	1419
AD-1527091	asuscugcCfcUfAfAfagucaagucuL96	830	asGfsacdTu(G2p)acuuuaGfgGfcagausgsu	1133	ACAUCUGCCCUAAAGUCAAGUCC	1420
AD-1527024	csuscugcAfcAfGfGfgaaccucuauL96	831	asUfsagdAg(G2p)uucccuGfuGfcagagsgsc	1134	GCCUCUGCACAGGGAACCUCUAC	1421
AD-1527025	uscsugcaCfaGfGfGfaaccucuacuL96	832	asGfsuadGa(G2p)guucccUfgUfgcagasgsg	1135	CCUCUGCACAGGGAACCUCUACC	1422
AD-1527026	ascsagggAfaCfCfUfcuaccuucuuL96	833	asAfsgadAg(G2p)uagaggUfuCfccugusgsc	1136	GCACAGGGAACCUCUACCUUCUC	1423
AD-1527027	csusgggaGfaGfAfUfaugccuucguL96	834	asCfsgadAg(G2p)cauaucUfcUfcccagscsa	1137	UGCUGGGAGAGAUAUGCCUUCGA	1424
AD-1527028	usgsggagAfgAfUfAfugccuucgauL96	671	asUfscgdAa(G2p)gcauauCfuCfucccasgsc	1138	GCUGGGAGAGAUAUGCCUUCGAG	1261
AD-1527029	asgsagauAfuGfCfCfuucgaggauuL96	835	asAfsucdCu(C2p)gaaggcAfuAfucucuscsc	1139	GGAGAGAUAUGCCUUCGAGGAUA	1425
AD-1527092	asgsauauGfcCfUfUfcgaggauauuL96	836	asAfsuadTc(C2p)ucgaagGfcAfuaucuscsu	1140	AGAGAUAUGCCUUCGAGGAUAUU	1426
AD-1527031	gsasuaugCfcUfUfCfgaggauauuuL96	676	asAfsaudAu(C2p)cucgaaGfgCfauaucsusc	1141	GAGAUAUGCCUUCGAGGAUAUUU	1266
AD-1527032	gsasagagAfaGfGfGfcaucugcaauL96	837	asUfsugdCa(G2p)augcccUfuCfucuucscsa	1142	UGGAAGAGAAGGGCAUCUGCAAC	1427
AD-1527033	gsasgcugCfuAfGfAfccaccugcguL96	838	asCfsgcdAg(G2p)uggucuAfgCfagcucsasu	1143	AUGAGCUGCUAGACCACCUGCGU	1428
AD-1527034	gsasccacCfuGfCfGfucucagcauuL96	839	asAfsugdCu(G2p)agacgcAfgGfuggucsusa	1144	UAGACCACCUGCGUCUCAGCAUC	1429
AD-1527035	csasccugCfgUfCfUfcagcauccuuL96	840	asAfsggdAu(G2p)cugagaCfgCfaggugsgsu	1145	ACCACCUGCGUCUCAGCAUCCUG	1430
AD-1527036	cscscuggGfaUfGfAfgagcauccuuL96	841	asAfsggdAu(G2p)cucucaUfcCfcagggscsa	1146	UGCCCUGGGAUGAGAGCAUCCUG	1431
AD-1527093	asgsguggAfuAfCfAfugagcaagauL96	842	asUfscudTg(C2p)ucauguAfuCfcaccususu	1147	AAAGGUGGAUACAUGAGCAAGAU	1432
AD-1527094	gsgsuggaUfaCfAfUfgagcaagauuL96	843	asAfsucdTu(G2p)cucaugUfaUfccaccsusu	1148	AAGGUGGAUACAUGAGCAAGAUU	1433
AD-1527095	csasugagCfaAfGfAfuuugcaacuuL96	844	asAfsgudTg(C2p)aaaucuUfgCfucaugsusa	1149	UACAUGAGCAAGAUUUGCAACUU	1434
AD-1527096	asusgagcAfaGfAfUfuugcaacuuuL96	845	asAfsagdTu(G2p)caaaucUfuGfcucausgsu	1150	ACAUGAGCAAGAUUUGCAACUUG	1435
AD-1527041	ususugcaAfcUfUfGfcuacccauuuL96	846	asAfsaudGg(G2p)uagcaaGfuUfgcaaasusc	1151	GAUUUGCAACUUGCUACCCAUUA	1436
AD-1527042	asusgcugCfcCfUfGfuacccugccuL96	847	asGfsgcdAg(G2p)guacagGfgCfagcaususa	1152	UAAUGCUGCCCUGUACCCUGCCU	1437
AD-1527097	gscscauuGfcGfAfUfuguccagaguL96	848	asCfsucdTg(G2p)acaaucGfcAfauggcsasg	1153	CUGCCAUUGCGAUUGUCCAGAGA	1438
AD-1527044	cscsauugCfgAfUfUfguccagagauL96	849	asUfscudCu(G2p)gacaauCfgCfaauggscsa	1154	UGCCAUUGCGAUUGUCCAGAGAC	1439
AD-1527045	asusugcgAfuUfGfUfccagagacuuL96	850	asAfsgudCu(C2p)uggacaAfuCfgcaausgsg	1155	CCAUUGCGAUUGUCCAGAGACUG	1440
AD-1527046	cscsagagAfcUfGfGfugacauggcuL96	851	asGfscdAu(G2p)ucaccaGfuCfucuggsasc	1156	GUCCAGAGACUGGUGACAUGGCU	1441
AD-1527047	gsascuggUfgAfCfAfuggcuuccauL96	852	asUfsggdAa(G2p)ccauguCfaCfcagucsusc	1157	GAGACUGGUGACAUGGCUUCCAG	1442

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1527098	usgsgugaCfaUfGfGfcuuccagauuL96	853	asAfsucdTg(G2p)aagccaUfgUfcaccasgsu	1158	ACUGGUGACAUGGCUUCCAGAUA	1443
AD-1527049	gsgsugacAfuGfGfCfuuccagauauL96	854	asUfsaudCu(G2p)gaagccAfuGfucaccsasg	1159	CUGGUGACAUGGCUUCCAGAUAU	1444
AD-1527050	usgsgcuuCfcAfGfAfuaugcccgauL96	855	asUfscgdGg(C2p)auaucuGfgAfagccasusg	1160	CAUGGCUUCCAGAUAUGCCCGAC	1445
AD-1527051	gsgscuucCfaGfAfUfaugcccgacuL96	856	asGfsucdGg(G2p)cauaucUfgGfaagccsasu	1161	AUGGCUUCCAGAUAUGCCCGACG	1446
AD-1527052	gsusugcaGfuGfGfGfugaccucacuL96	857	asGfsugdAg(G2p)ucacccAfcUfgcaacscsa	1162	UGGUUGCAGUGGGUGACCUCACA	1447
AD-1527099	cscsagguCfcCfAfAfaugccaguguL96	858	asCfsacdTg(G2p)cauuugGfgAfccuggsasg	1163	CUCCAGGUCCCAAAUGCCAGUGA	1448
AD-1527054	asgsguccAfgCfCfUfgaacuucuuuL96	756	asAfsagdAa(G2p)uucaggCfuGfgaccusgsa	1164	UCAGGUCCAGCCUGAACUUCUUC	1346
AD-1527100	gscsucucCfaCfCfUfuucccaguuuL96	859	asAfsacdTg(G2p)gaaaggUfgGfagagcscsc	1165	GGGCUCUCCACCUUUCCCAGUUU	1449
AD-1527056	asusucuuUfcAfGfAfggugcuaaauL96	860	asUfsuudAg(C2p)accucuGfaAfagaauscsu	1166	AGAUUCUUUCAGAGGUGCUAAAG	1450
AD-1527057	ususccccaUfcUfUfUfUfgugcagcuauL96	861	asUfsagdCu(G2p)cacaaaGfaUfgggaasasc	1167	GUUUCCCAUCUUUGUGCAGCUAC	1451
AD-1527058	usgsugcaGfcUfAfCfcucccgcauuuL96	766	asAfsaudGc(G2p)gagguaGfcUfgcacasasa	1168	UUUGUGCAGCUACCUCCCGCAUUG	1356
AD-1527059	gsascgugGfaGfGfAfucccagccuuL96	862	asAfsggdCu(G2p)ggauccUfcCfacgucsasc	1169	GUGACGUGGAGGAUCCCAGCCUC	1452
AD-1527060	usgsgaggAfuCfCfCfagccucugauL96	863	asUfscadGa(G2p)gcuggggAfuCfcuccascsg	1170	CGUGGAGGAUCCCAGCCUCUGAG	1453
AD-1527061	csuscugaGfcUfGfAfguugguuuuuL96	773	asAfsaadAc(C2p)aacucaGfcUfcagagsgsc	1171	GCCUCUGAGCUGAGUUGGUUUUA	1363
AD-1527101	usgsaguuGfgUfUfUfUfuaugaaaagL96	864	asCfsuudTu(C2p)auaaaaCfcAfacucasgsc	1172	GCUGAGUUGGUUUUAUGAAAAGC	1454
AD-1527102	gsgsuuuuAfuGfAfAfaagcuaggauL96	865	asUfsccdTa(G2p)cuuuucAfuAfaaaccsasa	1173	UUGGUUUUAUGAAAAGCUAGGAA	1455
AD-1527103	ususuaugAfaAfAfAfGfcuaggaagcuL96	866	asGfscudTc(C2p)uagcuuUfuCfauaaasasc	1174	GUUUUAUGAAAAGCUAGGAAGCA	1456
AD-1527104	ususaugaAfaAfGfCfuaggaagcauL96	867	asUfsgcdTu(C2p)cuagcuUfuUfcauaasasa	1175	UUUUAUGAAAAGCUAGGAAGCAA	1457
AD-1527105	asasuucaGfcUfGfGfuugggaaauuL96	868	asAfsuudTc(C2p)caaccaGfcUfgaauusasa	1176	UUAAUUCAGCUGGUUGGGAAAUG	1458
AD-1527067	csasgaggGfuCfCfCfuuacugacuuL96	869	asAfsgudCa(G2p)uaagggAfcCfcucugscsa	1177	UGCAGAGGGUCCCUUACUGACUG	1459
AD-1527068	usasuuaaUfgGfUfCfagacuguucuL96	870	asGfsaadCa(G2p)ucugacCfaUfuaauasgsg	1178	CCUAUUAAUGGUCAGACUGUUCC	1460
AD-1527106	asascaccUfuUfUfUfcaccuaacuuL96	871	asAfsgudTa(G2p)gugaaaAfaGfguguuscsu	1179	AGAACACCUUUUUCACCUAACUA	1461
AD-1527107	cscsuuuuUfcAfCfCfuaacuaaaauL96	872	asUfsuudTa(G2p)uuagguGfaAfaaaggsusg	1180	CACCUUUUUCACCUAACUAAAAU	1462
AD-1527108	ascscuguUfgAfaAfUfuuuguauuauL96	814	asUfsaadTa(C2p)aaaauuCfaAfcaggusasa	1181	UUACCUGUUUGAAUUUUUGUAUUAU	1404

Table 4. In Vitro Screen in Hep3B cells

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526763.1	43.68	7.66	181.42	13.60	136.21	17.31
AD-1527006.1	58.05	16.93	58.84	5.93	108.83	8.56
AD-1526764.1	114.18	3.92	155.68	23.31	76.32	36.13
AD-1526765.1	83.14	12.89	139.55	41.56	91.73	44.28
AD-1526766.1	122.68	26.64	138.64	9.78	123.48	58.96
AD-1526767.1	131.94	25.92	152.31	51.86	78.01	19.26
AD-1527090.1	35.21	6.06	58.54	9.71	60.78	14.27
AD-1526768.1	119.41	29.90	99.00	16.42	113.90	41.72
AD-1526769.1	153.69	16.54	195.82	75.85	64.62	14.63
AD-1526770.1	175.84	46.29	215.65	75.08	137.98	24.52
AD-1527008.1	43.03	14.31	69.68	4.54	88.86	26.56
AD-1526771.1	77.30	22.42	96.13	16.73	73.61	22.28
AD-1526772.1	68.48	8.41	85.29	26.44	57.61	29.56
AD-1527009.1	42.74	7.97	43.51	9.66	59.45	8.65
AD-1526773.1	74.79	14.85	80.19	9.48	40.34	16.76
AD-1527010.1	33.84	6.13	63.81	6.43	68.30	11.12
AD-1527072.1	77.73	5.92	75.34	5.27	49.69	12.24
AD-1527011.1	59.33	18.13	73.48	3.68	97.66	30.04
AD-1527073.1	33.26	12.21	60.52	13.44	40.99	15.37
AD-1527012.1	123.41	44.38	127.86	12.67	125.12	34.30
AD-1526776.1	72.77	15.21	81.61	8.25	57.82	17.75
AD-1526777.1	64.56	11.50	86.26	5.87	52.83	15.50
AD-1526778.1	148.34	35.79	136.17	20.96	138.39	20.82

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1527074.1	98.24	27.36	196.89	59.40	90.21	28.04
AD-1526780.1	87.94	27.44	117.41	35.81	85.29	26.99
AD-1526781.1	76.69	17.03	118.11	15.20	55.74	14.20
AD-1527013.1	66.62	8.03	67.19	11.53	89.46	25.63
AD-1526782.1	88.36	22.24	116.96	10.93	75.92	18.06
AD-1527075.1	62.63	11.22	73.33	3.57	48.35	7.83
AD-1527014.1	58.20	4.44	77.19	17.96	54.31	4.41
AD-1526784.1	73.82	20.16	96.48	26.47	64.25	9.86
AD-1526785.1	119.20	9.92	153.50	45.22	186.36	18.74
AD-1526786.1	96.65	22.25	111.90	18.07	129.07	19.15
AD-1526787.1	87.75	18.71	93.68	17.36	87.75	21.03
AD-1526788.1	64.55	12.37	82.56	20.24	40.65	8.51
AD-1526789.1	53.77	9.08	60.47	15.42	53.93	12.71
AD-1526790.1	74.54	11.91	114.45	9.13	76.82	7.11
AD-1526791.1	78.09	7.33	112.71	31.23	79.26	24.02
AD-1526792.1	77.89	19.83	69.58	4.59	59.03	15.77
AD-1526793.1	105.60	53.31	165.15	27.61	161.89	10.86
AD-1527015.1	41.17	1.93	40.76	15.19	53.79	8.32
AD-1527016.1	56.05	9.21	68.28	9.38	67.98	16.74
AD-1526794.1	58.79	15.75	78.21	13.17	67.27	10.53
AD-1527017.1	70.52	5.18	89.96	19.48	135.31	25.40
AD-1526795.1	51.99	12.25	57.65	4.96	40.95	17.51
AD-1526796.1	37.36	8.47	46.36	9.89	49.34	15.73
AD-1526797.1	65.01	12.64	80.03	5.10	59.17	11.40

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1527018.1	67.07	10.29	91.34	19.96	123.38	34.94
AD-1526798.1	67.32	14.00	96.68	14.84	89.78	26.78
AD-1526799.1	108.59	13.34	125.32	29.43	98.61	24.75
AD-1526800.1	171.82	23.70	152.44	24.55	117.88	30.09
AD-1526801.1	108.78	21.59	118.99	29.99	127.16	26.63
AD-1526802.1	96.99	22.67	127.81	21.15	62.19	13.93
AD-1526803.1	80.06	9.63	108.17	18.13	43.32	4.30
AD-1526804.1	79.14	8.73	88.77	16.65	74.12	22.19
AD-1526805.1	102.58	8.31	116.51	6.60	78.81	18.03
AD-1526806.1	68.15	13.14	92.96	24.34	72.95	18.24
AD-1526807.1	55.89	8.83	75.56	10.97	56.41	16.55
AD-1526808.1	96.45	12.43	96.21	17.45	101.04	21.38
AD-1526809.1	66.54	19.98	90.32	11.54	87.22	14.89
AD-1526810.1	66.39	18.78	91.36	28.16	108.70	24.37
AD-1526811.1	130.29	9.54	157.84	47.85	142.11	45.97
AD-1526812.2	34.47	7.82	43.52	11.77	41.81	11.97
AD-1526812.1	83.20	24.20	60.45	14.69	97.10	34.36
AD-1527019.1	76.17	23.27	77.37	13.08	78.31	20.20
AD-1527020.2	34.99	5.56	44.00	5.66	30.63	5.65
AD-1527020.1	26.42	2.01	34.55	6.72	46.67	11.48
AD-1526813.1	49.62	6.12	50.19	2.84	38.16	11.19
AD-1526814.1	54.03	13.48	65.30	19.73	47.10	6.60
AD-1526815.1	70.84	8.11	92.44	2.45	89.54	9.04
AD-1526816.1	41.99	8.84	56.84	14.62	46.95	5.29

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526817.1	36.23	6.41	60.26	13.19	39.94	5.18
AD-1526818.1	47.98	2.53	66.35	13.87	87.53	12.05
AD-1526819.1	119.04	42.58	124.80	21.09	148.12	32.92
AD-1527021.1	63.91	19.78	61.68	5.18	58.06	10.22
AD-1526820.2	38.33	8.91	37.64	8.13	35.09	4.39
AD-1526820.1	47.08	9.66	48.36	5.73	45.91	12.76
AD-1526821.1	53.23	7.75	62.76	9.56	50.12	7.90
AD-1527022.1	38.70	9.61	42.97	8.50	39.25	4.48
AD-1526822.1	50.62	5.87	50.70	6.50	49.37	11.99
AD-1526823.1	42.79	7.87	48.33	3.11	44.55	5.46
AD-1526824.1	40.67	13.82	63.95	11.12	51.37	11.24
AD-1526825.1	55.46	21.60	83.45	18.71	97.72	14.22
AD-1527091.1	67.10	10.74	64.51	9.14	70.62	21.66
AD-1526827.1	18.08	4.49	50.38	13.65	48.69	11.44
AD-1526826.1	50.58	13.77	65.79	12.67	59.92	27.86
AD-1526828.1	44.89	10.80	59.63	4.27	44.56	6.61
AD-1526830.1	70.82	12.22	75.57	6.87	58.52	11.57
AD-1526829.1	68.49	11.11	95.04	6.81	69.33	8.87
AD-1526831.1	61.84	13.89	80.85	9.40	57.43	13.70
AD-1527076.1	55.67	12.14	77.00	19.13	74.75	22.92
AD-1526833.1	55.81	9.84	52.49	4.27	47.54	21.50
AD-1527024.1	73.20	19.03	62.91	17.74	106.51	17.79
AD-1527025.1	66.36	6.24	67.19	10.66	84.57	20.21
AD-1526834.1	68.54	20.21	83.29	21.05	55.55	15.45

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526834.2	64.83	18.39	59.85	3.33	58.17	13.32
AD-1526835.2	28.10	2.16	43.83	9.76	26.75	7.30
AD-1526835.1	36.51	10.40	34.41	2.81	35.50	9.79
AD-1526836.1	33.12	10.43	46.01	7.36	23.30	9.57
AD-1526836.2	38.83	4.51	61.41	10.14	67.46	12.14
AD-1527026.1	34.85	4.42	42.91	12.61	35.45	6.74
AD-1527026.2	30.16	2.86	37.50	4.54	40.29	8.69
AD-1526837.2	48.94	15.13	56.90	11.70	70.50	13.24
AD-1526837.1	37.29	11.59	33.60	5.61	145.09	40.97
AD-1526838.1	41.27	3.44	59.42	10.16	49.44	18.79
AD-1526839.1	51.20	10.31	41.15	6.26	41.66	14.44
AD-1526839.2	52.99	10.71	64.23	13.34	51.30	20.44
AD-1527077.1	91.18	25.24	68.49	16.90	67.30	21.84
AD-1526841.1	76.63	18.36	87.08	23.10	85.23	28.21
AD-1526842.1	81.44	21.79	54.64	22.17	51.73	15.00
AD-1526843.1	75.45	25.32	62.39	25.42	75.59	12.99
AD-1526844.1	63.48	10.82	60.76	14.64	40.43	11.35
AD-1526845.1	36.44	5.86	32.87	8.30	39.88	6.95
AD-1527027.1	27.73	3.57	27.83	2.81	41.08	5.71
AD-1527027.2	42.42	7.52	46.72	9.13	60.97	15.27
AD-1527028.1	46.63	10.58	56.08	9.22	52.40	15.96
AD-1527028.2	52.54	9.22	59.69	5.15	74.14	11.24
AD-1526846.2	36.29	8.78	34.15	11.02	41.26	10.41
AD-1526846.1	36.91	5.36	28.18	8.59	48.85	7.12

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526847.1	38.66	9.93	32.54	11.74	47.82	14.06
AD-1526847.2						
AD-1526848.1	69.79	22.93	52.39	20.67	58.82	12.59
AD-1526848.2	73.31	20.03	48.45	10.41	69.40	12.04
AD-1526849.1	49.39	13.76	38.66	11.47	43.01	5.24
AD-1526849.2	46.07	7.22	37.03	3.90	46.50	11.14
AD-1527029.2	82.20	24.08	68.27	24.23	100.77	27.53
AD-1527029.1	71.48	27.82	60.44	3.58	108.03	31.28
AD-1526850.1	40.11	4.38	28.12	7.11	30.80	3.74
AD-1526850.2	38.79	7.13	26.24	9.22	31.19	9.61
AD-1527092.2	40.70	10.52	47.82	4.00	60.02	12.05
AD-1527092.1	46.11	7.40	52.04	7.08	73.20	9.59
AD-1527031.1	52.93	6.96	65.78	13.85	69.63	7.87
AD-1527031.2	63.79	8.94	57.89	6.78	69.69	18.40
AD-1526851.2	53.52	14.01	50.33	16.75	58.83	3.58
AD-1526851.1	61.27	15.99	50.00	10.67	61.87	5.70
AD-1526852.2	36.35	7.08	31.05	10.40	29.66	1.55
AD-1526852.1	52.95	15.70	29.39	11.18	31.03	4.92
AD-1526853.1	48.96	10.17	37.98	10.57	46.14	5.02
AD-1526854.1	93.11	9.69	82.00	5.90	86.61	13.23
AD-1526855.1	53.65	11.50	54.55	17.35	68.93	10.94
AD-1526856.1	59.22	5.88	49.39	11.67	80.53	9.48
AD-1526857.1	104.50	25.47	43.76	17.44	78.66	12.36
AD-1526859.1	62.18	11.24	69.00	7.48	73.58	9.69

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526858.1	98.49	15.02	93.59	23.59	109.28	29.05
AD-1526860.1	58.24	8.35	47.89	13.67	64.79	11.24
AD-1527032.1	38.57	3.50	38.91	8.27	44.81	2.41
AD-1526861.1	52.37	5.58	64.90	10.37	81.36	6.52
AD-1527078.1	47.57	5.68	51.55	16.61	94.51	5.09
AD-1526863.1	111.02	12.67	85.71	9.51	104.89	19.51
AD-1526864.1	89.20	14.44	74.31	7.92	90.32	22.05
AD-1526865.1	80.40	15.11	63.08	20.05	101.98	23.71
AD-1526866.1	84.08	9.33	59.60	19.08	92.28	16.88
AD-1526867.1	75.93	16.07	59.07	6.72	93.72	19.07
AD-1526868.1	62.35	7.36	70.09	12.15	70.05	3.61
AD-1526869.1	91.79	19.33	97.14	22.83	92.67	8.94
AD-1526870.1	52.26	6.97	61.78	16.63	74.55	11.34
AD-1527079.1	66.63	5.30	56.63	8.12	65.30	10.65
AD-1526872.1	81.52	21.99	97.98	23.74	97.53	20.83
AD-1527033.1	36.32	6.98	43.57	11.62	66.36	8.84
AD-1526873.1	50.66	17.75	76.37	25.11	75.14	15.36
AD-1526874.1	61.81	21.37	58.95	14.17	66.90	13.22
AD-1526875.1	69.98	18.02	72.67	12.59	73.62	5.71
AD-1527034.1	47.75	7.68	42.36	8.05	49.63	7.23
AD-1526876.1	65.23	12.07	65.93	4.99	80.48	13.12
AD-1527035.1	47.42	11.93	47.32	12.08	62.01	11.41
AD-1526877.1	39.19	12.06	33.81	10.57	44.37	9.48
AD-1526879.1	79.39	3.62	73.02	17.99	88.00	17.18

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1527080.1	85.10	11.96	99.12	15.42	115.01	14.90
AD-1526881.1	90.28	11.62	103.66	21.57	130.93	25.53
AD-1527036.1	48.44	9.35	53.17	8.93	81.56	14.38
AD-1526882.1	91.58	23.91	87.86	19.79	115.15	22.47
AD-1526883.1	70.76	10.26	65.66	22.65	89.94	18.26
AD-1526884.1	35.39	8.12	39.49	15.92	26.75	3.54
AD-1527081.1	85.03	13.16	97.43	16.77	97.22	10.69
AD-1526886.1	39.17	6.14	46.36	15.45	76.31	14.07
AD-1527082.1	48.33	9.22	42.60	13.98	68.22	11.57
AD-1526888.1	54.42	10.08	57.18	13.04	65.69	18.35
AD-1527093.1	73.36	8.54	54.15	4.59	70.52	24.04
AD-1527094.1	60.52	15.96	38.64	2.46	83.91	20.14
AD-1526889.1	46.40	6.96	59.71	11.32	71.80	18.46
AD-1526889.2	50.65	12.33	74.22	12.55	74.32	19.94
AD-1526890.1	59.61	10.37	97.12	11.00	73.13	3.94
AD-1526890.2	61.72	16.73	72.61	12.90	87.53	13.72
AD-1526891.2	26.30	3.55	38.57	8.83	38.00	9.87
AD-1526891.1	27.33	2.65	37.83	9.34	38.39	3.18
AD-1526892.1	26.27	8.01	33.30	5.34	43.32	7.28
AD-1526892.2	29.29	3.46	37.67	3.72	48.04	13.69
AD-1526893.1	73.13	8.49	103.27	22.31	97.55	16.62
AD-1527095.1	20.67	5.29	31.63	8.74	35.71	12.63
AD-1527096.1	19.75	5.12	18.43	5.22	26.09	8.19
AD-1526894.1	47.38	5.23	65.17	17.17	78.92	11.25

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526895.1	58.79	11.88	75.42	8.71	83.01	13.98
AD-1526896.1	37.60	5.77	48.70	7.47	64.91	8.35
AD-1526898.1	41.37	4.16	35.94	11.36	59.85	8.13
AD-1526897.1	36.62	8.02	44.33	7.62	68.90	4.70
AD-1527041.1	31.86	4.29	26.68	6.29	41.71	11.80
AD-1526899.1	52.76	9.00	56.94	6.25	81.20	14.10
AD-1526900.1	68.51	7.39	91.20	9.66	100.36	26.35
AD-1526901.1	58.91	12.19	73.47	9.70	92.78	27.89
AD-1526902.1	24.64	7.82	26.16	3.46	30.78	8.56
AD-1526903.1	25.18	5.60	28.52	8.58	37.75	4.38
AD-1193350.9	19.83	2.88	17.02	1.92	27.69	8.58
AD-519347.6	26.57	3.65	30.33	7.02	41.15	11.70
AD-1193350.10	15.97	1.12	18.84	2.78	26.52	7.81
AD-519347.7	21.74	5.32	28.47	5.19	38.39	7.80
AD-1193350.11	22.84	2.94	36.40	5.15	20.97	8.90
AD-519347.8	27.13	1.65	42.41	10.72	41.20	3.09
AD-1193350.12	14.56	4.45	17.60	2.96	29.05	2.48
AD-519347.9	25.72	4.25	32.93	10.71	33.65	5.33
AD-1193365.9	33.15	7.07	30.85	5.72	54.76	17.64
AD-1193365.10	31.48	5.87	64.54	10.60	43.82	14.24
AD-1193365.11	64.38	10.43	36.53	4.64	82.74	40.60
AD-1193365.12	33.51	8.14	42.26	6.34	38.39	7.06
AD-519351.16	26.85	7.10	32.60	3.49	36.91	8.64
AD-519351.17	28.37	3.16	39.80	3.44	43.55	4.19

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-519351.18	40.69	10.42	48.84	4.70	35.31	8.44
AD-519351.19	30.20	3.43	30.03	7.85	39.81	7.46
AD-1527042.1	69.28	11.64	70.88	13.22	88.52	8.89
AD-1526904.1	71.25	9.69	74.70	16.37	83.09	13.21
AD-1526905.1	66.61	4.88	69.11	15.35	88.21	13.41
AD-1526906.1	56.16	8.67	79.89	22.67	66.82	15.00
AD-1526907.1	48.94	6.87	74.13	14.64	78.81	17.98
AD-1526907.2	56.67	6.73	61.46	3.64	98.85	16.51
AD-1526908.2	35.29	12.25	45.50	9.46	26.51	10.59
AD-1526908.1	50.58	11.73	43.79	1.50	56.40	11.03
AD-1527097.2	35.86	6.58	46.39	5.52	67.17	24.56
AD-1527097.1	40.57	4.87	60.30	2.84	68.97	8.22
AD-1527044.1	57.85	16.21	81.64	22.04	109.62	26.86
AD-1527083.2	38.26	4.61	31.59	7.50	27.70	7.97
AD-1527083.1	42.67	14.90	30.03	10.63	30.67	10.77
AD-1527045.1	71.19	12.59	85.97	24.54	82.85	30.20
AD-1526910.1	23.93	4.40	25.72	4.82	33.59	12.10
AD-1527084.1	44.66	6.88	29.63	6.94	49.48	12.35
AD-1526912.1	70.21	22.31	77.54	30.44	87.11	16.30
AD-1526913.1	42.19	8.29	50.45	6.62	69.49	8.42
AD-1526914.1	104.95	16.06	77.35	13.27	90.73	4.65
AD-1526915.1	35.35	10.75	60.67	9.10	125.24	29.74
AD-1527046.1	52.52	6.48	62.17	8.66	74.69	17.20
AD-1526917.1	57.31	18.57	63.58	15.61	66.44	9.92

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526919.1	80.77	12.63	98.56	23.73	38.89	9.25
AD-1526918.1	74.94	6.02	58.52	10.31	46.49	7.42
AD-1527047.1	24.59	3.30	37.06	8.03	43.42	13.50
AD-1527047.2	30.55	3.44	35.86	10.15	45.17	8.64
AD-1526920.1	61.58	18.94	57.10	9.21	35.75	10.71
AD-1526921.1	102.19	29.02	85.36	21.12	145.13	6.40
AD-1527098.1	29.05	4.12	33.61	3.29	34.05	6.41
AD-1527049.1	33.44	4.86	39.91	1.34	41.11	6.52
AD-1527049.2	39.83	4.89	46.07	9.74	41.73	11.80
AD-1527086.1	31.72	10.14	49.16	11.72	57.41	16.44
AD-1527086.2	46.85	8.40	48.51	9.22	49.68	12.11
AD-1526923.1	51.84	16.26	51.24	8.13	83.67	13.66
AD-1526924.1	57.09	11.77	62.81	5.92	96.33	19.02
AD-1526925.2	44.01	14.29	36.79	2.99	59.96	14.22
AD-1526925.1	48.01	3.47	47.81	9.92	46.94	4.43
AD-1526926.1	70.07	5.64	52.70	5.35	70.70	7.13
AD-1526927.1	47.81	2.49	58.77	6.43	69.57	7.08
AD-1526927.2	56.29	0.00	46.16	5.64	80.25	15.03
AD-1526928.2	80.41	4.92	76.85	9.50	74.52	6.25
AD-1526928.1	120.37	13.77	125.17	17.23	129.65	18.69
AD-1527050.1	59.64	9.54	69.52	15.37	69.72	11.61
AD-1527051.1	29.34	4.64	39.39	8.75	34.97	5.60
AD-1526929.1	77.77	12.03	65.94	6.15	75.93	9.50
AD-1527052.1	38.34	4.16	39.46	8.19	38.07	7.92

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526930.1	74.06	5.92	86.66	7.80	96.25	11.80
AD-1526931.1	79.98	15.32	86.70	9.76	98.04	21.38
AD-1526933.1	91.83	22.91	86.26	21.32	114.31	6.72
AD-1526932.1	68.39	15.13	99.38	14.14	140.85	37.77
AD-1527099.1	30.77	4.98	47.96	3.67	58.78	4.10
AD-1526934.1	50.00	5.48	70.49	13.68	89.89	25.03
AD-1527087.1	60.71	6.79	44.82	9.21	57.70	12.06
AD-1527088.1	44.34	4.55	38.97	7.61	42.12	5.32
AD-1526937.1	35.46	5.10	32.39	7.46	51.22	3.94
AD-1526938.1	62.67	12.83	59.88	6.11	73.73	16.85
AD-1526939.1	41.41	8.36	42.00	7.05	38.97	8.50
AD-1526940.1	114.31	19.22	93.01	17.95	100.44	19.35
AD-1527054.1	77.09	3.84	68.84	7.98	64.71	17.39
AD-1526941.1	32.43	8.36	45.02	5.73	43.72	9.21
AD-1526942.1	90.78	8.41	73.93	7.12	87.90	15.68
AD-1526943.1	47.50	3.39	47.64	2.26	33.78	7.10
AD-1526945.1	63.06	10.10	57.15	10.06	82.33	13.93
AD-1526944.1	74.60	15.73	70.95	9.81	112.93	9.18
AD-1526946.1	69.13	6.02	64.43	13.35	86.59	13.92
AD-1527100.1	34.40	5.85	36.48	7.14	38.75	8.27
AD-1526947.1	89.86	15.67	90.06	26.39	162.30	14.02
AD-1527056.1	53.37	4.18	74.12	24.19	39.90	19.08
AD-1526948.1	75.94	12.20	77.85	19.57	138.95	33.74
AD-1527057.1	32.17	6.04	60.11	11.52	46.60	16.07

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526949.1	46.38	4.62	48.59	5.45	51.64	14.71
AD-1526951.1	70.86	9.36	73.91	9.88	80.51	17.22
AD-1526950.1	60.97	13.46	68.29	8.35	91.01	16.62
AD-1526952.1	83.27	18.16	66.43	5.00	74.40	15.55
AD-1527058.1	50.74	6.56	61.71	16.11	76.82	8.21
AD-1526953.1	33.40	6.71	45.82	7.55	72.96	13.99
AD-1526954.1	37.60	2.76	31.57	7.63	62.19	14.23
AD-1527059.1	59.96	12.38	79.54	10.50	100.56	30.82
AD-1527060.1	68.73	9.49	64.10	18.94	70.01	15.90
AD-1526955.1	47.36	6.72	66.12	8.74	71.33	16.21
AD-1526956.1	50.96	4.44	62.17	10.49	62.93	11.19
AD-1526957.1	54.20	5.52	65.17	12.50	65.39	7.84
AD-1526958.1	66.00	6.02	76.24	9.84	79.36	13.56
AD-1526959.1	65.35	17.34	62.64	10.41	66.10	12.72
AD-1527061.1	36.97	3.89	34.38	4.13	40.94	8.63
AD-1526960.1	34.21	7.72	38.13	5.60	44.04	4.30
AD-1526961.1	39.44	2.34	58.35	3.76	46.72	3.41
AD-1526962.1	37.16	11.86	33.93	1.71	40.87	2.62
AD-1526963.1	47.14	8.42	50.89	11.51	47.69	5.54
AD-1526964.1	44.73	7.56	59.95	12.88	56.96	8.29
AD-1526965.1	38.62	2.20	47.35	7.84	36.55	9.09
AD-1193373.2	78.34	7.12	61.65	18.85	87.36	12.80
AD-1527101.1	42.34	3.55	43.87	14.20	61.45	26.49
AD-1526967.1	53.76	10.19	50.61	12.76	79.97	22.48

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526968.1	45.80	8.92	43.88	10.04	55.49	11.76
AD-1526969.1	42.24	1.50	48.21	7.41	38.74	6.60
AD-1526970.1	41.97	3.62	44.01	9.64	41.95	6.14
AD-1526971.1	39.41	4.29	50.87	3.87	50.42	8.15
AD-1526972.1	45.05	2.05	53.79	5.79	63.66	12.77
AD-1527102.1	51.67	4.52	47.13	8.54	54.43	14.46
AD-1526973.1	50.42	6.93	65.74	12.16	61.14	10.65
AD-1526974.1	82.55	20.27	62.51	9.25	106.57	12.84
AD-1526975.1	57.05	7.78	37.85	1.77	81.29	16.19
AD-1526976.1	52.41	6.01	37.91	6.42	50.93	15.62
AD-1527103.1	41.12	4.05	75.42	19.02	70.06	31.99
AD-1527104.1	38.50	13.67	43.20	5.46	41.19	15.40
AD-1526977.1	59.65	9.85	49.94	9.40	40.05	6.98
AD-1527105.1	42.00	11.57	53.50	17.75	38.02	13.61
AD-1526978.1	59.23	8.36	49.05	5.18	68.40	13.06
AD-1526979.1	55.62	8.89	49.31	7.84	43.97	6.51
AD-1527089.1	59.84	20.28	51.08	5.71	60.93	11.78
AD-1526981.1	93.29	22.39	89.26	5.61	72.67	17.71
AD-1526982.1	90.26	9.70	55.21	13.50	91.27	21.17
AD-1526983.1	67.33	10.17	47.69	4.83	83.36	15.35
AD-1527067.1	54.11	13.15	75.54	14.14	51.97	21.89
AD-1527068.1	23.04	9.60	50.31	16.15	32.88	5.45
AD-1526984.1	45.16	6.61	34.84	9.72	40.42	11.68
AD-1526985.1	44.84	8.06	45.62	11.56	48.95	4.96

	Hep3B Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526986.1	72.17	7.57	69.28	13.46	87.93	25.79
AD-1527106.1	25.90	3.66	35.37	10.21	30.39	1.07
AD-1526987.1	35.67	4.70	19.52	2.84	32.75	11.24
AD-1526988.1	42.34	6.98	52.61	7.81	39.72	7.23
AD-1526989.1	28.65	9.51	38.74	4.90	65.47	13.64
AD-1527107.1	31.90	5.80	44.77	11.26	51.67	6.53
AD-1526990.1	28.23	10.55	26.21	10.15	33.94	9.09
AD-1526991.1	43.52	15.37	30.74	6.00	26.58	6.65
AD-1526992.1	63.96	7.22	34.20	6.99	29.03	2.60
AD-1526993.1	37.16	7.44	29.90	5.72	28.05	4.96
AD-1526994.1	45.33	12.92	39.80	11.37	45.51	7.36
AD-1526995.1	98.43	7.72	54.68	16.30	64.47	10.45
AD-1526996.1	115.72	19.07	77.30	11.14	44.02	14.08
AD-1526997.1	36.28	10.19	48.61	15.53	31.35	11.44
AD-1526999.1	34.13	8.43	58.17	16.48	23.79	4.41
AD-1526998.1	46.80	6.10	35.97	5.18	39.02	15.25
AD-1527000.1	38.25	12.96	39.42	17.08	41.33	13.32
AD-1527001.1	45.79	6.85	48.25	18.53	51.02	14.92
AD-1527002.1	37.87	8.90	58.60	5.53	54.16	20.12
AD-1527003.1	107.12	17.44	86.62	8.23	61.41	21.51
AD-1527108.1	43.45	6.21	53.56	18.85	52.10	7.89
AD-1527004.1	51.39	5.85	45.91	12.24	76.63	17.28
AD-1527005.1	41.64	2.88	50.23	8.91	40.48	4.44

Table 5. In Vitro Screen in HepG2 cells

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526763.1	72.04	9.39	119.91	24.51	68.49	N/A
AD-1527006.1	55.29	5.37	28.91	2.26	46.28	17.66
AD-1526764.1	49.22	11.45	89.17	20.23	55.33	4.33
AD-1526765.1	79.36	23.37	86.02	4.64	87.55	20.87
AD-1526766.1	72.02	20.36	106.42	8.65	87.89	29.50
AD-1526767.1	72.92	11.83	84.83	4.57	73.55	12.09
AD-1527090.1	45.42	12.72	28.98	9.62	65.06	18.55
AD-1526768.1	59.92	11.80	79.37	11.21	72.92	12.43
AD-1526769.1	93.82	5.83	98.84	11.12	82.25	21.00
AD-1526770.1	N/A	N/A	104.54	31.67	162.40	58.05
AD-1527008.1	52.67	11.12	81.35	16.70	88.60	20.09
AD-1526771.1	55.04	14.39	56.33	21.30	62.05	19.15
AD-1526772.1	51.13	22.42	53.35	15.15	38.57	7.07
AD-1527009.1	34.11	9.57	17.67	1.38	54.92	5.52
AD-1526773.1	59.16	15.64	67.56	24.76	62.18	21.73
AD-1527010.1	63.70	20.95	65.15	15.17	86.08	22.35
AD-1527072.1	62.25	4.38	74.58	33.48	77.46	18.97
AD-1527011.1	44.11	13.10	52.66	11.52	101.00	7.64
AD-1527073.1	30.59	3.40	45.31	11.87	39.29	10.81
AD-1527012.1	80.07	25.78	97.61	20.07	93.03	23.95
AD-1526776.1	37.90	6.06	74.86	29.35	58.77	19.20
AD-1526777.1	56.50	8.55	84.07	15.76	87.29	10.03
AD-1526778.1	91.04	21.97	183.41	53.42	114.15	17.32

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1527074.1	95.68	24.16	102.74	9.62	67.43	12.78
AD-1526780.1	73.95	17.72	102.90	6.41	66.93	8.57
AD-1526781.1	69.46	16.56	90.25	22.73	88.94	18.04
AD-1527013.1	53.68	16.59	60.11	11.02	68.24	9.38
AD-1526782.1	99.79	8.06	100.60	17.76	74.86	17.21
AD-1527075.1	43.17	14.75	76.65	14.04	62.52	11.00
AD-1527014.1	55.87	10.82	61.15	12.14	55.74	10.61
AD-1526784.1	54.51	11.27	78.04	8.06	69.94	17.79
AD-1526785.1	113.41	34.03	181.68	35.45	135.10	41.40
AD-1526786.1	75.41	30.23	46.94	4.90	61.79	3.29
AD-1526787.1	74.12	12.35	72.11	8.93	74.06	15.47
AD-1526788.1	94.88	26.29	89.76	20.21	88.91	16.65
AD-1526789.1	65.49	20.06	44.30	23.46	59.60	6.72
AD-1526790.1	79.64	16.42	104.56	19.14	71.60	7.93
AD-1526791.1	74.95	10.82	118.83	20.40	106.34	15.44
AD-1526792.1	60.00	9.53	122.31	37.01	121.90	16.40
AD-1526793.1	90.69	24.77	77.63	31.66	76.58	23.46
AD-1527015.1	39.54	6.49	47.06	6.26	56.24	11.18
AD-1527016.1	53.08	11.17	69.46	6.25	72.45	13.17
AD-1526794.1	63.91	23.28	61.80	16.67	51.54	10.41
AD-1527017.1	100.04	26.58	84.83	20.08	84.14	10.48
AD-1526795.1	47.15	1.25	47.63	5.68	48.92	5.70
AD-1526796.1	42.73	6.47	43.46	10.10	53.50	14.13
AD-1526797.1	59.67	15.43	82.50	32.98	67.28	3.76

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1527018.1	65.36	14.44	84.63	16.67	110.50	27.48
AD-1526798.1	67.10	22.85	83.72	28.00	84.19	1.67
AD-1526799.1	101.25	13.47	94.25	24.84	93.85	23.12
AD-1526800.1	142.08	41.50	234.02	38.41	133.77	35.44
AD-1526801.1	81.04	21.80	76.52	4.82	76.11	12.53
AD-1526802.1	87.50	9.48	74.53	4.15	71.64	4.58
AD-1526803.1	89.53	5.60	71.24	24.61	46.00	11.21
AD-1526804.1	125.39	24.45	79.25	9.16	75.65	6.12
AD-1526805.1	106.44	14.32	102.76	17.73	84.03	8.25
AD-1526806.1	77.54	8.78	78.23	6.72	82.32	11.17
AD-1526807.1	73.85	10.54	70.99	7.75	73.13	15.06
AD-1526808.1	96.82	9.29	103.27	18.47	83.40	10.94
AD-1526809.1	94.58	23.63	89.10	19.78	83.16	16.74
AD-1526810.1	94.33	16.60	94.87	19.76	103.19	22.62
AD-1526811.1	126.27	25.75	139.63	9.40	120.33	24.05
AD-1526812.2	34.26	7.33	38.95	4.01	34.41	4.43
AD-1526812.1	54.69	11.92	55.96	7.85	35.44	5.08
AD-1527019.1	70.23	4.96	56.39	16.47	55.17	16.00
AD-1527020.2	47.32	9.36	40.02	4.12	45.29	9.48
AD-1527020.1	39.09	5.34	34.96	4.83	35.18	6.18
AD-1526813.1	41.01	5.60	33.47	3.62	43.30	4.46
AD-1526814.1	54.68	11.71	45.54	8.90	47.53	7.83
AD-1526815.1	80.55	9.21	61.98	13.21	94.14	10.03
AD-1526816.1	39.65	11.30	35.31	3.51	47.02	4.42

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526817.1	53.82	17.16	43.38	1.71	45.59	10.05
AD-1526818.1	52.60	11.60	81.50	8.19	113.12	24.47
AD-1526819.1	96.29	19.64	98.77	13.64	70.94	12.64
AD-1527021.1	91.87	24.67	76.46	19.00	74.96	9.51
AD-1526820.2	52.84	4.55	47.75	4.30	38.33	10.66
AD-1526820.1	62.09	7.92	37.35	6.75	36.85	5.87
AD-1526821.1	64.45	10.73	55.19	10.05	51.99	8.64
AD-1527022.1	51.97	5.65	36.54	6.35	50.78	9.18
AD-1526822.1	54.41	2.55	53.56	11.74	56.34	15.43
AD-1526823.1	51.31	6.89	45.74	9.63	39.73	9.38
AD-1526824.1	47.33	8.32	66.96	16.86	55.75	16.38
AD-1526825.1	41.43	1.62	37.54	11.73	48.99	5.30
AD-1527091.1	82.10	5.99	77.11	16.44	104.47	14.85
AD-1526827.1	56.56	9.29	40.71	7.87	39.23	10.32
AD-1526826.1	53.59	9.34	41.64	8.29	44.37	10.50
AD-1526828.1	52.87	3.27	40.42	10.50	47.62	1.54
AD-1526830.1	83.65	22.87	60.40	9.57	63.55	11.89
AD-1526829.1	75.20	5.66	72.08	5.86	72.49	9.10
AD-1526831.1	43.75	10.90	65.23	19.86	54.76	13.46
AD-1527076.1	56.84	15.95	49.13	7.40	54.49	7.39
AD-1526833.1	42.76	14.74	27.90	5.96	35.09	5.54
AD-1527024.1	71.51	8.72	76.52	4.87	114.48	2.24
AD-1527025.1	39.31	10.29	56.24	5.12	42.03	8.96
AD-1526834.1	75.19	5.35	49.50	9.79	71.47	8.80

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526834.2	80.32	11.25	34.78	11.56	60.97	14.75
AD-1526835.2	39.50	3.96	34.48	12.59	44.45	6.41
AD-1526835.1	45.25	10.51	24.80	6.67	39.80	8.29
AD-1526836.1	46.40	7.55	38.77	6.39	28.90	4.03
AD-1526836.2	59.02	15.37	39.07	4.52	39.23	6.31
AD-1527026.1	43.86	11.91	23.57	5.67	38.55	5.99
AD-1527026.2	33.62	3.88	29.12	4.01	36.06	5.35
AD-1526837.2	33.14	7.45	32.48	5.17	35.33	6.60
AD-1526837.1	26.36	7.10	42.91	7.51	27.46	7.11
AD-1526838.1	29.70	5.60	26.44	5.68	39.84	3.18
AD-1526839.1	33.16	8.30	32.03	6.44	43.99	5.80
AD-1526839.2	40.43	7.83	29.97	3.51	54.17	11.19
AD-1527077.1	67.64	27.05	77.17	8.64	62.66	11.30
AD-1526841.1	64.91	18.66	60.65	14.05	65.53	9.68
AD-1526842.1	105.84	17.04	80.84	13.68	124.28	9.13
AD-1526843.1	67.21	7.42	90.56	23.36	51.54	14.46
AD-1526844.1	93.43	15.05	112.40	36.81	68.34	17.45
AD-1526845.1	35.15	9.38	30.92	3.63	31.90	8.81
AD-1527027.1	34.05	2.60	27.18	7.03	32.54	6.00
AD-1527027.2	48.23	8.67	56.91	3.13	52.37	8.16
AD-1527028.1	64.54	8.74	70.68	7.92	63.85	12.76
AD-1527028.2	74.30	3.69	69.07	8.79	64.89	9.62
AD-1526846.2	19.81	8.79	55.48	12.40	68.41	20.96
AD-1526846.1	14.19	3.38	39.64	6.00	50.37	11.50

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526847.1	54.81	19.46	38.91	15.76	42.03	17.92
AD-1526847.2	32.08	5.21	56.05	20.15	113.54	24.35
AD-1526848.1	41.17	5.83	90.45	21.90	102.64	5.03
AD-1526848.2	84.66	12.40	88.99	8.44	110.82	29.23
AD-1526849.1	67.89	21.05	80.06	17.67	68.23	20.79
AD-1526849.2	63.66	14.09	69.78	17.08	120.48	14.07
AD-1527029.2	67.13	20.75	80.64	15.04	65.11	10.54
AD-1527029.1	85.97	23.88	109.98	7.19	82.94	13.31
AD-1526850.1	59.86	20.44	58.11	5.64	78.03	23.35
AD-1526850.2	61.09	19.93	55.07	12.15	83.34	8.49
AD-1527092.2	53.90	9.46	62.03	6.41	58.41	7.85
AD-1527092.1	51.45	5.18	66.60	13.84	56.13	5.07
AD-1527031.1	82.19	7.41	84.87	21.78	79.23	17.70
AD-1527031.2	102.35	10.87	98.88	8.20	84.48	12.52
AD-1526851.2	156.84	48.56	70.79	N/A	121.00	38.56
AD-1526851.1	96.87	20.51	112.22	39.31	104.98	9.03
AD-1526852.2	61.47	14.92	35.30	7.54	55.34	8.60
AD-1526852.1	79.08	6.50	56.09	8.13	71.84	11.09
AD-1526853.1	69.89	9.72	66.12	3.41	83.57	15.97
AD-1526854.1	113.31	31.53	117.58	16.93	106.24	23.61
AD-1526855.1	82.44	16.24	115.21	13.88	112.38	12.31
AD-1526856.1	55.67	11.02	97.43	19.23	96.28	14.95
AD-1526857.1	124.85	12.29	118.66	18.74	113.64	16.65
AD-1526859.1	61.43	17.58	78.26	7.23	82.12	22.16

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526858.1	88.11	19.14	115.36	25.08	116.52	28.53
AD-1526860.1	77.90	12.12	68.28	19.44	83.26	9.46
AD-1527032.1	42.71	9.63	42.23	5.67	51.96	7.38
AD-1526861.1	72.00	17.72	93.92	21.32	100.44	9.64
AD-1527078.1	69.35	13.90	69.29	15.88	90.57	27.49
AD-1526863.1	118.92	17.99	122.09	27.91	146.26	27.51
AD-1526864.1	137.25	47.34	104.82	5.17	117.73	24.28
AD-1526865.1	96.13	12.92	125.19	18.90	128.06	2.66
AD-1526866.1	106.34	13.79	94.77	14.11	29.69	2.89
AD-1526867.1	77.23	19.16	99.39	13.03	86.04	16.68
AD-1526868.1	71.69	18.56	77.28	14.32	67.62	11.84
AD-1526869.1	134.64	18.79	129.87	20.05	98.61	17.00
AD-1526870.1	73.71	13.20	68.65	11.17	109.10	5.61
AD-1527079.1	72.48	13.01	76.56	12.66	73.32	11.09
AD-1526872.1	197.77	61.77	114.23	28.70	122.85	19.09
AD-1527033.1	60.59	8.70	54.07	5.24	60.53	2.34
AD-1526873.1	89.93	14.51	74.78	16.09	119.75	21.83
AD-1526874.1	29.46	11.75	44.48	5.80	62.32	17.72
AD-1526875.1	69.69	16.49	66.84	8.32	97.12	18.83
AD-1527034.1	51.72	6.35	68.16	10.22	73.47	13.27
AD-1526876.1	92.65	29.29	90.32	16.92	77.56	10.26
AD-1527035.1	66.74	9.75	65.18	6.17	69.83	4.21
AD-1526877.1	50.12	7.95	44.46	7.43	63.36	7.18
AD-1526879.1	93.50	9.62	80.47	26.57	87.55	11.21

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1527080.1	101.40	19.75	95.86	23.60	104.53	14.22
AD-1526881.1	114.83	16.49	90.61	19.52	108.60	12.76
AD-1527036.1	68.21	3.80	69.36	4.51	87.05	8.32
AD-1526882.1	78.06	7.99	97.66	5.45	115.74	10.14
AD-1526883.1	67.56	13.72	123.22	11.31	140.00	30.48
AD-1526884.1	44.97	14.01	40.30	13.38	59.62	11.52
AD-1527081.1	77.12	29.18	83.85	7.40	78.56	16.22
AD-1526886.1	54.60	12.49	53.33	11.65	63.83	8.07
AD-1527082.1	51.22	8.97	47.60	13.18	65.10	17.85
AD-1526888.1	44.74	14.10	48.37	10.69	62.68	3.73
AD-1527093.1	71.29	13.83	81.60	11.67	69.52	2.26
AD-1527094.1	62.26	15.66	57.03	11.73	30.53	7.70
AD-1526889.1	48.74	15.57	50.31	7.56	73.63	15.74
AD-1526889.2	64.21	2.70	56.28	7.31	72.95	18.52
AD-1526890.1	83.16	18.51	61.37	12.15	113.73	31.23
AD-1526890.2	70.45	15.83	93.38	13.91	98.14	18.54
AD-1526891.2	39.73	9.29	38.11	12.48	49.27	5.55
AD-1526891.1	45.34	13.91	33.08	10.03	53.22	4.11
AD-1526892.1	32.27	10.06	30.02	10.35	53.07	15.64
AD-1526892.2	31.49	4.69	35.33	7.11	61.99	10.14
AD-1526893.1	72.42	24.61	98.88	12.77	91.56	19.55
AD-1527095.1	34.37	4.84	32.69	3.02	36.26	6.42
AD-1527096.1	32.56	2.78	38.48	7.54	38.33	9.33
AD-1526894.1	75.53	8.91	64.50	12.26	97.18	35.53

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526895.1	75.08	5.29	46.33	7.72	83.49	10.45
AD-1526896.1	49.87	4.19	49.21	7.26	62.47	16.89
AD-1526898.1	34.54	10.71	42.97	4.95	53.88	7.99
AD-1526897.1	51.79	13.91	56.66	11.74	74.84	17.51
AD-1527041.1	44.44	9.02	35.68	4.54	45.70	10.68
AD-1526899.1	51.47	13.53	66.54	7.96	82.72	16.09
AD-1526900.1	64.65	13.05	78.22	12.08	81.93	13.37
AD-1526901.1	67.63	15.43	62.75	9.96	120.21	20.67
AD-1526902.1	23.65	3.15	27.97	7.17	41.11	4.41
AD-1526903.1	28.30	6.55	35.76	5.21	44.02	6.40
AD-1193350.9	20.68	3.97	16.51	0.87	29.05	4.81
AD-519347.6	25.67	4.87	39.90	7.45	44.46	9.12
AD-1193350.10	27.30	4.46	28.88	6.06	28.16	3.69
AD-519347.7	36.27	4.69	37.37	6.56	46.99	11.13
AD-1193350.11	28.91	2.99	28.76	2.84	34.68	7.69
AD-519347.8	31.01	3.63	36.43	5.87	40.90	10.08
AD-1193350.12	22.16	3.76	24.68	2.88	37.15	3.51
AD-519347.9	32.77	7.62	35.27	4.91	45.75	8.60
AD-1193365.9	37.65	13.28	34.29	9.29	40.76	1.77
AD-1193365.10	39.63	12.25	44.53	9.93	28.80	9.19
AD-1193365.11	43.71	16.38	41.31	13.94	38.34	8.54
AD-1193365.12	38.36	3.37	31.68	9.73	55.69	13.55
AD-519351.16	29.22	5.65	38.15	9.70	39.52	4.23
AD-519351.17	30.21	1.06	36.41	6.74	40.03	5.77

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-519351.18	39.12	14.84	32.70	9.62	25.65	2.54
AD-519351.19	30.10	2.93	32.27	12.67	46.65	3.60
AD-1527042.1	85.47	10.31	106.77	24.93	87.63	11.50
AD-1526904.1	68.16	12.90	68.36	4.28	68.63	1.50
AD-1526905.1	67.99	17.75	69.85	13.44	65.61	6.44
AD-1526906.1	61.73	16.58	58.29	22.12	78.85	9.94
AD-1526907.1	44.23	13.89	53.11	9.67	80.79	10.22
AD-1526907.2	44.78	5.60	49.08	4.21	57.21	8.42
AD-1526908.2	16.65	3.99	21.33	6.71	43.85	10.07
AD-1526908.1	69.29	14.22	53.81	3.09	65.13	11.02
AD-1527097.2	65.33	9.17	61.12	11.04	61.89	13.71
AD-1527097.1	49.78	6.31	68.26	3.70	64.08	10.67
AD-1527044.1	90.13	12.71	95.41	10.78	103.25	20.72
AD-1527083.2	30.49	4.80	28.38	2.37	43.88	5.87
AD-1527083.1	28.94	6.62	27.81	10.02	45.12	4.28
AD-1527045.1	52.07	12.94	76.77	13.35	63.04	14.69
AD-1526910.1	41.63	8.61	53.82	13.57	51.90	7.54
AD-1527084.1	36.92	4.33	44.16	8.47	54.17	5.93
AD-1526912.1	51.84	8.12	59.88	22.39	78.49	17.06
AD-1526913.1	44.72	7.90	72.64	11.98	54.79	14.20
AD-1526914.1	44.94	19.57	77.52	23.92	71.53	26.33
AD-1526915.1	54.39	16.39	66.70	16.14	83.83	26.69
AD-1527046.1	69.09	16.85	66.66	9.64	76.99	19.10
AD-1526917.1	49.98	14.45	61.88	5.84	81.45	16.66

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526919.1	64.64	19.69	78.96	21.82	75.86	11.18
AD-1526918.1	38.62	8.81	59.74	13.71	88.79	28.03
AD-1527047.1	52.53	4.34	51.19	11.48	51.11	15.73
AD-1527047.2	41.09	4.81	47.28	8.46	41.01	5.17
AD-1526920.1	46.98	6.69	41.70	14.11	39.10	4.98
AD-1526921.1	76.29	10.84	100.44	32.43	67.45	19.36
AD-1527098.1	47.13	7.24	44.45	9.17	34.66	5.78
AD-1527049.1	60.15	12.17	54.88	6.06	43.23	9.49
AD-1527049.2	48.37	5.70	50.53	4.90	54.03	6.09
AD-1527086.1	36.71	7.14	37.51	10.86	61.34	14.65
AD-1527086.2	36.75	7.80	42.79	5.35	61.66	7.86
AD-1526923.1	55.29	7.63	59.45	10.49	133.47	8.66
AD-1526924.1	50.61	13.65	76.32	9.40	97.91	17.43
AD-1526925.2	39.40	6.36	30.60	6.23	70.23	13.72
AD-1526925.1	36.38	8.74	37.13	8.66	81.75	14.09
AD-1526926.1	76.01	18.74	76.98	24.77	116.06	4.33
AD-1526927.1	53.12	14.93	66.25	16.23	65.67	9.19
AD-1526927.2	65.43	12.00	64.59	1.29	89.14	14.00
AD-1526928.2	73.45	13.40	83.74	13.31	100.15	12.13
AD-1526928.1	62.72	18.73	94.66	26.61	109.71	21.10
AD-1527050.1	59.29	12.88	66.90	7.24	55.00	3.77
AD-1527051.1	31.86	2.99	42.10	8.84	31.71	4.30
AD-1526929.1	62.38	3.85	80.12	14.74	111.12	15.12
AD-1527052.1	50.88	3.60	47.37	6.78	45.28	2.61

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526930.1	65.41	16.82	122.05	28.07	122.31	17.19
AD-1526931.1	79.41	12.54	89.14	6.07	114.36	6.89
AD-1526933.1	76.80	22.82	50.97	6.30	83.25	17.11
AD-1526932.1	72.60	15.38	92.45	1.48	134.89	25.86
AD-1527099.1	41.58	6.52	61.20	3.42	52.70	5.63
AD-1526934.1	43.90	5.59	47.77	12.31	67.36	16.11
AD-1527087.1	56.85	10.43	56.96	12.12	85.63	14.05
AD-1527088.1	47.52	5.25	50.25	12.29	54.99	4.37
AD-1526937.1	44.20	8.91	47.88	1.83	67.46	13.82
AD-1526938.1	54.00	9.43	**74.33**	18.89	103.14	23.62
AD-1526939.1	42.07	18.59	57.75	11.52	59.00	17.12
AD-1526940.1	72.58	16.32	72.16	12.45	76.29	16.08
AD-1527054.1	79.74	18.00	98.93	8.92	72.27	5.77
AD-1526941.1	54.12	12.89	55.96	2.02	77.41	9.83
AD-1526942.1	68.50	10.87	60.51	11.82	79.49	15.69
AD-1526943.1	55.90	14.56	56.85	8.29	81.36	14.79
AD-1526945.1	45.50	8.54	48.87	9.61	70.64	4.25
AD-1526944.1	46.65	21.17	53.27	16.49	90.96	9.65
AD-1526946.1	78.38	11.30	85.47	15.22	110.22	14.45
AD-1527100.1	36.40	5.71	48.44	4.23	45.70	2.90
AD-1526947.1	67.67	15.64	94.90	9.61	118.31	33.99
AD-1527056.1	34.72	6.01	55.06	7.96	44.18	3.52
AD-1526948.1	54.03	12.86	49.36	11.49	89.39	20.70
AD-1527057.1	45.64	3.49	42.30	5.15	40.75	6.88

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526949.1	51.99	11.22	40.56	8.10	49.31	4.91
AD-1526951.1	54.73	8.57	59.20	5.49	68.17	7.03
AD-1526950.1	57.95	9.53	60.74	3.30	69.44	15.48
AD-1526952.1	84.19	11.11	87.85	10.91	89.44	11.86
AD-1527058.1	57.03	3.64	52.85	6.86	39.49	1.95
AD-1526953.1	60.26	10.37	63.72	10.60	49.16	2.48
AD-1526954.1	58.97	10.67	63.12	10.57	60.61	6.41
AD-1527059.1	57.41	5.33	56.29	7.23	59.58	6.94
AD-1527060.1	67.72	6.72	89.94	16.03	64.39	9.96
AD-1526955.1	46.62	8.31	55.52	9.83	56.07	12.27
AD-1526956.1	48.87	8.78	50.68	3.66	63.65	14.00
AD-1526957.1	67.75	14.60	51.66	11.36	58.76	20.73
AD-1526958.1	66.82	17.50	61.92	13.39	87.99	19.94
AD-1526959.1	41.06	9.43	38.80	5.06	69.93	6.79
AD-1527061.1	50.79	11.11	43.10	8.65	30.91	6.40
AD-1526960.1	32.47	6.27	22.15	1.43	34.88	11.42
AD-1526961.1	35.95	9.75	36.11	2.97	54.29	12.43
AD-1526962.1	37.80	7.74	32.77	4.02	45.45	8.13
AD-1526963.1	41.80	10.46	39.64	4.04	54.54	8.94
AD-1526964.1	39.46	5.23	42.84	13.61	52.08	11.81
AD-1526965.1	38.58	9.77	33.55	3.81	38.36	6.62
AD-1193373.2	89.19	21.04	66.76	12.05	72.78	21.98
AD-1527101.1	44.99	8.54	57.13	10.30	49.59	4.03
AD-1526967.1	47.27	9.85	46.91	12.02	74.04	9.89

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526968.1	32.95	7.59	26.37	1.91	42.38	6.71
AD-1526969.1	31.53	12.80	37.27	6.68	37.52	12.94
AD-1526970.1	35.09	8.30	33.71	6.51	36.25	7.40
AD-1526971.1	45.31	8.62	35.84	7.93	39.24	14.20
AD-1526972.1	37.10	11.11	44.39	2.32	55.77	15.11
AD-1527102.1	53.14	12.38	47.35	6.08	42.83	10.78
AD-1526973.1	54.82	9.90	34.62	2.67	55.39	6.85
AD-1526974.1	47.57	11.14	41.72	8.02	58.37	3.14
AD-1526975.1	33.52	6.25	32.89	5.89	41.68	7.36
AD-1526976.1	36.03	7.29	29.55	6.75	48.13	9.07
AD-1527103.1	40.60	11.16	48.63	3.16	41.59	4.62
AD-1527104.1	34.77	3.61	49.84	8.70	38.08	5.74
AD-1526977.1	38.77	11.91	15.64	5.26	36.41	4.25
AD-1527105.1	54.73	9.93	63.20	16.32	50.10	8.59
AD-1526978.1	40.62	4.73	31.13	8.73	68.53	9.26
AD-1526979.1	46.09	11.74	43.69	6.02	52.84	14.23
AD-1527089.1	41.69	12.83	42.73	10.61	46.65	11.68
AD-1526981.1	59.45	13.82	57.97	14.58	75.33	22.34
AD-1526982.1	53.35	8.99	55.46	11.28	74.26	14.68
AD-1526983.1	52.50	9.57	51.42	5.46	65.63	6.73
AD-1527067.1	60.09	10.31	78.29	17.04	59.06	15.18
AD-1527068.1	35.32	4.98	44.61	4.50	41.89	4.73
AD-1526984.1	33.17	13.09	32.36	7.06	39.77	9.97
AD-1526985.1	35.56	7.24	34.94	8.94	41.34	5.80

	HepG2 Transfection					
	50 nM		10 nM		1 nM	
DuplexID	% of message remaining	St Dev	% of message remaining	St Dev	% of message remaining	St Dev
AD-1526986.1	54.14	10.17	43.25	10.42	63.80	7.67
AD-1527106.1	39.12	7.50	27.63	5.38	30.69	7.50
AD-1526987.1	25.56	7.50	18.83	5.39	35.02	4.42
AD-1526988.1	29.28	11.96	34.27	3.50	43.94	6.92
AD-1526989.1	21.41	6.72	27.94	9.08	34.06	4.64
AD-1527107.1	34.64	7.59	63.09	11.66	38.35	8.35
AD-1526990.1	20.26	6.19	16.02	2.88	28.64	8.79
AD-1526991.1	33.64	6.99	26.83	2.59	38.10	1.71
AD-1526992.1	39.73	10.60	36.00	7.96	45.37	7.03
AD-1526993.1	25.92	7.77	19.59	1.02	31.89	7.04
AD-1526994.1	31.17	7.10	41.45	4.94	35.73	3.39
AD-1526995.1	39.67	14.04	46.65	7.29	67.92	14.43
AD-1526996.1	41.58	3.43	57.50	3.08	48.20	8.40
AD-1526997.1	33.93	4.15	37.24	5.95	29.48	11.56
AD-1526999.1	24.56	4.55	37.31	4.43	36.13	2.97
AD-1526998.1	26.84	9.47	29.85	2.30	32.13	5.10
AD-1527000.1	30.27	8.27	32.00	8.16	35.84	11.10
AD-1527001.1	31.81	11.14	24.59	3.17	36.79	14.10
AD-1527002.1	30.46	11.56	24.48	4.62	37.85	14.87
AD-1527003.1	N/A	N/A	24.45	0.48	38.07	26.00
AD-1527108.1	38.59	5.45	39.66	13.53	39.49	5.84
AD-1527004.1	29.19	5.32	33.51	11.06	38.67	11.48
AD-1527005.1	30.69	3.17	35.60	10.58	45.66	5.78

Example 3. Additional Duplexes Targeting PNPLA3

Additional duplexes targeting human PNPLA3 gene were designed using custom R and Python scripts and synthesized as described above.

Detailed lists of the unmodified PNPLA3 sense and antisense strand nucleotide sequences are shown in Table 6. Detailed lists of the modified PNPLA3 sense and antisense strand nucleotide sequences are shown in Table 7.

Single dose screens of the additional agents are performed by free uptake and transfection as described above.

Table 6. Unmodifed Sense and Antisense Strand Sequences of PNPLA3 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5'-3'	SEQ ID NO:	Range in NM_025225.2
AD-1010714.3	AUUAGGAUAAUGUCUUAUGUA	1463	1215-1235	UACAUAAGACAUUAUCCUAAUGG	1500	1213-1235
AD-1010719.3	GCUGAGUUGGUUUUAUGAAAA	1464	1745-1765	UUUUCAUAAAACCAACUCAGCUC	1501	1743-1765
AD-1010732.4	CACCUUUUUCACCUAACUAAA	1465	2179-2199	UUUAGUUAGGUGAAAAAGGUGUU	1502	2177-2199
AD-1010734.3	UUUUUCACCUAACUAAAAUAA	1466	2183-2203	UUAUUUUAGUUAGGUGAAAAAGG	1503	2181-2203
AD-1010735.4	ACCUAACUAAAAUAAUGUUUA	1467	2189-2209	UAAACAUUAUUUUAGUUAGGUGA	1504	2187-2209
AD-1531673.2	GGGGUAACAAGAUGAUAAUCU	1468	2144-2164	AGAUUAUCAUCUUGUUACCCCCG	1505	2142-2164
AD-1531674.2	CUCCAUGGCGGGGGUAACAAA	1469	2134-2154	UUUGUUACCCCCGCCAUGGAGAC	1506	2132-2154
AD-1636724.1	UAGGAUAAUGUCUUAUGUAAU	1470	1217-1237	ATUACATAAGACAUUAUCCUAAU	1507	1215-1237
AD-1636725.1	CCUAACUAAAAUAAUGUUUAA	1471	2190-2210	UTAAACAUUAUTUUAGUUAGGUG	1508	2188-2210
AD-1636726.1	AUGUUAGUAGAAUAAGCCUUA	1472	2279-2299	UAAGGCTUAUUCUACUAACAUCU	1509	2277-2299
AD-1636727.1	CACCUUUUUCACCUAACUAAA	1465	2179-2199	UTUAGUTAGGUGAAAAAGGUGUU	1510	2177-2199
AD-1636728.1	UGAGUGAAGAAAUGAAAGACA	1473	1156-1176	UGUCTUTCAUUTCUUCACUCAGU	1511	1154-1176
AD-1636729.1	ACCUAACUAAAAUAAUGUUUA	1467	2189-2209	UAAACATUAUUTUAGUUAGGUGA	1512	2187-2209
AD-1636730.1	UUUUUCACCUAACUAAAAUAA	1466	2183-2203	UTAUTUTAGUUAGGUGAAAAAGG	1513	2181-2203
AD-1636731.1	GAUUUGCAACUUGCUACCCAU	1474	1196-1216	ATGGGUAGCAAGUUGCAAAUCUU	1514	1194-1216
AD-1636732.1	AUAAUGUCUUAUGUAAUGCUU	1475	1221-1241	AAGCAUTACAUAAGACAUUAUCC	1515	1219-1241
AD-1636733.1	AACUUGCUACCCAUUAGGAUA	1476	1203-1223	UAUCCUAAUGGGUAGCAAGUUGC	1516	1201-1223
AD-1636734.1	CUGAGUUGGUUUUAUGAAAAU	208	1746-1766	ATUUTCAUAAAACCAACUCAGCU	1517	1744-1766
AD-1636735.1	ACCUGUUGAAUUUUGUAUUAU	242	2245-2265	ATAATACAAAATUCAACAGGUAA	1518	2243-2265
AD-1636736.1	UUUUAGAACACCUUUUUCACU	1477	2171-2191	AGUGAAAAAGGTGUUCUAAAAUU	1519	2169-2191
AD-1636737.1	AUACAUGAGCAAGAUUUGCAA	1478	1184-1204	UTGCAAAUCUUGCUCAUGUAUCC	1520	1182-1204
AD-1636738.1	UCUGAGCUGAGUUGGUUUUAU	202	1740-1760	ATAAAACCAACTCAGCUCAGAGG	1521	1738-1760
AD-1636739.1	GCUGAGUUGGUUUUAUGAAAA	1464	1745-1765	UTUUCATAAAACCAACUCAGCUC	1522	1743-1765
AD-1636740.1	GGCCUUAUCCCUCCUUCCUUA	1479	630-650	UAAGGAAGGAGGGAUAAGGCCAC	1523	628-650
AD-1636741.1	CACCUUUUUCACCUAACUAAU	227	2179-2199	ATUAGUTAGGUGAAAAAGGUGUU	1524	2177-2199

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5'-3'	SEQ ID NO:	Range in NM_025225.2
AD-1636742.1	UGGAUACAUGAGCAAGAUUUA	1480	1181-1201	UAAATCTUGCUCAUGUAUCCACC	1525	1179-1201
AD-1636743.1	CUAUUAAUGGUCAGACUGUUA	1481	1901-1921	UAACAGTCUGACCAUUAAUAGGG	1526	1899-1921
AD-1636744.1	GCACAGGGAACCUCUACCUUA	1482	817-837	UAAGGUAGAGGTUCCCUGUGCAG	1527	815-837
AD-1636745.1	UUAUGUAAUGCUGCCCUGUAA	1483	1229-1249	UTACAGGGCAGCAUUACAUAAGA	1528	1227-1249
AD-1636746.1	GUGAGUGACAACGUACCCUUA	1484	678-698	UAAGGGTACGUTGUCACUCACUC	1529	676-698
AD-1636747.1	GUGCUAAAGUUUCCCAUCUUU	1485	1658-1678	AAAGAUGGGAAACUUUAGCACCU	1530	1656-1678
AD-1636748.1	UACCUGUUGAAUUUUGUAUUA	1486	2244-2264	UAAUACAAAAUTCAACAGGUAAC	1531	2242-2264
AD-1636749.1	GGGGUAACAAGAUGAUAAUCU	1468	2144-2164	AGAUTATCAUCTUGUUACCCCCG	1532	2142-2164
AD-1636750.1	CGACAUCUGCCCUAAAGUCAA	1487	746-766	UTGACUTUAGGGCAGAUGUCGUA	1533	744-766
AD-1636751.1	UGGUGACAUGGCUUCCAGAUA	1488	1288-1308	UAUCTGGAAGCCAUGUCACCAGU	1534	1286-1308
AD-1636752.1	UUGCUACCCAUUAGGAUAAUA	1489	1206-1226	UAUUAUCCUAATGGGUAGCAAGU	1535	1204-1226
AD-1636753.1	CAUGAGCAAGAUUUGCAACUU	272	1187-1207	AAGUTGCAAAUCUUGCUCAUGUA	562	1185-1207
AD-1636754.1	AAAUGAAAGACAAAGGUGGAU	1490	1165-1185	ATCCACCUUUGTCUUUCAUUUCU	1536	1163-1185
AD-1636755.1	UGGGAGAGAUAUGCCUUCGAA	1491	874-894	UTCGAAGGCAUAUCUCUCCCAGC	1537	872-894
AD-1636756.1	CUCCAUGGCGGGGGUAACAAA	1469	2134-2154	UTUGTUACCCCCGCCAUGGAGAC	1538	2132-2154
AD-1636757.1	AGCAUGAGGUUCUUAGAAUGU	1492	1923-1943	ACAUTCTAAGAACCUCAUGCUGG	1539	1921-1943
AD-1636758.1	AGGAAGCAACCUUUCGCCUGU	1493	1769-1789	ACAGGCGAAAGGUUGCUUCCUAG	1540	1767-1789
AD-1636759.1	UUGGUUUUAUGAAAAGCUAGA	1494	1751-1771	UCUAGCTUUUCAUAAAACCAACU	1541	1749-1771
AD-1636760.1	AUUAGGAUAAUGUCUUAUGUA	1463	1215-1235	UACATAAGACATUAUCCUAAUGG	1542	1213-1235
AD-1636761.1	UCACUUGAGGAGGCGAGUCUA	1495	1621-1641	UAGACUCGCCUCCUCAAGUGACU	1543	1619-1641
AD-1636762.1	CAAGAUUUGCAACUUGCUACA	1496	1193-1213	UGUAGCAAGUUGCAAAUCUUGCU	1544	1191-1213
AD-1636764.1	UGCCAAAACAACCAUCACCGU	1497	704-724	ACGGTGAUGGUTGUUUUGGCAUC	1545	702-724
AD-1636765.1	CCAUUAGGAUAAUGUCUUAUU	1498	1213-1233	AAUAAGACAUUAUCCUAAUGGGU	1546	1211-1233
AD-1636768.1	UUACCUGUUGAAUUUUGUAUU	1499	2243-2263	AAUACAAAAUUCAACAGGUAACA	1547	2241-2263
AD-518942.2	UGCCAAAACAACCAUCACCGU	1497	704-724	ACGGUGAUGGUUGUUUUGGCAUC	1548	702-724
AD-518942.3	UGCCAAAACAACCAUCACCGU	1497	704-724	ACGGUGAUGGUUGUUUUGGCAUC	1548	702-724

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5'-3'	SEQ ID NO:	Range in NM_025225.2
AD-518942.4	UGCCAAAACAACCAUCACCGU	1497	704-724	ACGGUGAUGGUUGUUUUGGCAUC	1548	702-724
AD-519346.5	CCAUUAGGAUAAUGUCUUAUU	1498	1213-1233	AAUAAGACAUUAUCCUAAUGGGU	1546	1211-1233
AD-519346.6	CCAUUAGGAUAAUGUCUUAUU	1498	1213-1233	AAUAAGACAUUAUCCUAAUGGGU	1546	1211-1233
AD-519346.7	CCAUUAGGAUAAUGUCUUAUU	1498	1213-1233	AAUAAGACAUUAUCCUAAUGGGU	1546	1211-1233
AD-519350.6	UAGGAUAAUGUCUUAUGUAAU	1470	1217-1237	AUUACAUAAGACAUUAUCCUAAU	1549	1215-1237
AD-519354.4	AUAAUGUCUUAUGUAAUGCUU	1475	1221-1241	AAGCAUUACAUAAGACAUUAUCC	1550	1219-1241
AD-519757.6	CUGAGUUGGUUUUAUGAAAAU	208	1746-1766	AUUUUCAUAAAACCAACUCAGCU	1551	1744-1766
AD-519780.2	AGGAAGCAACCUUUCGCCUGU	1493	1769-1789	ACAGGCGAAAGGUUGCUUCCUAG	1540	1767-1789
AD-519933.3	AGCAUGAGGUUCUUAGAAUGU	1492	1923-1943	ACAUUCUAAGAACCUCAUGCUGG	1552	1921-1943
AD-520053.7	UUUUAGAACACCUUUUUCACU	1477	2171-2191	AGUGAAAAAGGUGUUCUAAAAUU	1553	2169-2191
AD-520061.6	CACCUUUUUCACCUAACUAAU	227	2179-2199	AUUAGUUAGGUGAAAAAGGUGUU	1554	2177-2199
AD-67526.5	GGCCUUAUCCCUCCUUCCUUA	1479	630-650	UAAGGAAGGAGGGAUAAGGCCAC	1523	628-650
AD-67551.7	AUACAUGAGCAAGAUUUGCAA	1478	1184-1204	UUGCAAAUCUUGCUCAUGUAUCC	1555	1182-1204
AD-67554.11	UCUGAGCUGAGUUGGUUUUAU	202	1740-1760	AUAAAACCAACUCAGCUCAGAGG	490	1738-1760
AD-67560.7	CUAUUAAUGGUCAGACUGUUA	1481	1901-1921	UAACAGUCUGACCAUUAAUAGGG	1556	1899-1921
AD-67561.3	UGGAUACAUGAGCAAGAUUUA	1480	1181-1201	UAAAUCUUGCUCAUGUAUCCACC	1557	1179-1201
AD-67564.3	UCACUUGAGGAGGCGAGUCUA	1495	1621-1641	UAGACUCGCCUCCUCAAGUGACU	1543	1619-1641
AD-67565.3	AUGUUAGUAGAAUAAGCCUUA	1472	2279-2299	UAAGGCUUAUUCUACUAACAUCU	1558	2277-2299
AD-67567.3	UUGGUUUUAUGAAAAGCUAGA	1494	1751-1771	UCUAGCUUUUCAUAAAACCAACU	1559	1749-1771
AD-67568.3	GCACAGGGAACCUCUACCUUA	1482	817-837	UAAGGUAGAGGUUCCCUGUGCAG	1560	815-837
AD-67573.3	UGGUGACAUGGCUUCCAGAUA	1488	1288-1308	UAUCUGGAAGCCAUGUCACCAGU	1561	1286-1308
AD-67575.11	UUACCUGUUGAAUUUUGUAUU	1499	2243-2263	AAUACAAAAUUCAACAGGUAACA	1547	2241-2263
AD-67575.12	UUACCUGUUGAAUUUUGUAUU	1499	2243-2263	AAUACAAAAUUCAACAGGUAACA	1547	2241-2263
AD-67575.13	UUACCUGUUGAAUUUUGUAUU	1499	2243-2263	AAUACAAAAUUCAACAGGUAACA	1547	2241-2263
AD-67577.7	CGACAUCUGCCCUAAAGUCAA	1487	746-766	UUGACUUUAGGGCAGAUGUCGUA	1562	744-766
AD-67578.3	GUGAGUGACAACGUACCCUUA	1484	678-698	UAAGGGUACGUUGUCACUCACUC	1563	676-698

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_025225.2	Antisense Sequence 5'-3'	SEQ ID NO:	Range in NM_025225.2
AD-67582.6	GUGCUAAAGUUUCCCAUCUUU	1485	1658-1678	AAAGAUGGGAAACUUUAGCACCU	1530	1656-1678
AD-67583.7	CAAGAUUUGCAACUUGCUACA	1496	1193-1213	UGUAGCAAGUUGCAAAUCUUGCU	1544	1191-1213
AD-67584.8	CCUAACUAAAAUAAUGUUUAA	1471	2190-2210	UUAAACAUUAUUUUAGUUAGGUG	1564	2188-2210
AD-67586.3	UGGGAGAGAUAUGCCUUCGAA	1491	874-894	UUCGAAGGCAUAUCUCUCCCAGC	1565	872-894
AD-67589.6	AACUUGCUACCCAUUAGGAUA	1476	1203-1223	UAUCCUAAUGGGUAGCAAGUUGC	1516	1201-1223
AD-67605.10	ACCUGUUGAAUUUUGUAUUAU	242	2245-2265	AUAAUACAAAAUUCAACAGGUAA	532	2243-2265
AD-75247.4	AAAUGAAAGACAAAGGUGGAU	1490	1165-1185	AUCCACCUUUGUCUUUCAUUUCU	1566	1163-1185
AD-75265.5	CAUGAGCAAGAUUUGCAACUU	272	1187-1207	AAGUUGCAAAUCUUGCUCAUGUA	1567	1185-1207
AD-75269.3	UGAGUGAAGAAAUGAAAGACA	1473	1156-1176	UGUCUUUCAUUUCUUCACUCAGU	1568	1154-1176
AD-75270.4	UACCUGUUGAAUUUUGUAUUA	1486	2244-2264	UAAUACAAAAUUCAACAGGUAAC	1569	2242-2264
AD-75272.3	UUGCUACCCAUUAGGAUAAUA	1489	1206-1226	UAUUAUCCUAAUGGGUAGCAAGU	1570	1204-1226
AD-75274.6	UUAUGUAAUGCUGCCCUGUAA	1483	1229-1249	UUACAGGGCAGCAUUACAUAAGA	1571	1227-1249
AD-75275.3	GAUUUGCAACUUGCUACCCAU	1474	1196-1216	AUGGGUAGCAAGUUGCAAAUCUU	1572	1194-1216

Table 7. Modifed Sense and Antisense Strand Sequences of PNPLA3 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-1010714.3	asusuaggAfuAfAfUfgucuuauguaL96	1573	usAfscauAfaGfAfcauuAfuCfcuaausgsg	1652	CCAUUAGGAUAAUGUCUUAUGUA	1736
AD-1010719.3	gscsugagUfuGfGfUfuuuaugaaaaL96	1574	usUfsuucAfuAfAfaaccAfaCfucagcsusc	1653	GAGCUGAGUUGGUUUUAUGAAAA	1369
AD-1010732.4	csasccuuUfuUfCfAfccuaacuaaaL96	1575	usUfsuagUfuAfGfgugaAfaAfaggugsusu	1654	AACACCUUUUUCACCUAACUAAA	1389
AD-1010734.3	ususuuucAfcCfUfAfacuaaaauaaL96	1576	usUfsauuUfuAfGfuuagGfuGfaaaaasgsg	1655	CCUUUUUCACCUAACUAAAAUAA	1392
AD-1010735.4	ascscuaaCfuAfAfAfauaauguuuaL96	1577	usAfsaacAfuUfAfuuuuAfgUfuaggusgsa	1656	UCACCUAACUAAAAUAAUGUUUA	1398
AD-	gsgsgguaAfcAfAfGfaugauaaucuL96	1578	asGfsauuAfuCfAfucuuGfuUfaccccscsg	1657	CGGGGGUAACAAGAUGAUAAUCU	1737

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
1531673.2						
AD-1531674.2	csusccauGfgCfGfGfggguaacaaaL96	1579	usUfsuguUfaCfCfcccgCfcAfuggagsasc	1658	GUCUCCAUGGCGGGGGUAACAAG	1738
AD-1636724.1	usasggauaaUfGfUfcuuauguaauL96	1580	asdTsuadCadTaagadCaUfuauccuasasu	1659	AUUAGGAUAAUGUCUUAUGUAAU	1739
AD-1636725.1	cscsuaacuaAfAfAfuaauguuuaaL96	1581	usdTsaadAcdAuuaudTuUfaguuaggsusg	1660	CACCUAACUAAAAUAAUGUUUAA	1399
AD-1636726.1	asusguuaguAfGfAfauaagccuuaL96	1582	usdAsagdGcdTuauudCuAfcuaacauscsu	1661	AGAUGUUAGUAGAAUAAGCCUUA	1740
AD-1636727.1	csasccuuuuUfCfAfccuaacuaaaL96	1583	usdTsuadGudTaggudGaAfaaaggugsusu	1662	AACACCUUUUUCACCUAACUAAA	1389
AD-1636728.1	usgsagugaaGfAfAfaugaaagacaL96	1584	usdGsucdTudTcauudTcUfucacucasgsu	1663	ACUGAGUGAAGAAAUGAAAGACA	1741
AD-1636729.1	ascscuaacuAfAfAfauaauguuuaL96	1585	usdAsaadCadTuauudTuAfguuaggusgsa	1664	UCACCUAACUAAAAUAAUGUUUA	1398
AD-1636730.1	ususuuucacCfUfAfacuaaaauaaL96	1586	usdTsaudTudTaguudAgGfugaaaaasgsg	1665	CCUUUUUCACCUAACUAAAAUAA	1392
AD-1636731.1	gsasuuugcaAfCfUfugcuacccauL96	1587	asdTsggdGudAgcaadGuUfgcaaaucsusu	1666	AAGAUUUGCAACUUGCUACCCAU	1742
AD-1636732.1	asusaauguc UfUfAfuguaaugcuuL96	1588	asdAsgcdAudTacaudAaGfacauuauscsc	1667	GGAUAAUGUCUUAUGUAAUGCUG	1743
AD-1636733.1	asascuugcuAfCfCfcauuaggauaL96	1589	usdAsucdCudAauggdGuAfgcaaguusgsc	1668	GCAACUUGCUACCCAUUAGGAUA	1313
AD-1636734.1	csusgaguugGfUfUfuuaugaaaauL96	1590	asdTsuudTcdAuaaadAcCfaacucagscsu	1669	AGCUGAGUUGGUUUUAUGAAAAG	1370
AD-1636735.1	ascscuguugAfAfUfuuuguauuauL96	1591	asdTsaadTadCaaaadTuCfaacaggusasa	1670	UUACCUGUUGAAUUUUGUAUUAU	1404
AD-1636736.1	ususuuagaaCfAfCfcuuuuucacuL96	1592	asdGsugdAadAaaggdTgUfucuaaaasusu	1671	AAUUUUAGAACACCUUUUUCACC	1744
AD-1636737.1	asusacaugaGfCfAfagauuugcaaL96	1593	usdTsgcdAadAucuudGcUfcauguauscsc	1672	GGAUACAUGAGCAAGAUUUGCAA	1303
AD-1636738.1	uscsugagcuGfAfGfuugguuuuauL96	1594	asdTsaadAadCcaacdTcAfgcucagasgsg	1673	CCUCUGAGCUGAGUUGGUUUUAU	1364
AD-1636739.1	gscsugaguuGfGfUfuuuaugaaaaL96	1595	usdTsuudCadTaaaadCcAfacucagcsusc	1674	GAGCUGAGUUGGUUUUAUGAAAA	1369

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-1636740.1	gsgsccuuauCfCfCfuccuuccuuaL96	1596	usdAsagdGadAggagdGgAfuaaggccsasc	1675	GUGGCCUUAUCCCUCCUUCCUUC	1745
AD-1636741.1	csasccuuuuUfCfAfccuaacuaauL96	1597	asdTsuadGudTaggudGaAfaaaggugsusu	1676	AACACCUUUUUCACCUAACUAAA	1389
AD-1636742.1	usgsgauacaUfGfAfgcaagauuuaL96	1598	usdAsaadTcdTugcudCaUfguauccascsc	1677	GGUGGAUACAUGAGCAAGAUUUG	1746
AD-1636743.1	csusauuaauGfGfUfcagacuguuaL96	1599	usdAsacdAgdTcugadCcAfuuaauagsgsg	1678	CCCUAUUAAUGGUCAGACUGUUC	1747
AD-1636744.1	gscsacagggAfAfCfcucuaccuuaL96	1600	usdAsagdGudAgaggdTuCfccugugcsasg	1679	CUGCACAGGGAACCUCUACCUUC	1250
AD-1636745.1	ususauguaaUfGfCfugcccuguaaL96	1601	usdTsacdAgdGgcagdCaUfuacauaasgsa	1680	UCUUAUGUAAUGCUGCCCUGUAC	1748
AD-1636746.1	gsusgagugaCfAfAfcguacccuuaL96	1602	usdAsagdGgdTacgudTgUfcacucacsusc	1681	GAGUGAGUGACAACGUACCCUUC	1229
AD-1636747.1	gsusgcuaaaGfUfUfucccaucuuuL96	1603	asdAsagdAudGggaadAcUfuuagcacscsu	1682	AGGUGCUAAAGUUUCCCAUCUUU	1749
AD-1636748.1	usasccuguuGfAfAfuuuuguauuaL96	1604	usdAsaudAcdAaaaudTcAfacagguasasc	1683	GUUACCUGUUGAAUUUUGUAUUA	1750
AD-1636749.1	gsgsgguaacAfAfGfaugauaaucuL96	1605	asdGsaudTadTcaucdTuGfuuaccccscsg	1684	CGGGGGUAACAAGAUGAUAAUCU	1737
AD-1636750.1	csgsacaucuGfCfCfcuaaagucaaL96	1606	usdTsgadCudTuaggdGcAfgaugucgsusa	1685	UACGACAUCUGCCCUAAAGUCAA	1240
AD-1636751.1	usgsgugacaUfGfGfcuuccagauaL96	1607	usdAsucdTgdGaagcdCaUfgucaccasgsu	1686	ACUGGUGACAUGGCUUCCAGAUA	1443
AD-1636752.1	ususgcuaccCfAfUfuaggauaauaL96	1608	usdAsuudAudCcuaadTgGfguagcaasgsu	1687	ACUUGCUACCCAUUAGGAUAAUG	1751
AD-1636753.1	csasugagcaAfGfAfuuugcaacuuL96	1609	asdAsgudTgdCaaaudCuUfgcucaugsusa	1688	UACAUGAGCAAGAUUUGCAACUU	1434
AD-1636754.1	asasaugaaaGfAfCfaaagguggauL96	1610	asdTsccdAcdCuuugdTcUfuucauuuscsu	1689	AGAAAUGAAAGACAAAGGUGGAU	1752
AD-1636755.1	usgsggagagAfUfAfugccuucgaaL96	1611	usdTscgdAadGgcaudAuCfucucccasgsc	1690	GCUGGGAGAGAUAUGCCUUCGAG	1261
AD-1636756.1	csusccauggCfGfGfggguaacaaaL96	1612	usdTsugdTudAccccdCgCfcauggagsasc	1691	GUCUCCAUGGCGGGGGUAACAAG	1738
AD-	asgscaugagGfUfUfcuuagaauguL96	1613	asdCsaudTcdTaagadAcCfucaugcusgsg	1692	CCAGCAUGAGGUUCUUAGAAUGA	1753

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
1636757.1						
AD-1636758.1	asgsgaagcaAfCfCfuuucgccuguL96	1614	asdCsagdGcdGaaagdGuUfgcuuccusasg	1693	CUAGGAAGCAACCUUUCGCCUGU	1754
AD-1636759.1	ususgguuuuAfUfGfaaaagcuagaL96	1615	usdCsuadGcdTuuucdAuAfaaaccaascsu	1694	AGUUGGUUUUAUGAAAAGCUAGG	1374
AD-1636760.1	asusuaggauAfAfUfgucuuauguaL96	1616	usdAscadTadAgacadTuAfuccuaausgsg	1695	CCAUUAGGAUAAUGUCUUAUGUA	1736
AD-1636761.1	uscsacuugaGfGfAfggcgagucuaL96	1617	usdAsgadCudCgccudCcUfcaagugascsu	1696	AGUCACUUGAGGAGGCGAGUCUA	1755
AD-1636762.1	csasagauuuGfCfAfacuugcuacaL96	1618	usdGsuadGcdAaguudGcAfaaucuugscsu	1697	AGCAAGAUUUGCAACUUGCUACC	1756
AD-1636764.1	usgsccaaaaCfAfAfccaucaccguL96	1619	asdCsggdTgdAuggudTgUfuuuggcasusc	1698	GAUGCCAAAACAACCAUCACCGU	1757
AD-1636765.1	cscsauuaggAfUfAfaugucuuauuL96	1620	asdAsuadAgdAcauudAuCfcuaauggsgsu	1699	ACCCAUUAGGAUAAUGUCUUAUG	1758
AD-1636768.1	ususaccuguUfGfAfauuuuguauuL96	1621	asdAsuadCadAaauudCaAfcagguaascsa	1700	UGUUACCUGUUGAAUUUUGUAUU	1759
AD-518942.2	usgsccaaAfaCfAfAfccaucaccguL96	1622	asCfsgguGfaUfGfguugUfuUfuggcasusc	1701	GAUGCCAAAACAACCAUCACCGU	1757
AD-518942.3	usgsccaaAfaCfAfAfccaucaccguL96	1622	asCfsgguGfaUfGfguugUfuUfuggcasusc	1701	GAUGCCAAAACAACCAUCACCGU	1757
AD-518942.4	usgsccaaAfaCfAfAfccaucaccguL96	1622	asCfsgguGfaUfGfguugUfuUfuggcasusc	1701	GAUGCCAAAACAACCAUCACCGU	1757
AD-519346.5	cscsauuaGfgAfUfAfaugucuuauuL96	1623	asAfsuaaGfaCfAfuuauCfcUfaauggsgsu	1702	ACCCAUUAGGAUAAUGUCUUAUG	1758
AD-519346.6	cscsauuaGfgAfUfAfaugucuuauuL96	1623	asAfsuaaGfaCfAfuuauCfcUfaauggsgsu	1702	ACCCAUUAGGAUAAUGUCUUAUG	1758
AD-519346.7	cscsauuaGfgAfUfAfaugucuuauuL96	1623	asAfsuaaGfaCfAfuuauCfcUfaauggsgsu	1702	ACCCAUUAGGAUAAUGUCUUAUG	1758
AD-519350.6	usasggauAfaUfGfUfcuuauguaauL96	1624	asUfsuacAfuAfAfgacaUfuAfuccuasasu	1703	AUUAGGAUAAUGUCUUAUGUAAU	1739
AD-519354.4	asusaaugUfcUfUfAfuguaaugcuuL96	1625	asAfsgcaUfuAfCfauaaGfaCfauuauscsc	1704	GGAUAAUGUCUUAUGUAAUGCUG	1743
AD-519757.6	csusgaguUfgGfUfUfuuaugaaaauL96	780	asUfsuuuCfaUfAfaaacCfaAfcucagscsu	1705	AGCUGAGUUGGUUUUAUGAAAAG	1370

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-519780.2	asgsgaagCfaAfCfCfuuucgccugL96	1626	asCfsaggCfgAfAfagguUfgCfuuccusasg	1706	CUAGGAAGCAACCUUUCGCCUGU	1754
AD-519933.3	asgscaugAfgGfUfUfcuuagaaugL96	1627	asCfsauuCfuAfAfgaacCfuCfaugcusgsg	1707	CCAGCAUGAGGUUCUUAGAAUGA	1753
AD-520053.7	ususuuagAfaCfAfCfcuuuuucacuL96	1628	asGfsugaAfaAfAfggugUfuCfuaaaasusu	1708	AAUUUUAGAACACCUUUUUCACC	1744
AD-520061.6	csascccuuUfuUfCfAfccuaacuaauL96	799	asUfsuagUfuAfGfgugaAfaAfaggugsusu	1709	AACACCUUUUUCACCUAACUAAA	1389
AD-67526.5	gsgsccuuAfuCfCfCfuccuuccuuaL96	1629	usAfsaggAfaGfGfagggAfuAfaggccsasc	1710	GUGGCCUUAUCCCUCCUUCCUUC	1745
AD-67551.7	asusacauGfaGfCfAfagauuugcaaL96	1630	usUfsgcaAfaUfCfuugcUfcAfuguauscsc	1711	GGAUACAUGAGCAAGAUUUGCAA	1303
AD-67554.11	uscsugagCfuGfAfGfuugguuuuauL96	774	asUfsaaaAfcCfAfacucAfgCfucagasgsg	1712	CCUCUGAGCUGAGUUGGUUUUAU	1364
AD-67560.7	csusauuaAfuGfGfUfcagacuguuaL96	1631	usAfsacaGfuCfUfgaccAfuUfaauagsgsg	1713	CCCUAUUAAUGGUCAGACUGUUC	1747
AD-67561.3	usgsgauaCfaUfGfAfgcaagauuuaL96	1632	usAfsaauCfuUfGfcucaUfgUfauccascsc	1714	GGUGGAUACAUGAGCAAGAUUUG	1746
AD-67564.3	uscsacuuGfaGfGfAfggcgagucuaL96	1633	usAfsgacUfcGfCfcuccUfcAfagugascsu	1715	AGUCACUUGAGGAGGCGAGUCUA	1755
AD-67565.3	asusguuaGfuAfGfAfauaagccuuaL96	1634	usAfsaggCfuUfAfuucuAfcUfaacauscsu	1716	AGAUGUUAGUAGAAUAAGCCUUA	1740
AD-67567.3	ususgguuUfuAfUfGfaaaagcuagaL96	1635	usCfsuagCfuUfUfucauAfaAfaccaascsu	1717	AGUUGGUUUUAUGAAAAGCUAGG	1374
AD-67568.3	gscsacagGfgAfAfCfcucuaccuuaL96	1636	usAfsaggUfaGfAfgguuCfcCfugugcsasg	1718	CUGCACAGGGAACCUCUACCUUC	1250
AD-67573.3	usgsgugaCfaUfGfGfcuuccagauaL96	1637	usAfsucuGfgAfAfgccaUfgUfcaccasgsu	1719	ACUGGUGACAUGGCUUCCAGAUA	1443
AD-67575.11	ususaccuGfuUfGfAfauuuuguauuL96	1638	asAfsuacAfaAfAfuucaAfcAfgguaascsa	1720	UGUUACCUGUUGAAUUUUGUAUU	1759
AD-67575.12	ususaccuGfuUfGfAfauuuuguauuL96	1638	asAfsuacAfaAfAfuucaAfcAfgguaascsa	1720	UGUUACCUGUUGAAUUUUGUAUU	1759
AD-67575.13	ususaccuGfuUfGfAfauuuuguauuL96	1638	asAfsuacAfaAfAfuucaAfcAfgguaascsa	1720	UGUUACCUGUUGAAUUUUGUAUU	1759
AD-	csgsacauCfuGfCfCfcuaaagucaaL96	1639	usUfsgacUfuUfAfgggcAfgAfugucgsusa	1721	UACGACAUCUGCCCUAAAGUCAA	1240

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
67577.7						
AD-67578.3	gsusgaguGfaCfAfAfcguaccuuaL96	1640	usAfsaggGfuAfCfguugUfcAfcucacsusc	1722	GAGUGAGUGACAACGUACCCUUC	1229
AD-67582.6	gsusgcuaAfaGfUfUfucccaucuuuL96	1641	asAfsagaUfgGfGfaaacUfuUfagcacscsu	1723	AGGUGCUAAAGUUUCCCAUCUUU	1749
AD-67583.7	csasagauUfuGfCfAfacuugcuacaL96	1642	usGfsuagCfaAfGfuugcAfaAfucuugscsu	1724	AGCAAGAUUUGCAACUUGCUACC	1756
AD-67584.8	cscsuaacUfaAfAfAfuaauguuuaaL96	1643	usUfsaaaCfaUfUfauuuUfaGfuuaggsusg	1725	CACCUAACUAAAAUAAUGUUUAA	1399
AD-67586.3	usgsggagAfgAfUfAfugccuucgaaL96	1644	usUfscgaAfgGfCfauauCfuCfucccasgsc	1726	GCUGGGAGAGAUAUGCCUUCGAG	1261
AD-67589.6	asascuugCfuAfCfCfcauuaggauaL96	1645	usAfsuccUfaAfUfggguAfgCfaaguusgsc	1727	GCAACUUGCUACCCAUUAGGAUA	1313
AD-67605.10	ascscuguUfgAfAfUfuuuguauuauL96	814	asUfsaauAfcAfAfAfaauuCfaAfcaggusasa	1728	UUACCUGUUGAAUUUUGUAUUAU	1404
AD-75247.4	asasaugaAfaGfAfCfaaagguggauL96	1646	asUfsccaCfcUfUfuguCfuUfcauuuscsu	1729	AGAAAUGAAAGACAAAGGUGGAU	1752
AD-75265.5	csasugagCfaAfGfAfuuugcaacuuL96	844	asAfsguuGfcAfAfaucuUfgCfucaugsusa	1730	UACAUGAGCAAGAUUUGCAACUU	1434
AD-75269.3	usgsagugAfaGfAfAfaugaaagacaL96	1647	usGfsucuUfuCfAfuuucUfuCfacucasgsu	1731	ACUGAGUGAAGAAAUGAAAGACA	1741
AD-75270.4	usasccugUfuGfAfAfuuuuguauuaL96	1648	usAfsauaCfaAfAfauucAfaCfagguasasc	1732	GUUACCUGUUGAAUUUUGUAUUA	1750
AD-75272.3	ususgcuaCfcCfAfUfuaggauaauaL96	1649	usAfsuuaUfcCfUfaaugGfgUfagcaasgsu	1733	ACUUGCUACCCAUUAGGAUAAUG	1751
AD-75274.6	ususauguAfaUfGfCfugcccuguaaL96	1650	usUfsacaGfgGfCfagcaUfuAfcauaasgsa	1734	UCUUAUGUAAUGCUGCCCUGUAC	1748
AD-75275.3	gsasuuugCfaAfCfUfugcuacccauL96	1651	asUfsgggUfaGfCfaaguUfgCfaaaucsusu	1735	AAGAUUUGCAACUUGCUACCCAU	1742

Example 4. *In vivo* screening of dsRNA Duplexes in Mice

Duplexes of interest, identified from the above *in vitro* studies, were evaluated *in vivo*. In particular, at pre-dose day -14 wild-type mice (C57BL/6) were transduced with 2 x 10^{10} viral particles of an adeno-associated virus 8 (AAV8) vector encoding human PNPLA3 by intravenous administration. In particular, mice were administered an AAV8 encoding a portion of human PNPLA3 mRNA encoding the open reading frame and 3' UTR of human PNPLA3 mRNA referenced as NM_025225.2 , referred to as AAV8-TBG-PI-PNPLA3.

At day 0, groups of three mice were subcutaneously administered a single 10 mg/kg dose of the agents of interest or PBS control. Table 8 provides the treatment groups and Table 9 provides the duplexes of interest. At day 14 post-dose animals were sacrificed, liver samples were collected and snap-frozen in liquid nitrogen. Liver mRNA was extracted and analyzed by the RT-QPCR method.

Human PNPLA3 mRNA levels were compared to a housekeeping gene, GAPDH. The values were then normalized to the average of PBS vehicle control group. The data were expressed as percent of baseline value, and presented as mean plus standard deviation. The results, listed in Table 10 and shown in Figure 2, demonstrate that the exemplary duplex agents tested effectively reduce the level of the human PNPLA3 messenger RNA *in vivo*.

Table 8. Treatment Groups

Group #	Duplex ID	Treatment
1	PBS	n/a
2	AD-519347.20	10 mg/kg siRNA Day 14
3	AD-1526902.2	
4	AD-1526891.3	
5	AD-1526820.3	
6	AD-1526960.2	
7	AD-1526996.2	
8	AD-1526999.2	
9	AD-1526987.2	
10	AD-1526846.3	
11	AD-1526993.2	
12	AD-1526961.2	
13	AD-1526989.2	
14	AD-1526988.2	

Table 9. Duplexes of Interest

Duplex ID	Range in NM_025225.2
AD-519347.20	
AD-1526902.2	1201-1223
AD-1526891.3	1182-1204
AD-1526820.3	687-709
AD-1526960.2	1738-1760

Duplex ID	Range in NM_025225.2
AD-1526996.2	2186-2208
AD-1526999.2	2122-2144
AD-1526987.2	2176-2198
AD-1526846.3	872-894
AD-1526993.2	2183-2205
AD-1526961.2	1739-1761
AD-1526989.2	2178-2200
AD-1526988.2	2177-2199

The unmodified nucleotide sequences of the sense and antisense strands of duplex AD-519347 are: Sense 5'-CAUUAGGAUAAUGUCUUAUGU-3'; Antisense 5'-ACAUAAGACAUUAUCCUAAUGGG-3'.

The modified nucleotide sequences of the sense and antisense strands of duplex AD-519347 are: Sense 5'-csasuuagGfaUfAfAfugucuuauguL96-3'; Antisense 5'-asCfsauaAfgAfCfauuaUfcCfuaaugsgsg-3'.

Table 10.

Groups	% message remaining	SD
PBS	100.00	14.10
AD-519347.20	28.57	9.24
AD-1526902.2	32.75	13.07
AD-1526891.3	34.84	7.36
AD-1526820.3	43.33	3.45
AD-1526960.2	53.52	16.89
AD-1526996.2	53.71	23.02
AD-1526999.2	62.92	13.49
AD-1526987.2	79.77	5.36
AD-1526846.3	86.01	32.26
AD-1526993.2	91.77	19.92
AD-1526961.2	100.00	15.56
AD-1526989.2	108.14	57.72
AD-1526988.2	133.47	43.79

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

5

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding PNPLA3, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2, 3, 6, and 7.

2. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 187-209; 214-238; 219-245; 283-305; 351-379; 361-391; 395-419; 416-439; 472-494; 483-506; 570-598; 618-649; 631-654; 636-659; 640-662; 643-677; 676-710; 740-772; 782-805; 803-825; 810-842; 864-905; 905-927; 910-934; 919-942; 953-983; 1062-1087; 1069-1097; 1078-1108; 1094-112; 1164-1187; 1170-1199; 1180-1212; 1196-1224; 1234-1262; 1259-1297; 1278-1318; 1326-1351; 1382-1411; 1518-1545; 1543-1568; 1549-1574; 1575-1597; 1621-1643; 1644-1692; 1676-1700; 1712-1734; 1719-1745; 1733-1778; 1733-1760; 1739-1770; 1749-1778; 1829-1856; 1865-1890; 1900-1925; 2076-2098; 2121-2148; 2175-2208; or 2243-2265 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

3. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 687-709, 1182-1204, 1201-1223, 1738-1760, or 2186-2208 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

4. The dsRNA agent of any one of claims 1-3, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of an agent selected from the group consisting of AD-1526902.2, AD-1526891.3, AD-1526820.3, AD-1526960.2, and AD-1526996.2.

5. The dsRNA agent of any one of claims 1-3, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense

strand nucleotide sequences of an agent selected from the group consisting of AD-1526902.2, AD-1526891.3, AD-1526820.3, and AD-1526960.2.

6. The dsRNA agent of any one of claims 1-3, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than two nucleotides from any one of the antisense strand nucleotide sequences of an agent selected from the group consisting of AD-1526902.2, AD-1526891.3, AD-1526820.3, and AD-1526960.2.

7. The dsRNA agent of any one of claims 1-3, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than one nucleotide from any one of the antisense strand nucleotide sequences of an agent selected from the group consisting of AD-1526902.2, AD-1526891.3, AD-1526820.3, and AD-1526960.2.

8. The dsRNA agent of any one of claims 1-3, wherein the sense and the antisense strand comprise at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the sense and the antisense strand nucleotide sequences of an agent selected from the group consisting of AD-1526902.2, AD-1526891.3, AD-1526820.3, and AD-1526960.2.

9. The dsRNA agent of any one of claims 1-3, wherein the sense and the antisense strand comprise at least 15 contiguous nucleotides differing by no more than two nucleotides from any one of the sense and the antisense strand nucleotide sequences of an agent selected from the group consisting of AD-1526902.2, AD-1526891.3, AD-1526820.3, and AD-1526960.2.

10. The dsRNA agent of any one of claims 1-3, wherein the sense and the antisense strand comprise at least 15 contiguous nucleotides differing by no more than one nucleotide from any one of the sense and the antisense strand nucleotide sequences of an agent selected from the group consisting of AD-1526902.2, AD-1526891.3, AD-1526820.3, and AD-1526960.2.

11. The dsRNA agent of any one of claims 1-3, wherein the sense and the antisense strand comprise the sense and the antisense strand nucleotide sequences of an agent selected from the group consisting of AD-1526902.2, AD-1526891.3, AD-1526820.3, and AD-1526960.2.

12. The dsRNA agent of any one of claims 1-3, wherein the sense and the antisense strand consist of the sense and the antisense strand nucleotide sequences of an agent selected from the group consisting of AD-1526902.2, AD-1526891.3, AD-1526820.3, and AD-1526960.2.

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 substantially all of the nucleotides of the sense strand; substantially all of the nucleotides of the antisense strand are modified nucleotides; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides.

15. The dsRNA agent of claim 14, wherein all of the nucleotides of the sense strand care modified nucleotides; all of the nucleotides of the antisense strand are modified nucleotides; or all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.

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, 2'-hydroxly-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 thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), a nucleotide comprising a 2' phosphate, and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

17. The dsRNA agent of any one of claims 13-15, wherein the modified nucleotides are selected from the group consisting of LNA modified nucleotides, HNA modified nucleotides, CeNA modified nucleotides, 2′-methoxyethyl modified nucleotides, 2′-O-alkyl modified nucleotides, 2′-O-allyl modified nucleotides, 2′-C- allyl modified nucleotides, 2′-fluoro modified nucleotides, 2′-deoxy modified nucleotides, 2'-hydroxyl modified nucleotides, 2'-O-methyl modified nucleotides, 2'-halo modified nucleotides, and glycol; modified nucleotides and combinations thereof.

18. The dsRNA 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 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), a nucleotide comprising a 2' phosphate, a vinyl-phosphonate nucleotide; and combinations thereof.

19. The dsRNA of any one of claims 13-15, wherein at least one of the modified nucleotides is a thermally destabilizing nucleotide modification.

20. The dsRNA of claim 19, wherein the thermally destabilizing nucleotide modification is selected from the group consisting of an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), and a glycerol nucleic acid (GNA).

21. The dsRNA agent of any one of claims 1-20, wherein the double stranded region is 19-30 nucleotide pairs in length.

22. The dsRNA agent of claim 21, wherein the double stranded region is 19-25 nucleotide pairs in length.

23. The dsRNA agent of claim 21, wherein the double stranded region is 19-23 nucleotide pairs in length.

24. The dsRNA agent of claim 21, wherein the double stranded region is 23-27 nucleotide pairs in length.

25. The dsRNA agent of claim 21, wherein the double stranded region is 21-23 nucleotide pairs in length.

26. The dsRNA agent of any one of claims 1-25, wherein each strand is independently no more than 30 nucleotides in length.

27. The dsRNA agent of any one of claims 1-26, wherein the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

28. The dsRNA agent of any one of claims 1-27, wherein the region of complementarity is at least 17 nucleotides in length.

29. The dsRNA agent of any one of claims 1-27, wherein the region of complementarity is between 19 and 23 nucleotides in length.

30. The dsRNA agent of any one of claims 1-28, wherein the region of complementarity is 19 nucleotides in length.

31. The dsRNA agent of any one of claims 1-30, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

32. The dsRNA agent of any one of claims 1-30, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

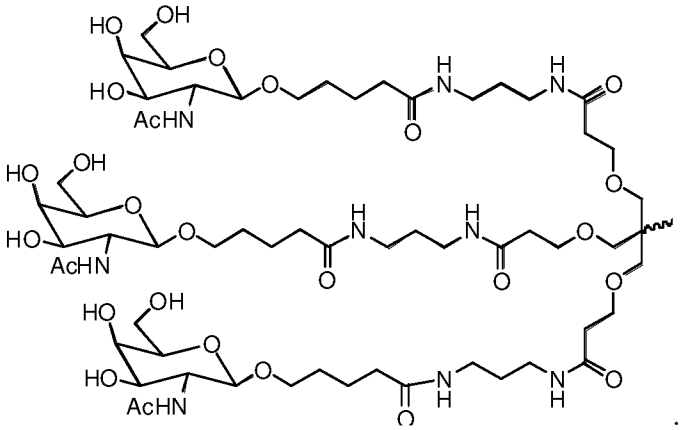
33. The dsRNA agent of any one of claims 1-32, further comprising a ligand.

34. The dsRNA agent of claim 33, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

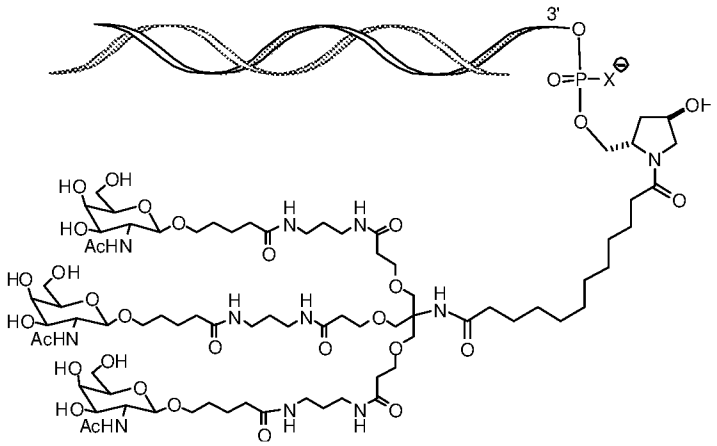
35. The dsRNA agent of claim 33 or 34, wherein the ligand comprises an N-acetylgalactosamine (GalNAc) derivative.

36. The dsRNA agent of any one of claims 33-35, wherein the ligand comprises one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

37. The dsRNA agent of claim 35 or 36, wherein the ligand comprises



38. The dsRNA agent of claim 37, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

39. The dsRNA agent of claim 38, wherein the X is O.

40. The dsRNA agent of any one of claims 1-39, wherein the dsRNA agent comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

41. The dsRNA agent of claim 40, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

42. The dsRNA agent of claim 41, wherein the strand is the antisense strand.

43. The dsRNA agent of claim 41, wherein the strand is the sense strand.

44. The dsRNA agent of claim 40, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

45. The dsRNA agent of claim 44, wherein the strand is the antisense strand.

46. The dsRNA agent of claim 44, wherein the strand is the sense strand.

47. The dsRNA agent of claim 40, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand.

48. The dsRNA agent of claim 47, wherein the strand is the antisense strand.

49. The dsRNA agent of any one of claims 1-48, wherein the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

50. A cell containing the dsRNA agent of any one of claims 1-49.

51. A pharmaceutical composition for inhibiting expression of a gene encoding Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) comprising the dsRNA agent of any one of claims 1-49.

52. The pharmaceutical composition of claim 51, wherein dsRNA agent is in an unbuffered solution.

53. The pharmaceutical composition of claim 52, wherein the unbuffered solution is saline or water.

54. The pharmaceutical composition of claim 51, wherein said dsRNA agent is in a buffer solution.

55. The pharmaceutical composition of claim 54, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

56. The pharmaceutical composition of claim 54, wherein the buffer solution is phosphate buffered saline (PBS).

57. A method of inhibiting expression of a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene in a cell, the method comprising contacting the cell with the dsRNA agent of any one of claims 1-49, or the pharmaceutical composition of any one of claims 51-56, thereby inhibiting expression of the PNPLA3 gene in the cell.

58. The method of claim 57, wherein the cell is within a subject.

59. The method of claim 58, wherein the subject is a human.

60. The method of claim 58, wherein the subject has a PNPLA3-associated disorder.

61. The method of claim 60, wherein the PNPLA3-associated disorder is selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, and nonalcoholic fatty liver disease (NAFLD).

62. The method of any one of claims 57-61, wherein contacting the cell with the dsRNA agent inhibits the expression of PNPLA3 by at least 50%, 60%, 70%, 80%, 90%, or 95%.

63. The method of any one of claims 57-62, wherein inhibiting expression of PNPLA3 decreases PNPLA3 protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

64. A method of treating a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression, comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-49, or the pharmaceutical composition of any one of claims 51-56, thereby treating the subject having the disorder that would benefit from reduction in PNPLA3 expression.

65. A method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression, comprising administering to the subject a prophylactically effective amount of the dsRNA agent of any one of claims 1-49, or the pharmaceutical composition of any one of claims 51-56, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in PNPLA3 expression.

66. The method of claim 64 or 65, wherein the disorder is a PNPLA3-associated disorder.

67. The method of claim 66, wherein the PNPLA3-associated disorder is selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, and nonalcoholic fatty liver disease (NAFLD).

68. The method of claim 66, wherein the PNPLA3-associated disorder is NAFLD.

69. The method of claim 66, wherein the subject is human.

70. The method of claim 64 or 65, wherein the administration of the agent to the subject causes a decrease in PNPLA3 protein accumulation.

71. The method of any one of claims 64-70, wherein the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

72. The method of any one of claims 64-71, wherein the dsRNA agent is administered to the subject subcutaneously.

73. The method of any one of claims 64-72, further comprising determining the level of PNPLA3 in a sample(s) from the subject.

74. The method of claim 73, wherein the level of PNPLA3 in the subject sample(s) is a PNPLA3 protein level in a blood or serum sample(s).

75. The method of any one of claims 64-74, further comprising administering to the subject an additional therapeutic agent for treatment of a PNPLA3-associated disorder.

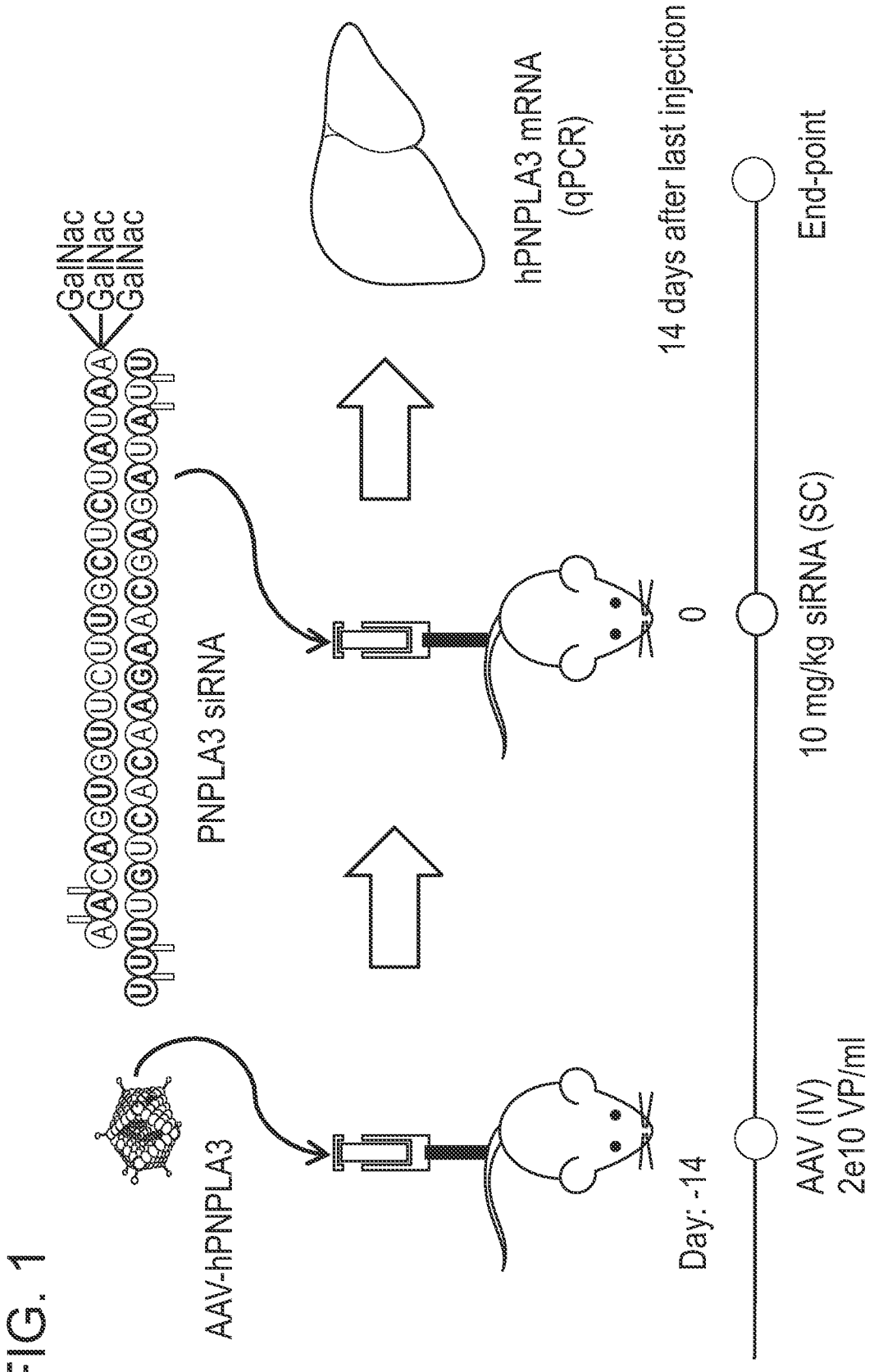
76. A kit comprising the dsRNA agent of any one of claims 1-49 or the pharmaceutical composition of any one of claims 51-56.

77. A vial comprising the dsRNA agent of any one of claims 1-49 or the pharmaceutical composition of any one of claims 51-56.

78. A syringe comprising the dsRNA agent of any one of claims 1-49 or the pharmaceutical composition of any one of claims 51-56.

79. An RNA-induced silencing complex (RISC) comprising an antisense strand of any of the dsRNA agents of claims 1-49.

FIG. 1


AAV-hPNPLA3
PNPLA3 siRNA
GalNac
GalNac
GalNac
hPNPLA3 mRNA
(qPCR)
Day: -14
0
14 days after last injection
AAV (IV)
2e10 VP/ml
10 mg/kg siRNA (SC)
End-point

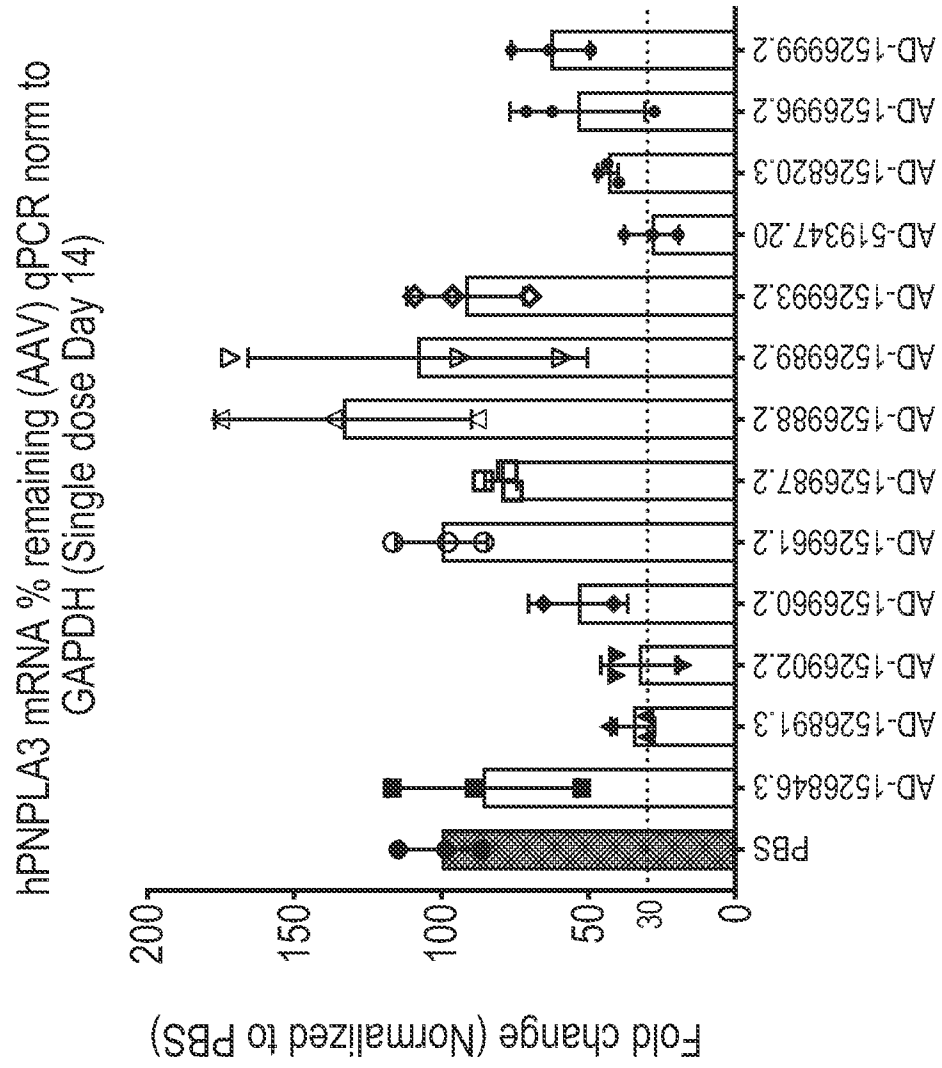

hPNPLA3 mRNA % remaining (AAV) qPCR norm to GAPDH (Single dose Day 14)
Fold change (Normalized to PBS)
200
150
100
50
30
0
PBS
AD-1526846.3
AD-1526891.3
AD-1526902.2
AD-1526960.2
AD-1526961.2
AD-1526987.2
AD-1526988.2
AD-1526989.2
AD-1526993.2
AD-519347.20
AD-1526820.3
AD-1526996.2
AD-1526999.2

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/031755

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/713
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/130806 A2 (ALNYLAM PHARMACEUTICALS INC [US]) 18 August 2016 (2016-08-18) pages 130,137; tables 4,5,6; sequences 470,219,972,721	1,2, 13-79
A	WO 2020/123508 A2 (AMGEN INC [US]) 18 June 2020 (2020-06-18) the whole document	1,2, 13-79

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 August 2022	21/10/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Romano, Alper

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/031755

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

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

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

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

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

1, 2, 13-79(all partially)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 2, 13-79(all partially)

A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding PNPLA3, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from target position 187-209

2-50. claims: 1-79(partially)

dsRNA agents defined as above wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from other target position defined in tables 2 and 6.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2022/031755

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		WO 2020123508 A2	18-06-2020