

BETA-CATENIN (CTNNB1) iRNA COMPOSITIONS AND METHODS OF USE THEREOF**Related Applications**

This application claims the benefit of priority to U.S. Provisional Application No. 63/224,901, filed on July 23, 2021, and U.S. Provisional Application No. 63/293,851, filed on December 27, 2021. The entire contents of each of the foregoing applications are incorporated herein by reference.

Background of the Invention

Wnt/ β -catenin signaling is an evolutionarily conserved and versatile pathway that is known to be involved in embryonic development, tissue homeostasis and a wide variety of human diseases. Aberrant activation of this pathway gives rise to the accumulation of β -catenin in the nucleus and promotes the transcription of many oncogenes such as c-Myc and CyclinD-1. As a result, it contributes to carcinogenesis and tumor progression of several cancers, including hepatocellular carcinoma, colon cancer, pancreatic cancer, lung cancer and ovarian cancer (Khrantsov AI, *et al. Am J Pathol.* 2010;176:2911–2920; Tao J, *et al. Gastroenterology.* 2014;147:690–701; Kobayashi M, *et al. Br J Cancer.* 2000;82:1689–1693; Damsky WE, *et al. Cancer Cell.* 2011;20:741–754; Gekas C, *et al. Leukemia.* 2016;30:2002–2010).

β -catenin, encoded by the CTNNB1 gene, is a multifunctional protein with a central role in physiological homeostasis. β -catenin acts both as a transcriptional co-regulator and an adaptor protein for intracellular adhesion. Wnt is the chief regulator of β -catenin, which is a family of 19 glycoproteins to regulate both the β -catenin-dependent (canonical Wnt) and -independent (non-canonical Wnt) signaling pathways (van Ooyen A, Nusse R. *Cell.* 1984;39:233–240).

In canonical Wnt pathway, Dsh, β -catenin, Glycogen Synthase Kinase 3 beta (GSK3 β), adenomatous polyposis coli (APC), AXIN, and T-cell factor (TCF)/lymphoid enhancement factor (LEF) have been identified as signal transducers of the canonical Wnt pathway, in which β -catenin is a core molecule (Behrens J, *et al. Nature.* 1996;382:638–642; Peifer M, *et al. Dev Biol.* 1994;166:543–556; Rubinfeld B, *et al. Science.* 1996;272:1023–1026; Yost C, *et al., Genes Dev.* 1996;10:1443–1454). In the absence of Wnt ligands, β -catenin is kept at a low level through the ubiquitin proteasome system (UPS) which results in the ubiquitylation and proteasomal degradation of β -catenin. Upon Wnt activation or genetic mutations of Wnt components, β -catenin accumulates in the cytoplasm and then translocates into the nucleus. Consequently, it binds to other proteins, such as LEF-1/TCF4, to promote the transcription of target genes, such as Jun, c-Myc and CyclinD-1 in a tissue specific manner, most of which encode oncoproteins. As a result, aberrant high expression of β -catenin leads to various diseases including cancer.

In addition, a high-level cytoplasm expression and nuclear localization of β -catenin also induces tumorigenic traits and promotes cancer cell proliferation and survival (Valkenburg KC, *et al. Cancers (Basel)* 2011;3:2050–2079). Moreover, β -catenin promotes the progression of tumors via suppressing the T-cell responses (Hong Y, *et al. Cancer Res.* 2015;75:656–665).

Current treatments for cancer include surgery, radiation and chemotherapy. However, these methods are not fully effective and may result in serious side effects. Accordingly, there is a need in the art for alternative treatments for subjects having a CTNNB1-associated disorder, such as cancer, *e.g.*, hepatocellular carcinoma.

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Summary of the Invention

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding beta-catenin (CTNNB1). The CTNNB1 gene may be within a cell, *e.g.*, a cell within a subject, such as a human subject. The present invention also provides methods of using the iRNA compositions of the invention for inhibiting the expression of a CTNNB1 gene and/or for treating a subject who would benefit from inhibiting or reducing the expression of a CTNNB1 gene, *e.g.*, a subject suffering or prone to suffering from a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma.

Accordingly, in an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of beta-catenin (CTNNB1) 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, or 20, 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 corresponding portion of the nucleotide sequence of SEQ ID NO:2.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of beta-catenin (CTNNB1) 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 CTNNB1, 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, 5, or 6.

In one embodiment, the dsRNA agent comprises a sense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, or 6 and an antisense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, or 23 contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, or 6.

In one embodiment, the dsRNA agent comprises a sense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, or 6 and an antisense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous

nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, or 6.

In one embodiment, the dsRNA agent comprises a sense strand comprising or consisting of a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, and 6 and an antisense strand comprising or consisting of a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, and 6.

In one embodiment, the dsRNA agent comprises a sense strand comprising a contiguous nucleotide sequence which has at least 85%, *e.g.*, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, nucleotide sequence identity over its entire length to any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, or 6 and an antisense strand comprising a contiguous nucleotide sequence which has at least 85%, *e.g.*, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, nucleotide sequence identity over its entire length to any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, or 6.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of beta-catenin (CTNNB1) 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 three, *e.g.*, 3, 2, 1, or 0, nucleotides from any one of the nucleotide sequence of nucleotides 603-625, 1489-1511, or 1739-1761 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 three, *e.g.*, 3, 2, 1, or 0, nucleotides, from the corresponding nucleotide sequence of SEQ ID NO:2.

In one embodiment, 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 a duplex selected from the group consisting of AD-1548393, AD-1548488, and AD-1548459.

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

In one embodiment, substantially all of the nucleotides of the sense strand are modified nucleotides; 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 deoxythymidine (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'-hydroxyl-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.

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, and glycol; 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 with a 2' phosphate, *e.g.*, G2p, C2p, A2p or U2p, a nucleotide comprising a phosphorothioate group, and a vinyl-phosphonate nucleotide; and combinations thereof.

In another embodiment, at least one of the modified nucleotides is a nucleotide with 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; a destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acid (UNA), and a glycerol nucleic acid (GNA).

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

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 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.

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.

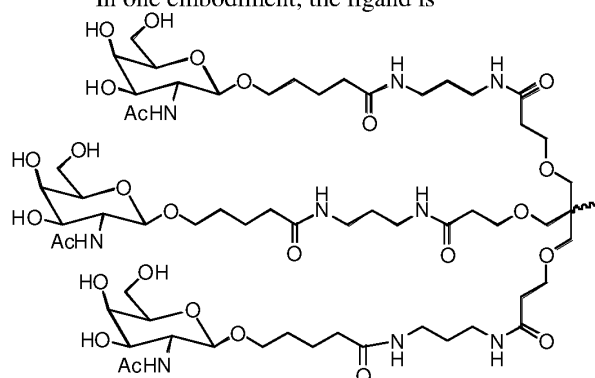
5 In one embodiment, the dsRNA agent further comprises a ligand.

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

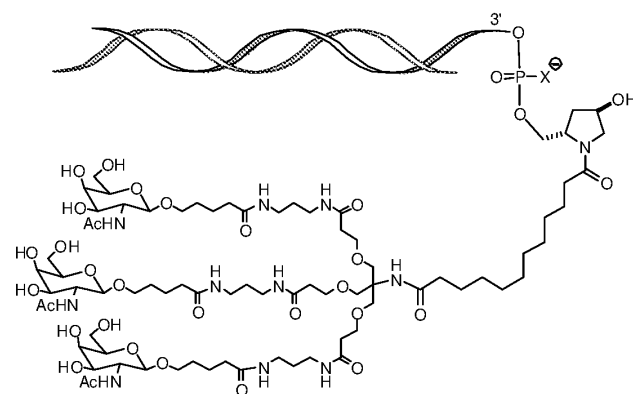
In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

10 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 is



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



15 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.

20 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.

5 In one embodiment, 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 3, *e.g.*, 3, 2, 1, or 0, nucleotides from the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

In one embodiment, the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 3, *e.g.*, 3, 2, 1, or 0, nucleotides from the
10 nucleotide sequence 5' - UACUGUUGGAUUGAUUCGAAA -3' 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 3, *e.g.*, 3, 2, 1, or 0, nucleotides from the nucleotide sequence 5' -
UTUCGAAUCAATCCAACAGUAGC -3'.

In one embodiment, the antisense strand comprises the nucleotide sequence 5' -
15 UTUCGAAUCAATCCAACAGUAGC -3'.

In one embodiment, the sense strand comprises the nucleotide sequence 5' -
UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprises the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

In one embodiment, the sense strand differs by no more than 4, *e.g.*, 4, 3, 2, 1, or 0, bases
20 from the nucleotide sequence 5' -usascuguugGfAfUfugauucgasasa-3' and the antisense strand differs by no more than 4, *e.g.*, 4, 3, 2, 1, or 0, bases from the nucleotide sequence 5' -
VPudTucdGadAucaadTcCfaacaguasgsc -3', wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; s is a
phosphorothioate linkage; VP is a vinyl phosphonate; dT is 2'-deoxythymidine -3'-phosphate; dG is
25 2'-deoxyguanosine-3'-phosphate; and dA is 2'-deoxyadenosine-3'-phosphate.

In one aspect, the present invention provides a double stranded RNA (dsRNA) agent for
inhibiting expression of beta-catenin (CTNNB1) in a cell, comprising a sense strand comprising the
nucleotide sequence 5' -usascuguugGfAfUfugauucgasasa-3' and an antisense strand comprising the
nucleotide sequence 5' -VPudTucdGadAucaadTcCfaacaguasgsc -3',
30 wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are
2'-fluoro A, G, C and U respectively; s is a phosphorothioate linkage; VP is a vinyl phosphonate; dT
is 2'-deoxythymidine -3'-phosphate; dG is 2'-deoxyguanosine-3'-phosphate; and dA is 2'-
deoxyadenosine-3'-phosphate.

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

35 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

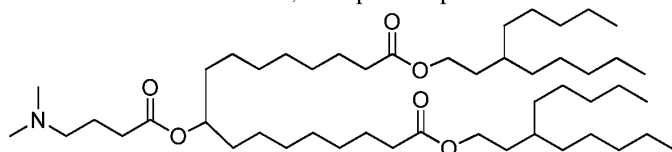
include the dsRNA agent is in a buffer solution, *e.g.*, a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof; or phosphate buffered saline (PBS).

In one aspect, the present invention provides a pharmaceutical composition for inhibiting expression of a gene encoding beta-catenin (CTNNB1) comprising the dsRNA agent of any one of claims 1-47 and a lipid.

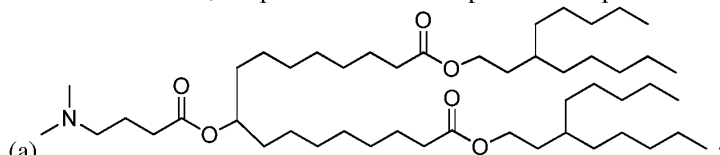
In one embodiment, the lipid is a cationic lipid.

In one embodiment, the cationic lipid comprises one or more biodegradable groups.

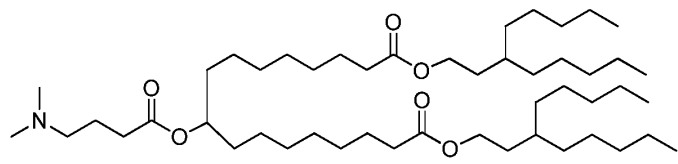
In one embodiment, the lipid comprises the structure



In one embodiment, the pharmaceutical composition comprises

- (a)  ;
 (b) cholesterol;
 (c) DSPC; and
 (d) PEG-DMG.

In one embodiment, the



DSPC, cholesterol, and PEG-DMG are present in a molar ratio of 50:12:36:2, respectively.

In one aspect, the present invention provides a pharmaceutical composition for inhibiting expression of a gene encoding beta-catenin (CTNNB1) comprising, a dsRNA agent for inhibiting expression of a gene encoding beta-catenin (CTNNB1), comprising a sense strand and an antisense strand forming a double stranded region, wherein 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 3, *e.g.*, 3, 2, 1, or 0, nucleotides from the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3', and a lipid.

In one embodiment, the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 3, *e.g.*, 3, 2, 1, or 0, nucleotides from the nucleotide sequence 5' - UACUGUUGGAUUGAUUCGAAA -3' 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 3, *e.g.*, 3, 2, 1, or 0, nucleotides from the nucleotide sequence 5' -

UTUCGAAUCAATCCAACAGUAGC -3'.

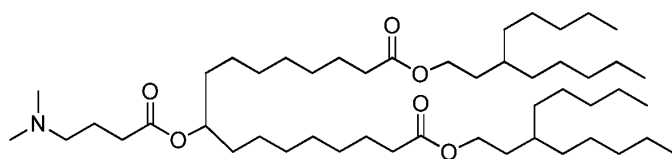
In one embodiment, the antisense strand comprises the nucleotide sequence 5' -

UTUCGAAUCAATCCAACAGUAGC -3'.

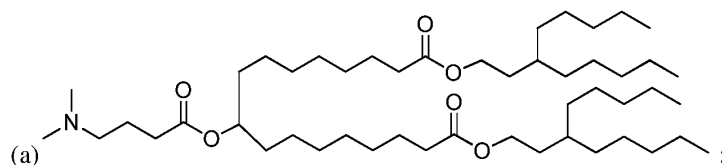
In one embodiment, the sense strand comprises the nucleotide sequence 5'-UACUGUUGGAUUGAUUCGAAA-3' and the antisense strand comprises the nucleotide sequence 5'-UTUCGAAUCAATCCAACAGUAGC-3'.

In one embodiment, the sense strand differs by no more than 4, *e.g.*, 4, 3, 2, 1, or 1, bases from the nucleotide sequence 5'-usascuguugGfAfUfugauucgasasa-3' and the antisense strand differs by no more than 4, *e.g.*, 4, 3, 2, 1, or 0, bases from the nucleotide sequence 5'-VPudTucdGadAucaadTcCfaacaguasgsc-3', wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; s is a phosphorothioate linkage; VP is a vinyl phosphonate; dT is 2'-deoxythymidine-3'-phosphate; dG is 2'-deoxyguanosine-3'-phosphate; and dA is 2'-deoxyadenosine-3'-phosphate.

In one embodiment, the lipid comprises the structure



In one embodiment, the pharmaceutical composition comprises



- (b) cholesterol;
(c) DSPC; and
(d) PEG-DMG.

In one embodiment, the DSPC, cholesterol, and PEG-DMG are present in a molar ratio of 50:12:36:2, respectively.

In one aspect, the present invention provides a method of inhibiting expression of a beta-catenin (CTNNB1) 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 CTNNB1 gene in the cell.

In one embodiment, the cell is within a subject, *e.g.*, a human subject, *e.g.*, a subject having a beta-catenin (CTNNB1)-associated disorder, such as cancer, *e.g.*, hepatocellular carcinoma.

In certain embodiments, the CTNNB1 expression is inhibited by at least about 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%. In one embodiment, inhibiting expression of CTNNB1

decreases CTNNB1 protein level in serum of the subject by at least 30%, 40%, 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 beta-catenin (CTNNB1) expression. The method includes
5 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 CTNNB1 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 beta-catenin (CTNNB1)
10 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 CTNNB1 expression.

In certain embodiments, the disorder is a beta-catenin (CTNNB1)-associated disorder, *e.g.* a
15 cancer.

In some embodiments, the CTNNB1-associated disorder is hepatocellular carcinoma.

In certain embodiments, administration of the dsRNA to the subject causes a decrease CTNNB1 protein accumulation in the subject.

In a further aspect, the present invention also provides methods of inhibiting the expression
20 of CTNNB1 in a subject. The methods include administering to the subject a therapeutically effective amount of any of the dsRNAs provided herein, thereby inhibiting the expression of CTNNB1 in the subject.

In one embodiment, the subject is human.

In one embodiment, the dsRNA agent is administered to the subject at a dose of about
25 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 dsRNA agent is administered to the subject intravenously.

In one embodiment, the methods of the invention include further determining the level of CTNNB1 in a sample(s) from the subject.

30 In one embodiment, the level of CTNNB1 in the subject sample(s) is a CTNNB1 protein level in a blood or serum or liver tissue sample(s).

In certain embodiments, the methods of the invention further comprise administering to the subject an additional therapeutic agent.

In certain embodiments, the additional therapeutic agent is selected from the group consisting
35 of a chemotherapeutic agent, a growth inhibitory agent, an anti-angiogenesis agent, an anti-neoplastic composition and a combination of any of the foregoing.

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. In one embodiment, the invention provides a kit for performing a method of inhibiting expression of

CTNNB1 gene in a cell by contacting a cell with a double stranded RNAi agent of the invention in an amount effective to inhibit expression of the CTNNB1 in the cell. The kit comprises an RNAi agent and instructions for use and, optionally, means for administering the RNAi agent to a subject.

- The present invention further provides an RNA-induced silencing complex (RISC)
 5 comprising an antisense strand of any of the dsRNA agents of the invention.

Brief Description of the Drawings

FIG. 1A is a graph depicting the effect of a single 0.1 mg/kg or 0.3 mg/kg intravenously administered dose of AD-1548393 at Days 5, 15, and 29 post-dose. The percent of CTNNB1 mRNA
 10 remaining relative to pre-dose levels of CTNNB1 mRNA are shown.

FIG. 1B is a graph depicting the effect of a single 0.1 mg/kg or 0.3 mg/kg intravenously administered dose of AD-1548459 at Days 5, 15, and 29 post-dose. The percent of CTNNB1 mRNA remaining relative to pre-dose levels of CTNNB1 mRNA are shown.

FIG. 1C is a graph depicting the effect of a single 0.1 mg/kg or 0.3 mg/kg intravenously
 15 administered dose of AD-1548488 at Days 5, 15, and 29 post-dose. The percent of CTNNB1 mRNA remaining relative to pre-dose levels of CTNNB1 mRNA are shown.

Detailed Description of the Invention

The present invention provides iRNA compositions which effect the RNA-induced silencing
 20 complex (RISC)-mediated cleavage of RNA transcripts of a beta-catenin (CTNNB1) 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 (CTNNB1) in mammals.

The iRNAs of the invention have been designed to target the human beta-catenin (CTNNB1) gene, including portions of the gene that are conserved in the CTNNB1 orthologs of other mammalian
 25 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 a beta-catenin (CTNNB1)-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma, using iRNA
 30 compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a CTNNB1 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,
 35 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of a CTNNB1 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 a CTNNB1 gene. In some embodiments, such iRNA agents having longer length antisense strands may, for example, 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 (CTNNB1 gene) in mammals. Using *in vitro* assays, the present inventors have demonstrated that iRNAs targeting a CTNNB1 gene can potentially mediate RNAi, resulting in significant inhibition of expression of a CTNNB1 gene. Thus, methods and compositions including these iRNAs are useful for treating a subject having a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma.

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 a CTNNB1 gene, *e.g.*, a beta-catenin (CTNNB1)-associated disease, such as cancer, *e.g.*, hepatocellular carcinoma, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a CTNNB1 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 CTNNB1 gene, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a CTNNB1 gene as well as compositions, uses, and methods for treating subjects that would benefit from inhibition and/or reduction of the expression of a CTNNB1 gene, *e.g.*, subjects susceptible to or diagnosed with a CTNNB1-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 $\pm 10\%$. In certain embodiments, about means $\pm 5\%$. When about is present before a series of numbers or a range, it is understood that “about” can modify each of the numbers in the series or range.

The term “at least”, “no less than”, or “or more” 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 “or less” 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, “beta-catenin,” used interchangeably with the term “CTNNB1,” refers to a structure protein in the cadherin mediated cell-cell adhesive system, and is also known as a pivotal transcriptional activator of the Wnt signaling pathway. The Wnt/ β -catenin signaling pathway, also called the canonical Wnt signaling pathway, is a conserved signaling axis participating in diverse physiological processes such as proliferation, differentiation, apoptosis, migration, invasion and tissue homeostasis (Choi B, *et al.*, *Cell Rep.* 2020;31(5):107540). Dysregulation of the Wnt/ β -catenin cascade contributes to the development and progression of some solid tumors and hematological malignancies, such as hematocellular carcinoma (HCC) (Ge X, *et al. Journal of hematology & oncology.* 2010;3:33; He S, *et al., Biomed Pharmacother.* 2020;132:110851; Gajos-Michniewicz A, *et al., Int J Mol Sci* 2020, 21(14); Suzuki T, *et al., J Gastroenterol Hepatol.* 2002;17:994–1000). Indeed, beta-catenin plays important roles in promoting tumor progression by stimulating tumor cell proliferation and reducing the activity of cell adhesion systems and is associated with a poor prognosis, especially in patients with poorly differentiated HCCs (Inagawa S, *et al., Clin Cancer Res.* 2002;8:450–456). CTNNB1 is also known as catenin beta, Armadillo, NEDSDV; MRD19; or EVR7.

The sequence of a human CTNNB1 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519314571 (NM_001904.4; SEQ ID NO:1; reverse complement, SEQ ID NO: 2). The sequence of mouse CTNNB1 mRNA can be found at, for example, GenBank Accession No. GI: 260166638 (NM_007614.3; SEQ ID NO:3; reverse complement, SEQ ID NO:4). The sequence of

rat CTNNB1 mRNA can be found at, for example, GenBank Accession No. GI: 46048608 (NM_053357.2; SEQ ID NO:5; reverse complement, SEQ ID NO: 6). The sequence of *Macaca fascicularis* CTNNB1 mRNA can be found at, for example, GenBank Accession No. GI: 985482040 (NM_001319394.1; SEQ ID NO:7; reverse complement, SEQ ID NO: 8). The sequence of *Macaca mulatta* CTNNB1 mRNA can be found at, for example, GenBank Accession No. GI: 383872646 (NM_001257918.1; SEQ ID NO:9; reverse complement, SEQ ID NO:10).

Additional examples of CTNNB1 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, and the *Macaca* genome project web site.

Further information on CTNNB1 can be found, for example, at
 10 www.ncbi.nlm.nih.gov/gene/?term=CTNNB1.

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 CTNNB1, as used herein, also refers to variations of the CTNNB1 gene including variants provided in the SNP database. Numerous sequence variations within the CTNNB1 gene have
 15 been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=CTNNB1, 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 CTNNB1 gene, including mRNA that is a
 20 product of RNA processing of a primary transcription product. In one embodiment, 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 CTNNB1 gene.

The target sequence may be from about 19-36 nucleotides in length, *e.g.*, about 19-30
 25 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
 30 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,
 35 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
5 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
10 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 CTNNB1 gene in a cell, *e.g.*, a liver cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNA that
15 interacts with a target RNA sequence, *e.g.*, a CTNNB1 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
20 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
25 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 CTNNB1 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
30 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
35 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 CTNNB1 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 CTNNB1 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 CTNNB1 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 CTNNB1 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 CTNNB1 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 CTNNB1 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 CTNNB1 gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a CTNNB1 gene is important, especially if the particular region of complementarity in a CTNNB1 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, 50°C or 70°C 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, in vitro or in vivo. 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 Hoogsteen 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 two oligonucleotides or polynucleotides, such as 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 CTNNB1 gene). For example, a polynucleotide is complementary to at least a part of a CTNNB1 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding a CTNNB1 gene.

Accordingly, in some embodiments, the antisense polynucleotides disclosed herein are fully complementary to the target CTNNB1 sequence. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target CTNNB1 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, or 9, or a fragment of any one of SEQ ID NOs:1, 3, 5, 7, or 9, 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 CTNNB1 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, 5, or 6, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2, 3, 5, or 6, 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 CTNNB1 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, or 10, or a fragment of any one of SEQ ID NOs: 2, 4, 6, 8, or 10, 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 CTNNB1 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, 5, or 6, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 2, 3, 5, or 6, 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 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 “cationic lipid” includes those lipids having one or two fatty acid or fatty aliphatic chains and an amino acid containing head group that may be protonated to form a cationic lipid at physiological pH. In some embodiments, a cationic lipid is referred to as an “amino acid conjugate cationic lipid.”

The term “biodegradable cationic lipid” refers to a cationic lipid having one or more biodegradable groups located in the mid- or distal section of a lipidic moiety (*e.g.*, a hydrophobic chain) of the cationic lipid. The incorporation of the biodegradable group(s) into the cationic lipid results in faster metabolism and removal of the cationic lipid from the body following delivery of the active pharmaceutical ingredient to a target area.

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 CTNNB1 expression; a human at risk for a disease or disorder that would benefit from reduction in CTNNB1 expression; a human having a disease or disorder that would benefit from reduction in CTNNB1 expression; or human being treated for a disease or disorder that would benefit from reduction in CTNNB1 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 CTNNB1-associated disorder in a subject. Treatment also includes a reduction of one or more sign or symptoms associated with unwanted CTNNB1 expression; diminishing the extent of unwanted CTNNB1 activation or stabilization; amelioration or palliation of unwanted CTNNB1 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 CTNNB1 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%, 35%, 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 CTNNB1 in a subject is a decrease 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. The term “lower” can also be used in association with normalizing a symptom of a disease or condition, *i.e.* decreasing the difference between a level in a subject suffering from a CTNNB1-associated disorder towards or to a level in a normal subject not suffering from a CTNNB1-associated disorder. As used herein, if a disease is associated with an elevated value for a symptom, “normal” is considered to be the upper limit of normal. If a disease is associated with a decreased value for a symptom, “normal” is considered to be the lower limit of normal.

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 a CTNNB1 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 a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma. 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 "beta-catenin-associated disorder" or "CTNNB1-associated disorder," is a disease or disorder that is caused by, or associated with, CTNNB1 gene expression or CTNNB1 protein production. The term "CTNNB1-associated disorder" includes a disease, disorder or condition that would benefit from a decrease in CTNNB1 gene expression, replication, or protein activity. In some embodiments, the CTNNB1-associated disorder is cancer, *e.g.*, hepatocellular carcinoma.

The term "cancer" is used herein to refer to a group of cells that exhibit abnormally high levels of proliferation and growth. A cancer may be benign (also referred to as a benign tumor), pre-malignant, or malignant. Cancer cells may be solid cancer cells or leukemic cancer cells. The term "cancer growth" is used herein to refer to proliferation or growth by a cell or cells that comprise a cancer that leads to a corresponding increase in the size or extent of the cancer.

Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, myeloma and leukemia. In some embodiments, the cancer comprises a solid tumor cancer. In other embodiments, the cancer comprises a blood based cancer, *e.g.*, leukemia, lymphoma or myeloma. More particular nonlimiting examples of such cancers include squamous cell cancer, small-cell lung cancer, pituitary cancer, esophageal cancer, astrocytoma, soft tissue sarcoma, non-small cell lung cancer (including squamous cell non-small cell lung cancer), adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cell carcinoma, hepatocellular carcinoma, hepatoblastomas, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, brain cancer, endometrial cancer, testis cancer, cholangiocarcinoma, gallbladder carcinoma, gastric cancer, melanoma, and various types of head and neck cancer (including squamous cell carcinoma of the head and neck).

In some embodiments, the CTNNB1-associated disorder is hepatocellular carcinoma (HCC). As used herein, the term "hepatocellular carcinoma" refers to a major type of primary liver cancer and one of the rare human neoplasms etiologically linked to viral factors. Chronic infections with the hepatitis B virus (HBV) and the hepatitis C virus (HCV) have been implicated in about 80% of cases worldwide (Wang W, *et al.*, J Gastroenterol. 2017 Apr; 52(4):419-431). Genetic mutations and abnormal activation of signal transduction pathways involved in cell proliferation, apoptosis, metabolism, splicing, and the cell cycle are known to contribute to the development of HCC. In particular, the Wnt/ β -catenin signaling pathway was known to be activated in up to 50% of HCC (Lee JM, *et al.* Cancer Lett. 2014 Feb 1; 343(1):90-7; Vilchez V, *et al.* World J Gastroenterol. 2016 Jan 14; 22(2):823-32). The Wnt/ β -catenin pathway regulates multiple cellular processes that are involved in the initiation, growth, survival, migration, differentiation, and apoptosis of HCC (Wang Z, *et al.*, Mol Clin Oncol. 2015 Jul; 3(4):936-940). Mutations in β -catenin have been identified in these tumors, and β -catenin mutation has also been shown to affect the prognosis of HCC. (Prange W, *et al.*, J Pathol. 2003;201:250-259; Torbenson M, *et al.*, Am J Clin Pathol. 2004;122:377-382).

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a CTNNB1-associated disorder, 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 CTNNB1-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.

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II. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of a CTNNB1 gene. In certain embodiments, the iRNA includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a CTNNB1 gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human susceptible to developing a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma. 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 CTNNB1 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).

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Upon contact with a cell expressing the CTNNB1 gene, the iRNA inhibits the expression of the CTNNB1 gene (*e.g.*, a human, a primate, a non-primate, or a rat CTNNB1 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 certain 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 certain 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.

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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 CTNNB1 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.

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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 embodiments, the

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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 CTNNB1 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, 5, and 6, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 2, 3, 5, and 6. 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 CTNNB1 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, 5, or 6, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 2, 3, 5, or 6.

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.

It will be understood that, although the sequences in, for example, Table 2, 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, 5, or 6 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, 5, or 6 which are un-modified, un-conjugated, modified, or conjugated, as described herein. For example, although the sense strands of the agents of the invention shown in Table 5 are conjugated to an L96 ligand, these agents may be unconjugated 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, 5, or 6. 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, 5, or 6 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, 5, or 6, and differing in their ability to inhibit the expression of a CTNNB1 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.

- 5 In addition, the RNAs provided in Tables 2, 3, 5, or 6 identify a site(s) in a CTNNB1 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
- 10 from any one of the sequences provided in any one of Tables 2, 3, 5, or 6 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a CTNNB1 gene.

III. Modified iRNAs of the Invention

- In certain embodiments, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is unmodified, 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
- 15 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
- 20 of an iRNA are modified, *i.e.*, not more than 5, 4, 3, 2, or 1 unmodified 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
- 25 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'-
- 30 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
- 35 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
5 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
10 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 phosphorothioate 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
15 the sodium salt form, sodium ions are present in the agent as counterions for all of the phosphodiester and/or phosphorothioate 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;
20 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.

25 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;
30 formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S, and CH₂ component parts.

Representative U.S. Patents that teach the preparation of the above oligonucleosides include,
35 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 --CH₂--NH--CH₂--, --CH₂--N(CH₃)--O--CH₂--[known as a methylene (methylimino) or MMI backbone], --CH₂--O--N(CH₃)--CH₂--, --CH₂--N(CH₃)--N(CH₃)--CH₂-- and --N(CH₃)--CH₂--CH₂-- 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. The native phosphodiester backbone can be represented as O-P(O)(OH)-OCH₂--.

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 C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Exemplary suitable modifications include O[(CH₂)_nO]_mCH₃, O(CH₂)_nOCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, 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--CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminoethoxy, *i.e.*, a O(CH₂)₂ON(CH₃)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--CH₂--O--CH₂--N(CH₃)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'-OCH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂) 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.

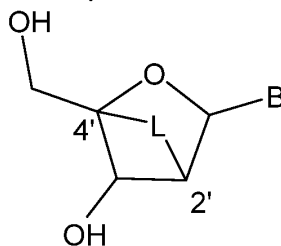
An iRNA can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxythymidine (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 and other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, 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.

In some embodiments, an RNAi agent of the disclosure can also be modified to include one or more bicyclic sugar moieties. A “bicyclic sugar” is a furanosyl ring modified by a ring formed by the bridging of two carbons, whether adjacent or non-adjacent. A “bicyclic nucleoside” (“BNA”) is a nucleoside having a sugar moiety comprising a ring formed by bridging two carbons, whether adjacent or non-adjacent, 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, optionally, via the 2'-acyclic oxygen atom. 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'-CH₂-O-2' bridge. This structure effectively “locks” the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, 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.

A locked nucleoside can be represented by the structure (omitting stereochemistry),



wherein B is a nucleobase or modified nucleobase and L is the linking group that joins the 2'-carbon to the 4'-carbon of the ribose ring. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to 4'-(CH₂)—O-2' (LNA); 4'-(CH₂)—S-2'; 4'-(CH₂)₂—O-2' (ENA); 4'-CH(CH₃)—O-2' (also referred to as “constrained ethyl” or “cEt”) and 4'-CH(CH₂OCH₃)—O-2' (and analogs thereof; see, *e.g.*, U.S. Patent No. 7,399,845); 4'-C(CH₃)(CH₃)—O-2' (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,283); 4'-CH₂—N(OCH₃)-2' (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,425); 4'-CH₂—O—N(CH₃)-2' (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4'-CH₂—N(R)—O-2', wherein R is H, C1-C12 alkyl, or a nitrogen protecting group (see, *e.g.*, U.S. Patent No. 7,427,672); 4'-CH₂—C(H)(CH₃)-2' (see, *e.g.*, Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009,

74, 118-134); and 4'-CH₂—C(=CH₂)-2' (and analogs thereof; see, *e.g.*, U.S. 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.

5 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.

10 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(CH₃)-O-2' bridge (*i.e.*, L in the preceding structure). In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to
15 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
20 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.

25 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
30 *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;
35 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'-O-deoxythymidine (ether), N-

(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3'-phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

Other modifications of 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. As shown herein and in WO2013/075035, one or more motifs of three identical modifications on three consecutive nucleotides may be introduced 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.*, CTNNB1 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 not 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 (*i.e.*, 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 blunt-ended 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, and 13 from the 5' end.

In other embodiments, the dsRNAi agent is a double blunt-ended 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, and 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, and 13 from the 5' end.

In yet other embodiments, the dsRNAi agent is a double blunt-ended 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, and 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, and 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, and 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, and 13 from the 5' end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang. In one embodiment, 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 (such as, GalNAc₃).

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 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 then the chemistries can be the same or different. Two or more wing modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

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...", "AAABAAABAAB...", "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
 5 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
 10 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
 15 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
 20 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
 25 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
 30 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)
 35 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 deoxythymidine (dT) or the nucleotide at the 3'-end of the antisense strand is deoxythymidine (dT). For example, there is a short sequence of deoxythymidine 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):



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;

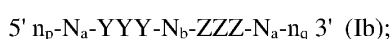
wherein N_b and Y do not have the same modification; and

XXX, YYY, and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. In one embodiment, YYY is all 2'-F modified nucleotides.

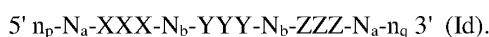
In some embodiments, the N_a or N_b comprises modifications of alternating pattern.

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



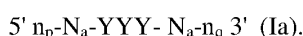
When the sense strand is represented by formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

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.

- 5 When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. In one embodiment, 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.

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



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.

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



wherein:

k and l are each independently 0 or 1;

- 20 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;

- 25 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.

- 30 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. In one embodiment, 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 '-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. In one embodiment, 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.

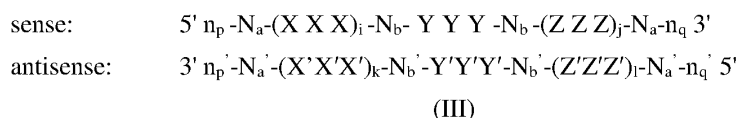
Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, CRN, UNA, cEt, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 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):



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;

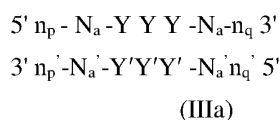
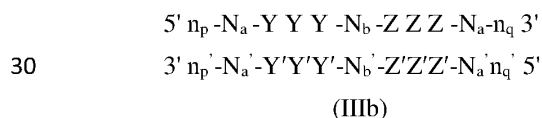
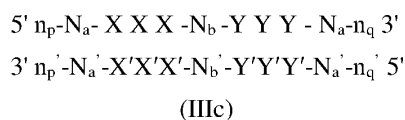
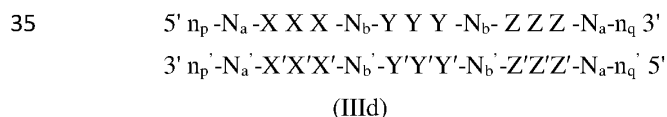
each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

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; or 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:



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.

5 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.

10 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.

15 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.

20 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.

25 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.

30 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.

35 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 *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
5 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
10 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
15 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
20 the duplexes can target same gene at two different target sites.

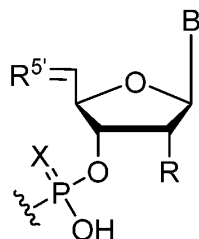
In one embodiment, two dsRNAi agents represented by at least one of formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends, and are optionally conjugated to a ligand. Each of the agents can target the same gene or two different genes;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

- 5 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 5' vinyl phosphonate modified nucleotide of the disclosure has the structure:



wherein X is O or S;

- 10 R is hydrogen, hydroxy, fluoro, or C₁₋₂₀alkoxy (*e.g.*, methoxy or n-hexadecyloxy);
 R^{5'} is =C(H)-P(O)(OH)₂ and the double bond between the C5' carbon and R^{5'} is in the *E* or *Z* orientation (*e.g.*, *E* orientation); and
 B is a nucleobase or a modified nucleobase, optionally where B is adenine, guanine, cytosine, thymine, or uracil.

- 15 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 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.

- 20 Vinyl phosphonate modifications are also contemplated for the compositions and methods of the instant disclosure. An exemplary vinyl phosphonate structure includes the preceding structure, where R^{5'} is =C(H)-OP(O)(OH)₂ and the double bond between the C5' carbon and R^{5'} is in the *E* or *Z* orientation (*e.g.*, *E* orientation).

- 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 (such as, 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
- 25
30

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,” such as, 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. In one embodiment, 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. In one embodiment, the acyclic group is a serinol backbone or diethanolamine backbone. PCT/US12/068491, ,

i. Thermally Destabilizing Modifications

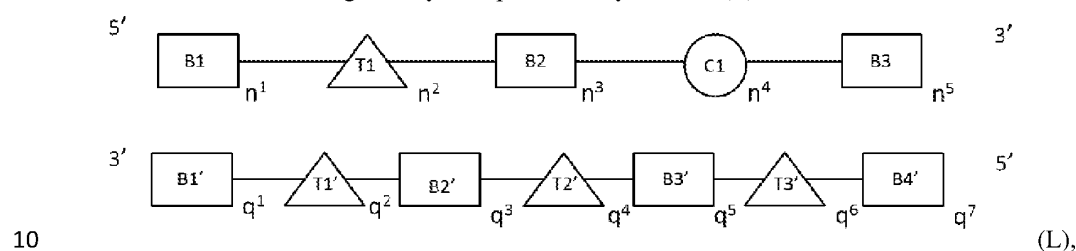
In certain embodiments, a dsRNA molecule can be optimized for RNA interference by incorporating thermally destabilizing modifications in the seed region of the antisense strand. As used herein “seed region” means at positions 2-9 of the 5'-end of the referenced strand or at positions 2-8 of the 5'-end of the referenced strand. For example, thermally destabilizing modifications can be incorporated in the seed region of the antisense strand to reduce or inhibit off-target gene silencing.

The term “thermally destabilizing modification(s)” includes modification(s) that would result with a dsRNA with a lower overall melting temperature (T_m) than the T_m of the dsRNA without having such modification(s). For example, the thermally destabilizing modification(s) can decrease the T_m of the dsRNA by 1 – 4 °C, such as one, two, three or four degrees Celsius. And, the term “thermally destabilizing nucleotide” refers to a nucleotide containing one or more thermally destabilizing modifications.

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'

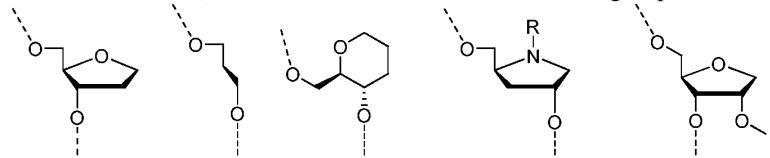
region of the antisense strand. In some embodiments, one or more thermally destabilizing modification(s) of the duplex is/are located in positions 2-9, such as, positions 4-8, from the 5'-end of the antisense strand. In some further embodiments, the thermally destabilizing modification(s) of the duplex 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. 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):

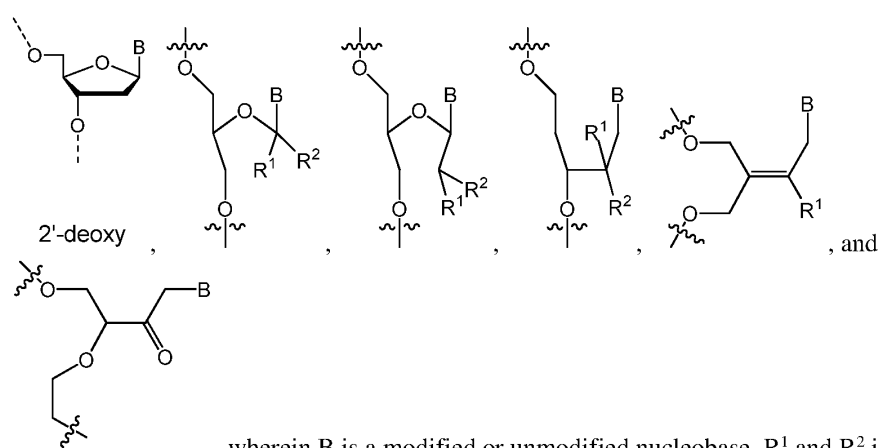


In formula (L), B1, B2, B3, B1', B2', B3', and B4' each are independently a nucleotide containing a modification selected from the group consisting of 2'-O-alkyl, 2'-substituted alkoxy, 2'-substituted alkyl, 2'-halo, ENA, and BNA/LNA. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 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, 2'-O-CH₂C(O)N(Me)H) modification.

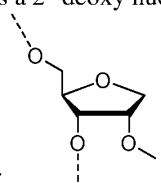
C1 is a thermally destabilizing nucleotide placed at a site opposite to the seed region of the antisense strand (*i.e.*, at positions 2-8 of the 5'-end of the antisense strand, or at positions 2-9 of the 5'-end of the referenced strand). For example, C1 is at a position of the sense strand that pairs 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:



selected from the group consisting of:



H, halogen, OR₃, or alkyl; and R₃ 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.

n¹, n³, and q¹ are independently 4 to 15 nucleotides in length.

n⁵, q³, and q⁷ are independently 1-6 nucleotide(s) in length.

n⁴, q², and q⁶ are independently 1-3 nucleotide(s) in length; alternatively, n⁴ is 0.

q⁵ is independently 0-10 nucleotide(s) in length.

n² and q⁴ are independently 0-3 nucleotide(s) in length.

Alternatively, n⁴ is 0-3 nucleotide(s) in length.

In one embodiment, n⁴ can be 0. In one example, n⁴ is 0, and q² and q⁶ are 1. In another example, n⁴ is 0, and q² and q⁶ 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.

5 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

10 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.

15 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).

20 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.

25 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.

30 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.

35 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.

5 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
10 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
15 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).
20

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.
25 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
30 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.
35

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.

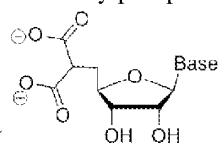
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 5, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4'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

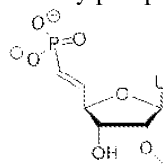
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe,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

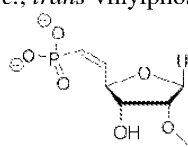
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and 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'-PS₂), 5'-end vinylphosphonate (5'-VP), 5'-end methylphosphonate (MePhos), or 5'-deoxy-5'-C-malonyl (). When the 5'-end phosphorus-containing group is 5'-end vinylphosphonate (5'-VP), the 5'-VP can be either

5'-E-VP isomer (*i.e.*, *trans*-vinylphosphonate,



5'-Z-VP isomer (*i.e.*, *cis*-



vinylphosphonate, or mixtures thereof.

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.

5 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.

10 In one embodiment, the RNAi agent comprises a 5'-PS₂. In one embodiment, the RNAi agent comprises a 5'-PS₂ in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS₂. 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, 15 T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-P. 20

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination 25 thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS₂.

30 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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 35

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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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.

5 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'-PS₂.

15 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.

25 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.

30 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand
 5 (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'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, 10 q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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'-
 15 C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The dsRNAi RNA agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ 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'-PS₂.

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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

5 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

10 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

15 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-PS₂.

20 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

25 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ 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.

30 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ 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.

35 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ 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'-PS₂.

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'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ 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

5 (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.

10 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
15 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
20 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'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ 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
30 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'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ 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'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ 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:

- 15 (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);
- 20 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);
- 25 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.
- 30

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);
- 35 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:

- 5 (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
- 10 (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:

- 15 (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
 - 20 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
- 25 (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
 - 30 (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.

35 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);

5 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);

10

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.

15

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);

20

25 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);

30

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.

35

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

5 (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;

10 (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

15 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:

20 (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

25 (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;

30 (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 deoxy-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

35 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, 5, or 6. 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. Acad. Sci. USA*, 1989, 86: 6553-6556). In other embodiments, the ligand is cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Lett.*, 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306-309; Manoharan *et al.*, *Biorg. Med. Chem. Lett.*, 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J*, 1991, 10:1111-1118; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327-330; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or 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 some embodiments a ligand provides an enhanced affinity for a

selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. In some embodiments, 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-glycolide) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic acid), N-isopropylacrylamide polymers, or polyphosphazene. 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- κ B.

5 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.

10 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,
15 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
20 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
25 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
30 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
35 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.

In one embodiment, such a lipid or lipid-based molecule 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. In one embodiment, it binds HSA with a sufficient affinity such that the conjugate will be 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. In one embodiment, the conjugate will be 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, such as, a helical cell-permeation agent. In one embodiment, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopodia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. In one embodiment, the helical agent is an alpha-helical agent, which 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.

- 5 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
- 10 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
- 15 (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
- 20 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
- 25 cyclic, and may be modified, *e.g.*, glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidomimetics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand, *e.g.*, PECAM-1 or VEGF.

- A “cell permeation peptide” is capable of permeating a cell, *e.g.*, a microbial cell, such as a
- 30 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
- 35 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.

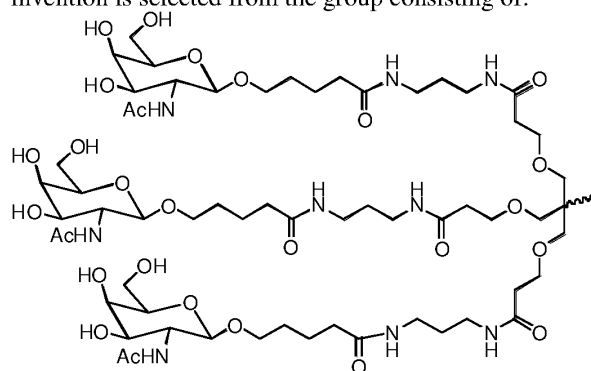
In certain embodiments, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc

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

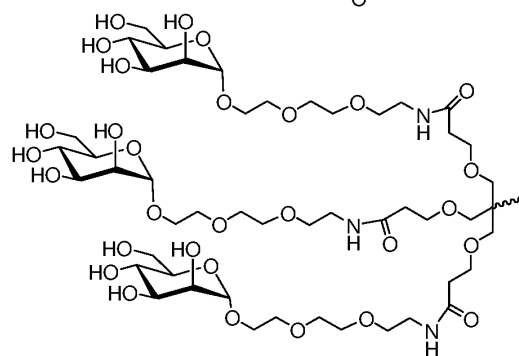
In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of 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.

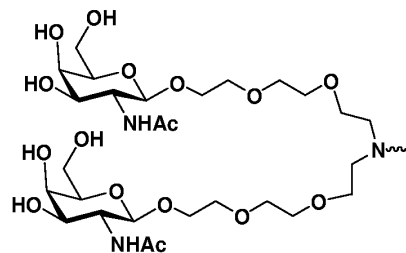
In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



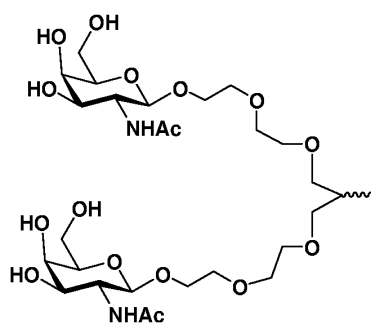
Formula II,



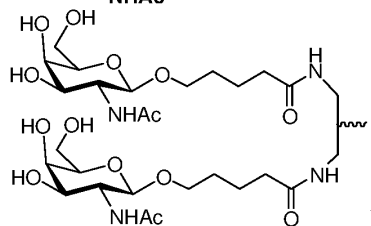
Formula III,



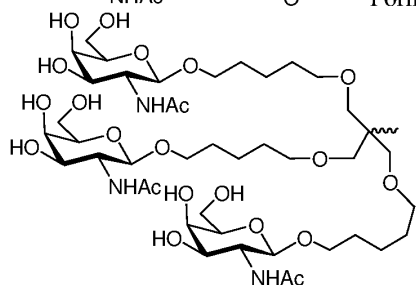
Formula IV,



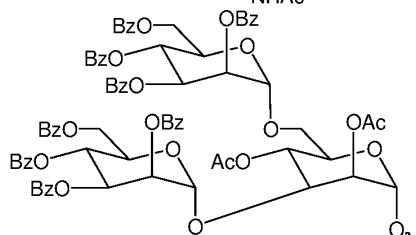
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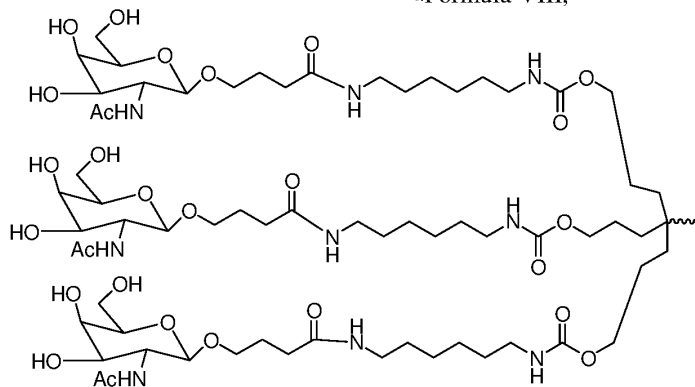
Formula VI,



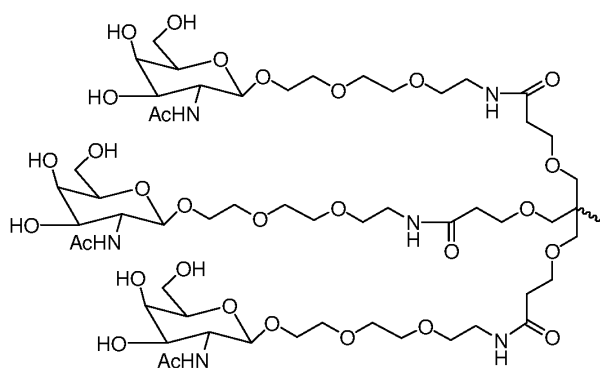
Formula VII,



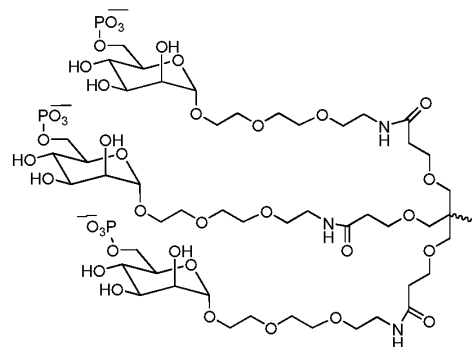
Formula VIII,



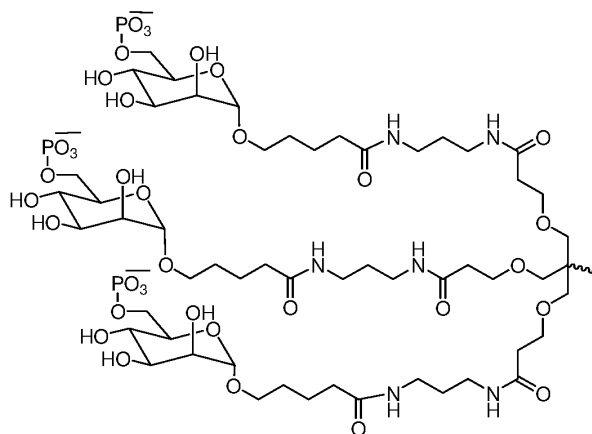
Formula IX,



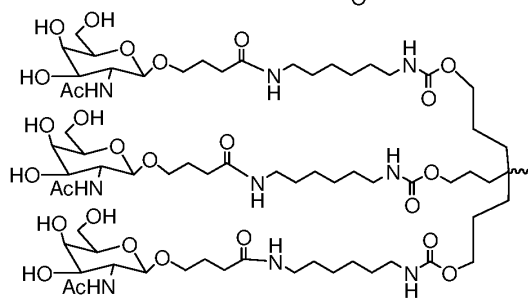
Formula X,



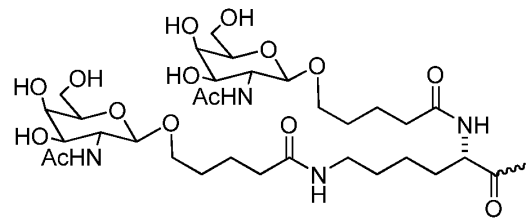
Formula XI,



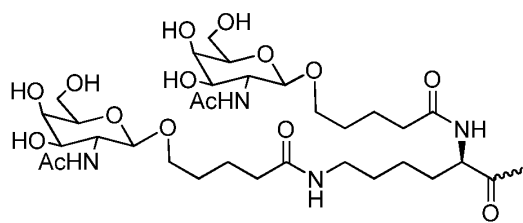
Formula XII,



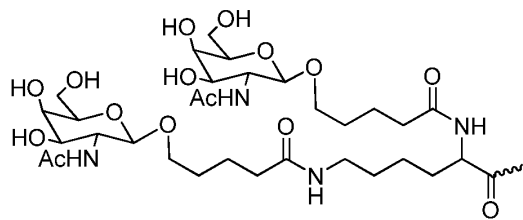
Formula XIII,



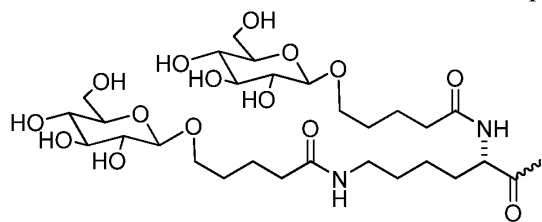
Formula XIV,



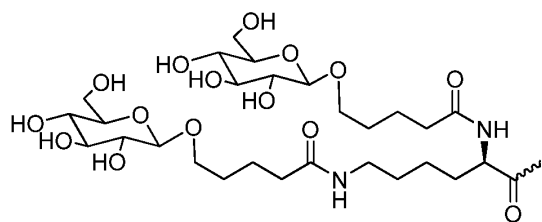
Formula XV,



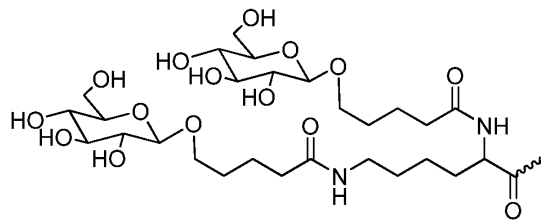
Formula XVI,



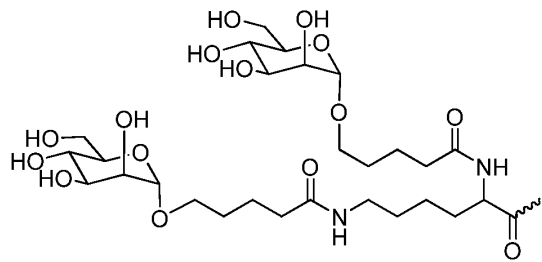
Formula XVII,



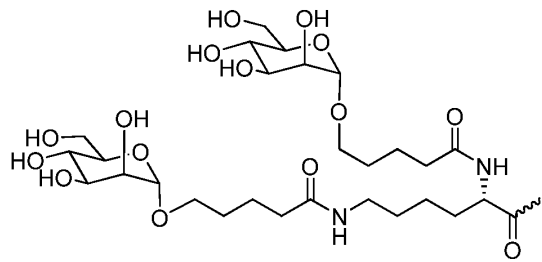
Formula XVIII,



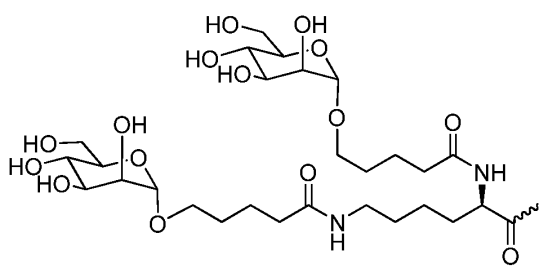
Formula XIX,



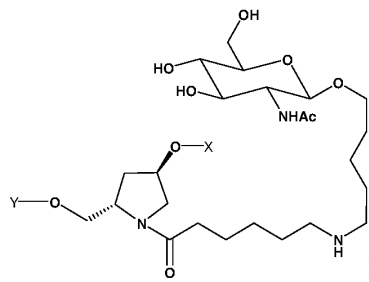
Formula XX,



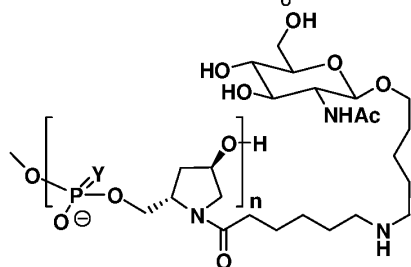
Formula XXI,



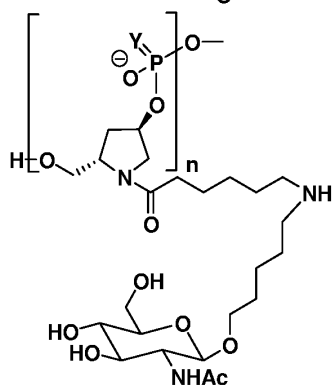
Formula XXII,



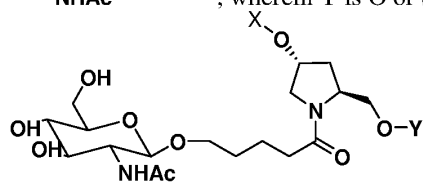
Formula XXIII;



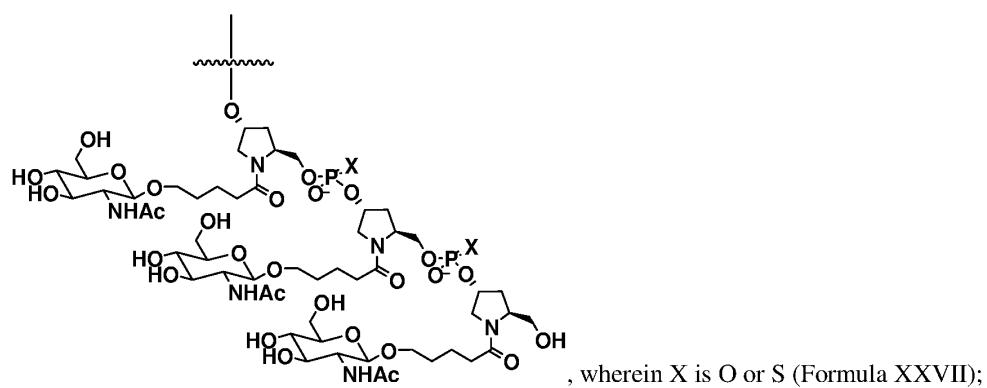
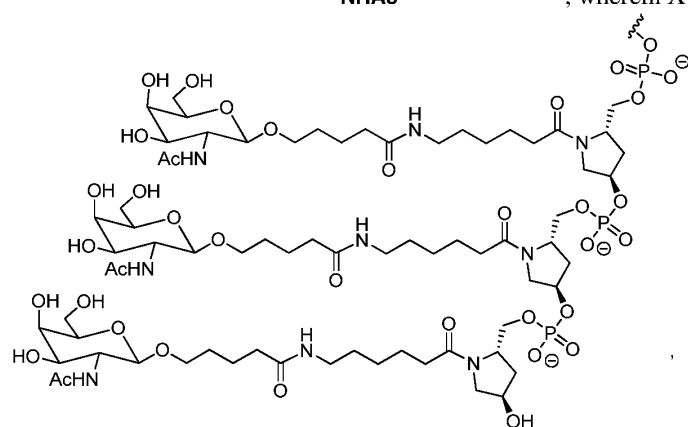
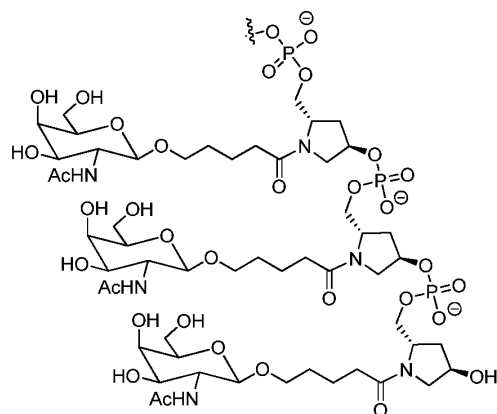
, wherein Y is O or S and n is 3 -6 (Formula XXIV);



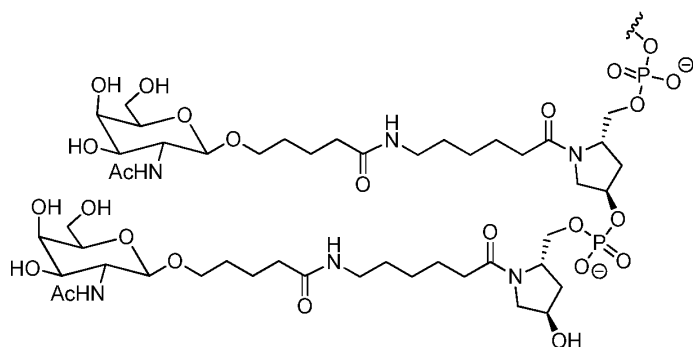
, wherein Y is O or S and n is 3-6 (Formula XXV);



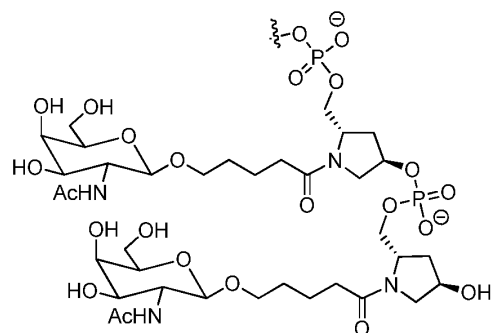
Formula XXVI;



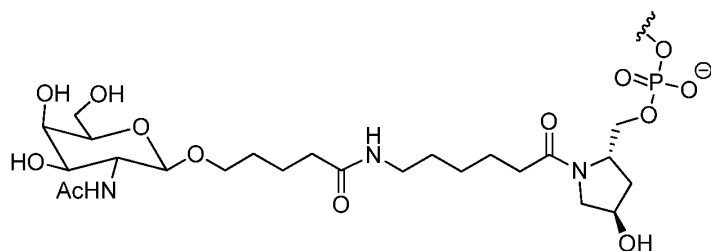
Formula XXVII; Formula XXIX;



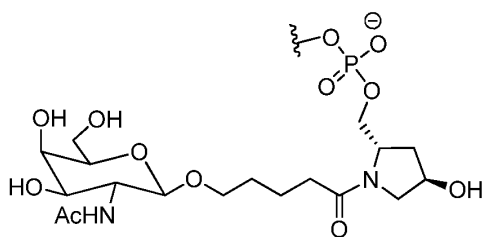
Formula XXXI;



Formula XXX;

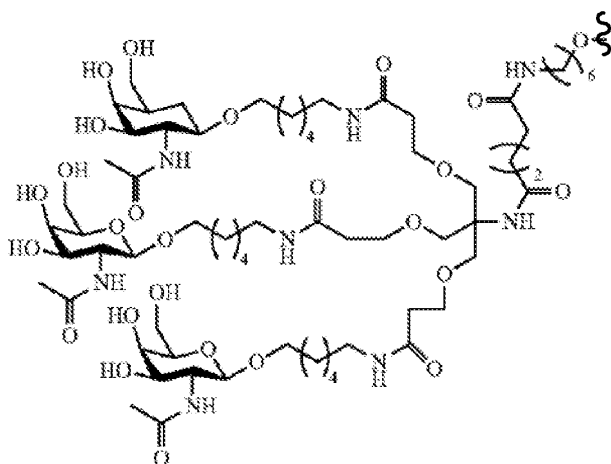


, and



Formula XXXII;

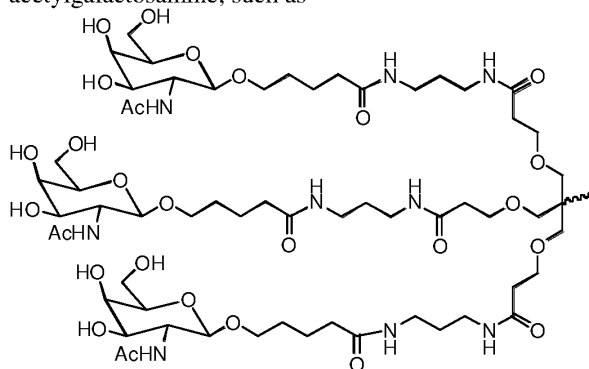
5 Formula XXXIII.



Formula XXXIV.

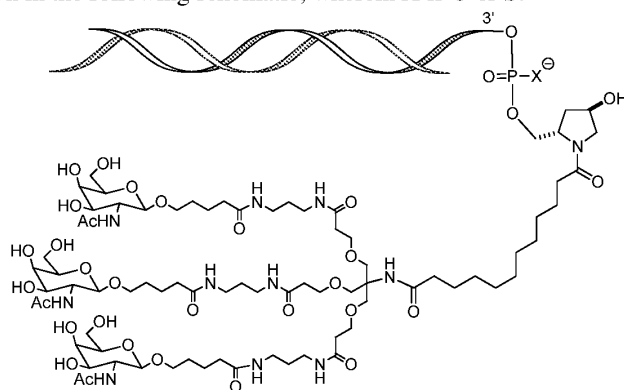
In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In one embodiment, the monosaccharide is an N-

5 acetylglucosamine, such as

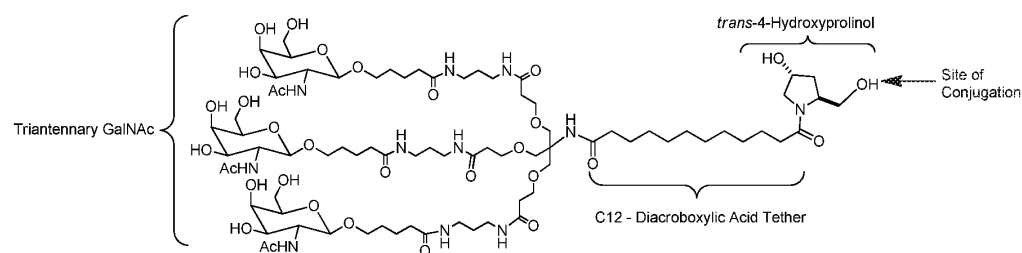


Formula II.

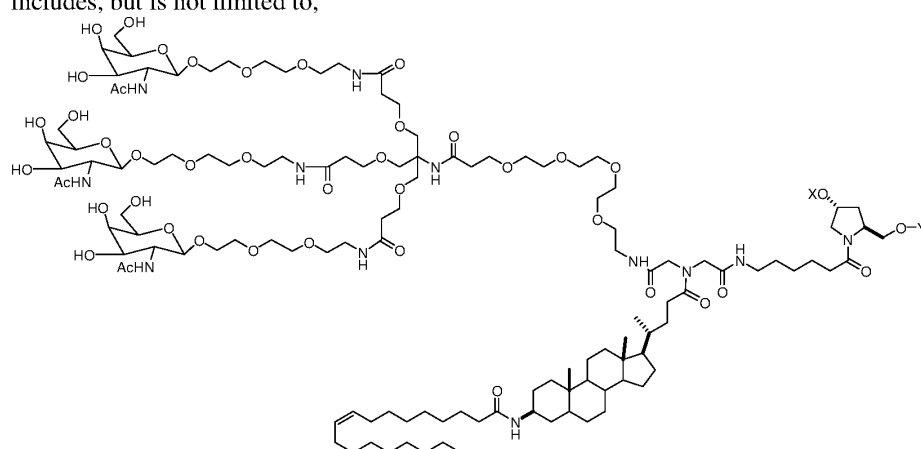
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate *via* a linker as shown in the following schematic, wherein X is O or S.



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,

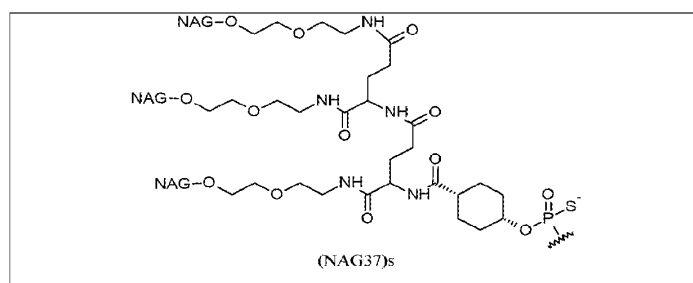


(Formula XXXVI), when one of X or Y is an oligonucleotide, the other is a hydrogen.

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:

15

20



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 antisense 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.

10 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.

20 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

25 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, SO₂, SO₂NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclalkyl, heterocyclalkenyl, heterocyclalkynyl, aryl, heteroaryl, heterocycl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclalkyl, alkylheterocyclalkenyl, alkylheterocyclalkynyl, alkenylheterocyclalkyl, alkenylheterocyclalkenyl, alkenylheterocyclalkynyl, alkynylheterocyclalkyl,

alkynylheterocyclalkenyl, alkynylheterocyclalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylheteroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO₂, N(R₈), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or substituted or unsubstituted heterocyclic; where R₈ is

5 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 an exemplary embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30

10 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

15 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

20 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.

25 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 selected 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

30 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.

35 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 certain 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 known in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

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-, wherein Rk at each occurrence can be, independently, C1-C20 alkyl, C1-C20 haloalkyl, C6-C10 aryl, or C7-C12 aralkyl. Exemplary embodiments include -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-. In certain embodiments a phosphate-based linking group is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

5 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 certain 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
10 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). An exemplary 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
15 to those described above.

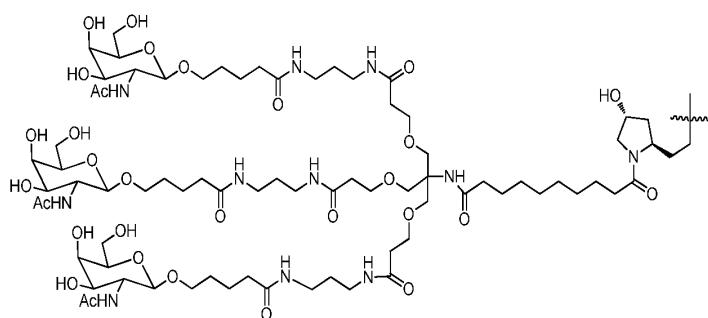
iv. Ester-based linking groups

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.
20 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.

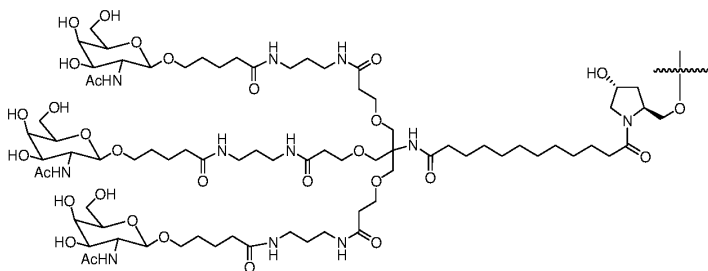
v. Peptide-based cleaving groups

25 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
30 between any alkylene, alkenylene or alkynylene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula -NHCHRAC(O)NHCHRBC(O)-, where RA and RB are the
35 R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

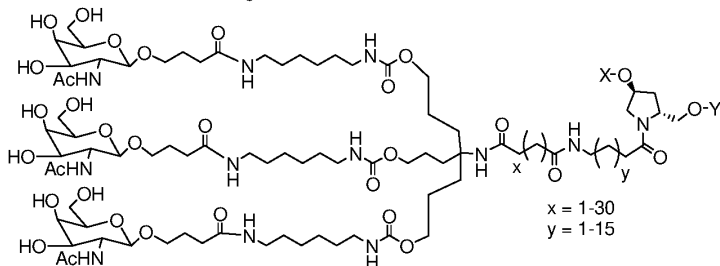
In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



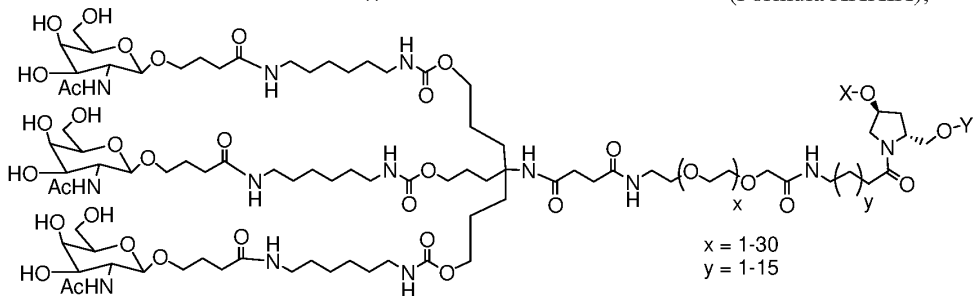
(Formula XXXVII),



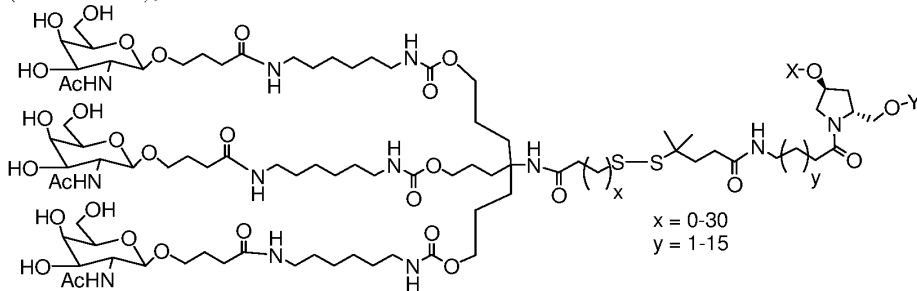
(Formula XXXVIII),



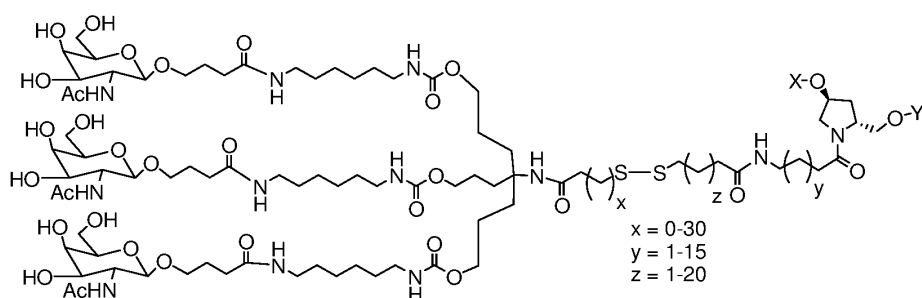
(Formula XXXIX),



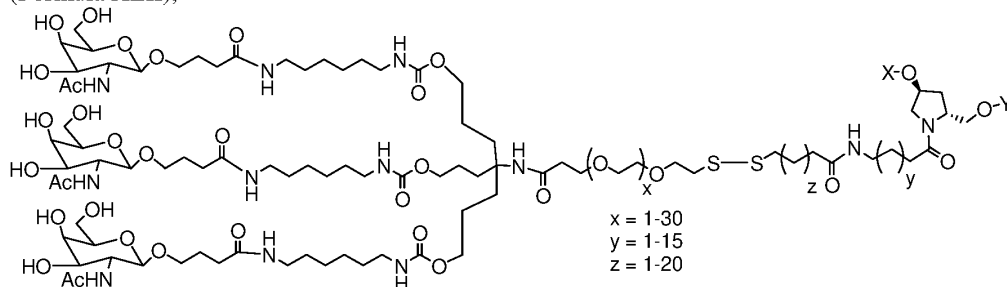
(Formula XL),



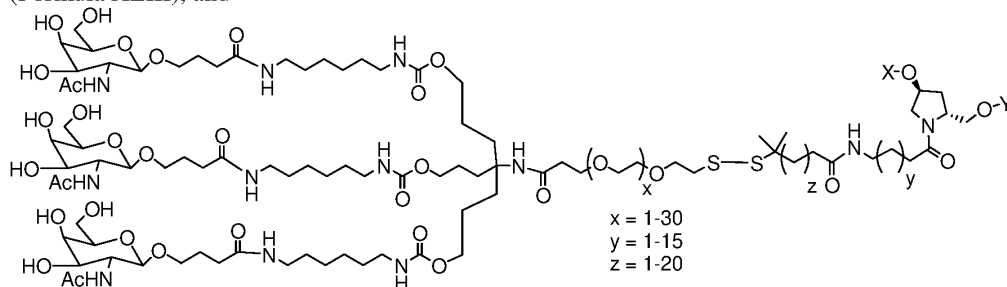
(Formula XLI),



(Formula XLII),



(Formula XLIII), and

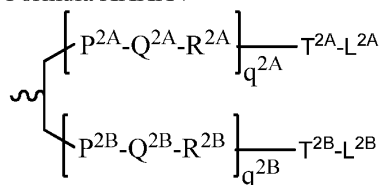


(Formula XLIV), when one of X or Y is an oligonucleotide, the other is a hydrogen.

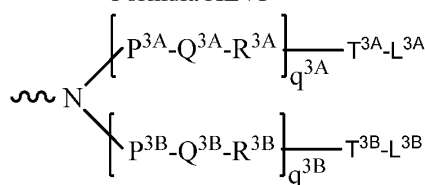
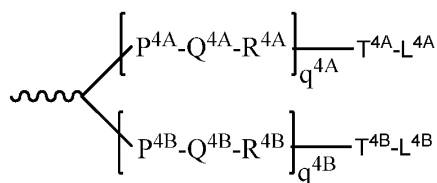
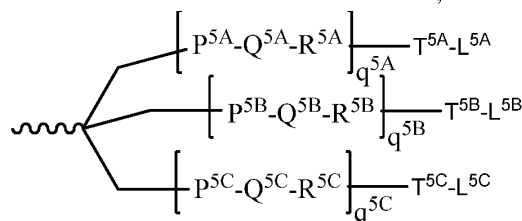
In certain embodiments of the compositions and methods of the invention, a ligand is one or more “GalNAc” (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

10 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



Formula XLVI



, or

;

Formula XLVII

Formula XLVIII

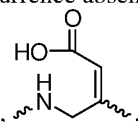
5 wherein:

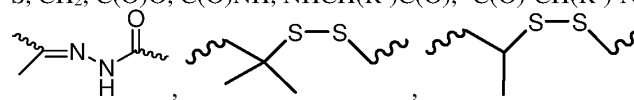
q^{2A}, q^{2B}, q^{3A}, q^{3B}, q^{4A}, q^{4B}, q^{5A}, q^{5B} and q^{5C} represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

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^{5A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH₂, CH₂NH or CH₂O;

10 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), SO₂, 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,

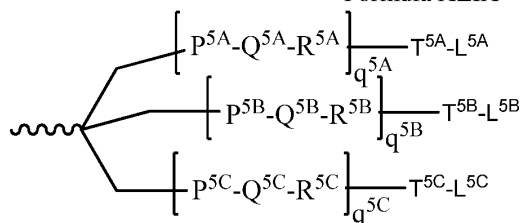
S, CH₂, C(O)O, C(O)NH, NHCH(R^a)C(O), -C(O)-CH(R^a)-NH-, CO, CH=N-O, ,

15 , 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

20 target gene, such as those of formula (XLIX):

Formula XLIX



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.

5 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; 10 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.

15 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 20 compounds, such as, 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 25 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 30 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 35 available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. *et al.*, *Biochem. Biophys. Res. Comm.*, 2007, 365(1):54-61; Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553), cholic acid (Manoharan *et al.*, *Bioorg. Med. Chem. Lett.*, 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan *et al.*, *Bioorg. Med. Chem. Lett.*, 1993, 3:2765), a thiocholesterol (Oberhauser *et*

al., *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J.*, 1991, 10:111; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 1996, 277:923). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

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 CTNNB1-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma) 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) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR, *et al* (2003) *J. Mol. Biol* 327:761-766; Verma, UN, *et al* (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS *et al* (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of iRNAs include DOTAP (Sorensen, DR., *et al* (2003), *supra*; Verma, UN, *et al* (2003), *supra*), "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. Certain aspects of the instant disclosure relate to a method of reducing the expression of a CTNNB1 gene in a cell, comprising contacting said cell with the double-stranded RNAi agent of the disclosure. In one embodiment, the cell is a hepatic cell, optionally a hepatocyte. In one embodiment, the cell is an extrahepatic cell.

A. Vector encoded iRNAs of the Invention

iRNA targeting the CTNNB1 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
5 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
10 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

15 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 CTNNB1-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma.

20 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 CTNNB1 gene.

25 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 CTNNB1 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
30 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, such as, 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
35 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 pharmaceutical compositions of the present disclosure can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral, or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; subdermal, *e.g.*, via an implanted device; or intracranial, *e.g.*, by intraparenchymal, intrathecal or intraventricular, administration.

The iRNA can be delivered in a manner to target a particular tissue, such as the liver.

Pharmaceutical compositions and formulations for topical administration can include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable. Coated condoms, gloves and the like can also be useful. Suitable topical formulations include those in which the RNAi agents featured in the disclosure are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Suitable lipids and liposomes include neutral (*e.g.*, dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (*e.g.*, dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.*, dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). RNAi agents featured in the disclosure can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, RNAi agents can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, monoolein, dilaurin, glyceryl 1-monocaprinate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C1-20 alkyl ester (*e.g.*, isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in US 6,747,014, which is incorporated herein by reference.

In one embodiment, the siRNAs, double stranded RNA agents of the invention, are administered to a cell in a pharmaceutical composition by a topical route of administration.

In one embodiment, the pharmaceutical composition may include an siRNA compound mixed with a topical delivery agent. The topical delivery agent can be a plurality of microscopic vesicles. The microscopic vesicles can be liposomes. In some embodiments the liposomes are cationic liposomes.

In another embodiment, the dsRNA agent is admixed with a topical penetration enhancer. In one embodiment, the topical penetration enhancer is a fatty acid. The fatty acid can be arachidonic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, monolein, dilaurin, glyceryl 1-monocaprinate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C1-10 alkyl ester, monoglyceride, diglyceride or pharmaceutically acceptable salt thereof.

In another embodiment, the topical penetration enhancer is a bile salt. The bile salt can be cholic acid, dehydrocholic acid, deoxycholic acid, glucolic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate, sodium glycodihydrofusidate, polyoxyethylene-9-lauryl ether or a pharmaceutically acceptable salt thereof.

In another embodiment, the penetration enhancer is a chelating agent. The chelating agent can be EDTA, citric acid, a salicylate, a N-acyl derivative of collagen, laureth-9, an N-amino acyl derivative of a beta-diketone or a mixture thereof.

In another embodiment, the penetration enhancer is a surfactant, *e.g.*, an ionic or nonionic surfactant. The surfactant can be sodium lauryl sulfate, polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether, a perfluorchemical emulsion or mixture thereof.

In another embodiment, the penetration enhancer can be selected from a group consisting of unsaturated cyclic ureas, 1-alkyl-alkones, 1-alkenylazacyclo-alkanones, steroidal anti-inflammatory agents and mixtures thereof. In yet another embodiment the penetration enhancer can be a glycol, a pyrrol, an azone, or a terpenes.

In one aspect, the invention features a pharmaceutical composition including an siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, (*e.g.*, a precursor, *e.g.*, a larger siRNA compound which can be processed into a ssiRNA compound, or a DNA which encodes an siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, or precursor thereof) in an injectable dosage form. In one embodiment, the injectable dosage form of the pharmaceutical composition includes sterile aqueous solutions or dispersions and sterile powders. In some embodiments the sterile solution can include a diluent such as water; saline solution; fixed oils, polyethylene glycols, glycerin, or propylene glycol.

The iRNA molecules of the invention can be incorporated into pharmaceutical compositions. Such compositions typically include one or more species of iRNA and a pharmaceutically acceptable carrier. As used herein the language “pharmaceutically acceptable carrier” is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration to a cell, *e.g.*, a liver cell. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound,

use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

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.

iRNAs featured in the invention can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, iRNAs can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, monoolein, dilaurin, glyceryl 1-monocaprinate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C₁₋₂₀ alkyl ester (*e.g.*, isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof). Topical formulations are described in detail in U.S. Patent No. 6,747,014, which is incorporated herein by reference.

A. iRNA Formulations Comprising Membranous Molecular Assemblies

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

A liposome containing a RNAi agent can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic.

Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The RNAi agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the RNAi agent and condense around the RNAi agent to form a liposome. After condensation, the detergent is removed, *e.g.*, by dialysis, to yield a liposomal preparation of RNAi agent.

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

Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as structural components of the delivery vehicle, are further described in, *e.g.*, WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Felgner, P. L. *et al.*, *Proc. Natl. Acad. Sci., USA* 8:7413-7417, 1987; U.S. Pat. No. 4,897,355; U.S. Pat. No. 5,171,678; Bangham, *et al. M. Mol. Biol.* 23:238, 1965; Olson, *et al. Biochim. Biophys. Acta* 557:9, 1979; Szoka, *et al. Proc. Natl. Acad. Sci.* 75: 4194, 1978; Mayhew, *et al. Biochim. Biophys. Acta* 775:169, 1984; Kim, *et al. Biochim. Biophys. Acta* 728:339, 1983; and Fukunaga, *et al. Endocrinol.* 115:757, 1984. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, *e.g.*, Mayer, *et al. Biochim. Biophys. Acta* 858:161, 1986). Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew, *et al. Biochim. Biophys. Acta* 775:169, 1984). These methods are readily adapted to packaging RNAi agent preparations into liposomes.

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

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

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic

liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

5 Examples of other methods to introduce liposomes into cells *in vitro* and *in vivo* include U.S. Pat. No. 5,283,185; U.S. Pat. No. 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Felgner, *J. Biol. Chem.* 269:2550, 1994; Nabel, *Proc. Natl. Acad. Sci.* 90:11307, 1993; Nabel, *Human Gene Ther.* 3:649, 1992; Gershon, *Biochem.* 32:7143, 1993; and Strauss *EMBO J.* 11:417, 1992.

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

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

 Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos *et al. (Ann. N.Y. Acad. Sci.*, 1987, 507, 64) reported the ability of monosialoganglioside G_{M1},
30 galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon *et al. (Proc. Natl. Acad. Sci. U.S.A.*, 1988, 85, 6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen *et al.*, disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb *et al.*) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-
35 sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim *et al.*).

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

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

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

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

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

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

Liposomal formulations are particularly suited for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer RNAi agent into the skin. In some implementations, liposomes are used for delivering RNAi agent to epidermal cells and also to enhance the penetration of RNAi agent into dermal tissues, e.g., into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, e.g., Weiner *et al.*, *Journal of Drug Targeting*, 1992, vol. 2, 405-410 and du Plessis *et al.*, *Antiviral Research*, 18, 1992, 259-265; Mannino, R. J. and Fould-Fogerite, S., *Biotechniques* 6:682-690, 1988; Itani, T. *et al.* *Gene* 56:267-276, 1987; Nicolau, C. *et al.* *Meth. Enz.* 149:157-176, 1987; Straubinger, R. M. and Papahadjopoulos, D. *Meth. Enz.* 101:512-527, 1983; Wang, C. Y. and Huang, L., *Proc. Natl. Acad. Sci. USA* 84:7851-7855, 1987).

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

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

Other formulations amenable to the present invention are described in United States provisional application serial Nos. 61/018,616, filed January 2, 2008; 61/018,611, filed January 2, 2008; 61/039,748, filed March 26, 2008; 61/047,087, filed April 22, 2008 and 61/051,528, filed May 8, 2008. PCT application no PCT/US2007/080331, filed October 3, 2007 also describes formulations that are amenable to the present invention.

Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g., they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add

surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

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

10 If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated
15 esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

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

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

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

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

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

A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of the siRNA composition, an alkali metal C₈ to C₂₂ alkyl sulphate, and a micelle forming compounds. Exemplary micelle forming compounds include

lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof. The micelle forming compounds may be added at the same time or after addition of the alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients but vigorous mixing in order to provide smaller size micelles.

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

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

For delivery of the micellar formulation as a spray, the formulation can be put into an aerosol dispenser and the dispenser is charged with a propellant. The propellant, which is under pressure, is in liquid form in the dispenser. The ratios of the ingredients are adjusted so that the aqueous and propellant phases become one, *i.e.*, there is one phase. If there are two phases, it is necessary to shake the dispenser prior to dispensing a portion of the contents, *e.g.*, through a metered valve. The dispensed dose of pharmaceutical agent is propelled from the metered valve in a fine spray.

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

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

B. Lipid particles

iRNAs, *e.g.*, dsRNAs of in the invention may be fully encapsulated in a lipid formulation, *e.g.*, a LNP, *e.g.*, to form a SPLP, pSPLP, SNALP, or other nucleic acid-lipid particle.

As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle, including SPLP. As used herein, the term "SPLP" refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle. SNALPs and SPLPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate).

SNALPs and SPLPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (*e.g.*, sites physically separated from the administration site). SPLPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No.

- 5 WO 00/03683. The particles of the present invention typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid-lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.*, U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 10 6,815,432; U.S. Publication No. 2010/0324120 and PCT Publication No. WO 96/40964.

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

- The cationic lipid can be, for example, N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleoyloxypropylamine 20 (DODMA), 1,2-Dilinoleoyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleoyloxy-3-(dimethylamino)acetoxyp propane (DLin-DAC), 1,2-Dilinoleoyl-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleoyloxy-3-dimethylaminopropane (DLin-2-DMA), 1,2-Dilinoleoyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleoyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleoyloxy-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediy)l)didodecan-2-ol (Tech G1), or a mixture 35 thereof. The cationic lipid can comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

In another embodiment, the compound 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane can be used to prepare lipid-siRNA nanoparticles. Synthesis of 2,2-Dilinoleyl-4-dimethylaminoethyl-

[1,3]-dioxolane is described in United States provisional patent application number 61/107,998 filed on October 23, 2008, which is herein incorporated by reference.

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

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

The conjugated lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkylxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl (C_{12}), a PEG-dimyristyloxypropyl (C_{14}), a PEG-dipalmitoxypropyl (C_{16}), or a PEG-distearoxypropyl (C_{18}). The conjugated lipid that prevents aggregation of particles can be from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

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

In one embodiment, the lipidoid ND98·4HCl (MW 1487) (see U.S. Patent Application No. 12/056,230, filed 3/26/2008, which is incorporated herein by reference), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid-dsRNA nanoparticles (*i.e.*, LNP01 particles).

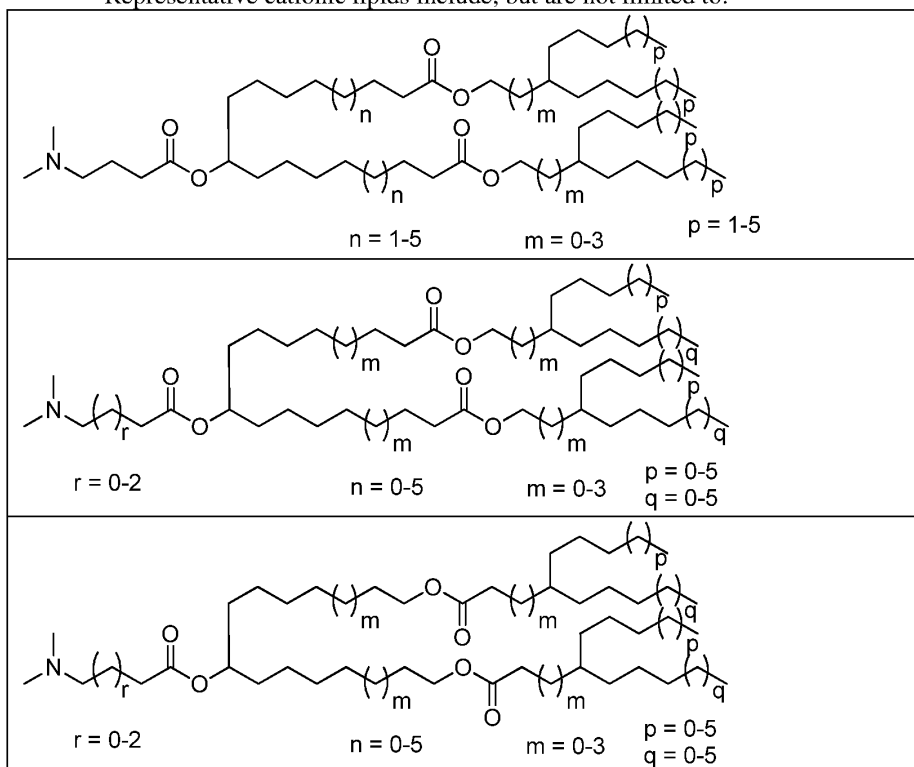
In certain embodiments of the invention, suitable cationic lipids suitable for use in the compositions of the invention are those described in U.S. Patent No. 9,061,063, and PCT Publication No. WO 2013/086354, the entire contents of each of which are incorporated herein by reference. In some embodiments, suitable cationic lipids include one or more biodegradable groups. The biodegradable group(s) include one or more bonds that may undergo bond breaking reactions in a biological environment, *e.g.*, in an organism, organ, tissue, cell, or organelle. Functional groups that contain a biodegradable bond include, for example, esters, dithiols, and oximes. Biodegradation can be a factor that influences the clearance of the compound from the body when administered to a subject. Biodegradation can be measured in a cell based assay, where a formulation including a cationic lipid is exposed to cells, and samples are taken at various time points. The lipid fractions can

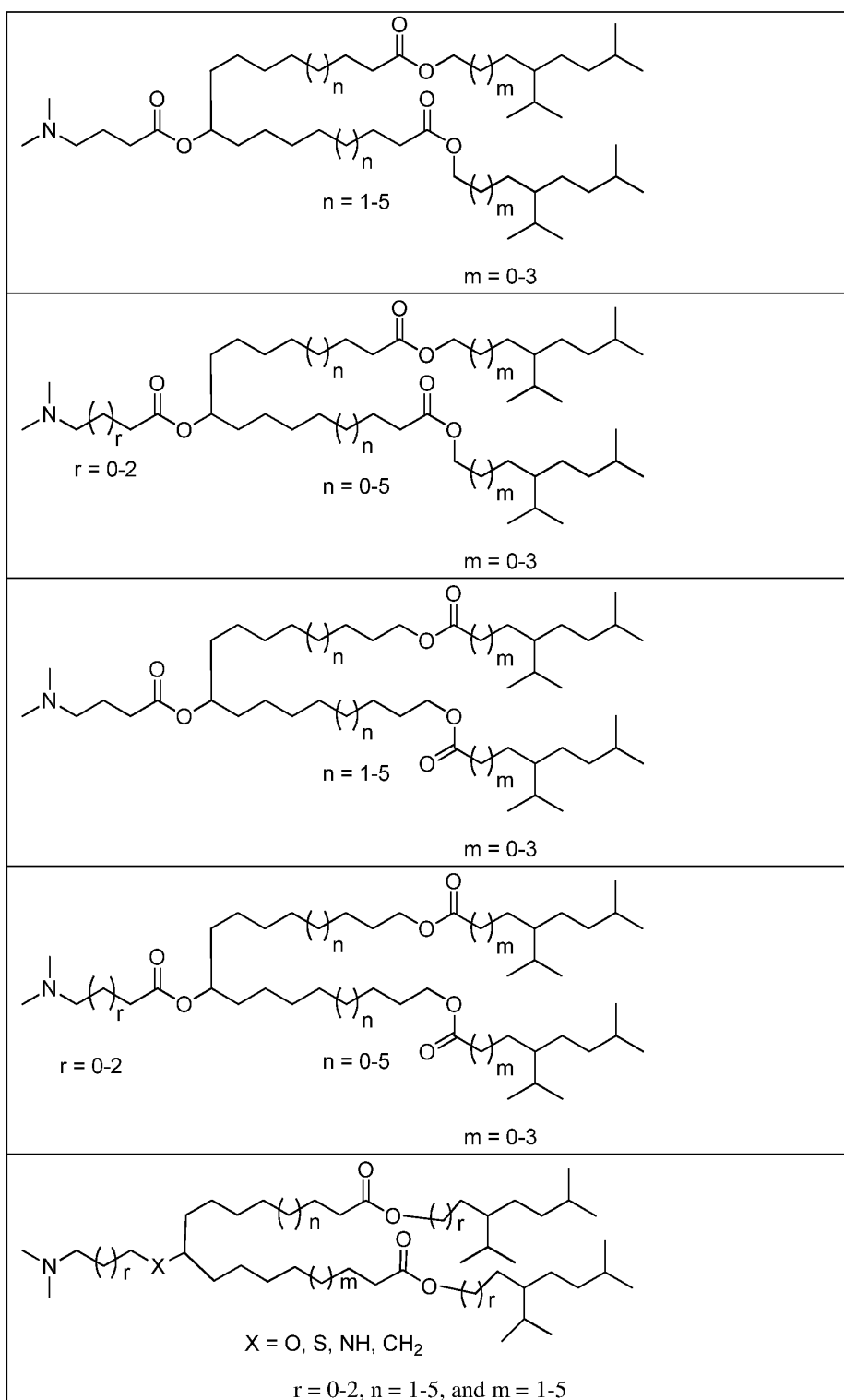
be extracted from the cells and separated and analyzed by LC-MS. From the LC-MS data, rates of biodegradation (e.g., as $t_{1/2}$ values) can be measured. the cationic lipid comprises a biodegradable group.

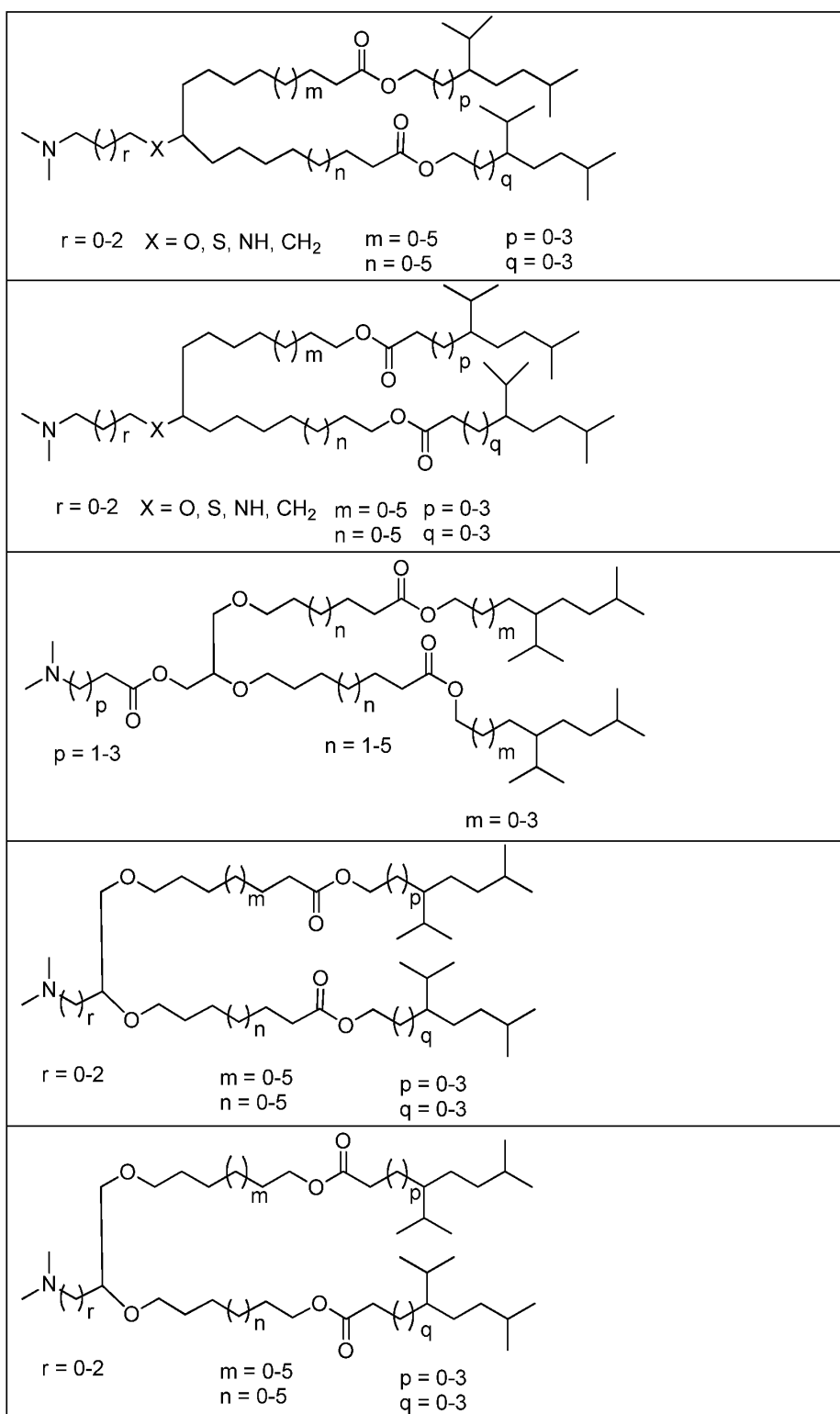
- In one embodiment, a cationic lipid of any of the embodiments described herein has an in vivo half life ($t_{1/2}$) (e.g., in the liver, spleen or plasma) of less than about 3 hours, such as less than about 2.5 hours, less than about 2 hours, less than about 1.5 hours, less than about 1 hour, less than about 0.5 hour or less than about 0.25 hours. The cationic lipid preferably remains intact, or has a half-life sufficient to form a stable lipid nanoparticle which effectively delivers the desired active pharmaceutical ingredient (e.g., a nucleic acid) to its target but thereafter rapidly degrades to minimize any side effects to the subject. For instance, in mice, the cationic lipid preferably has a $t_{1/2}$ in the spleen of from about 1 to about 7 hours.

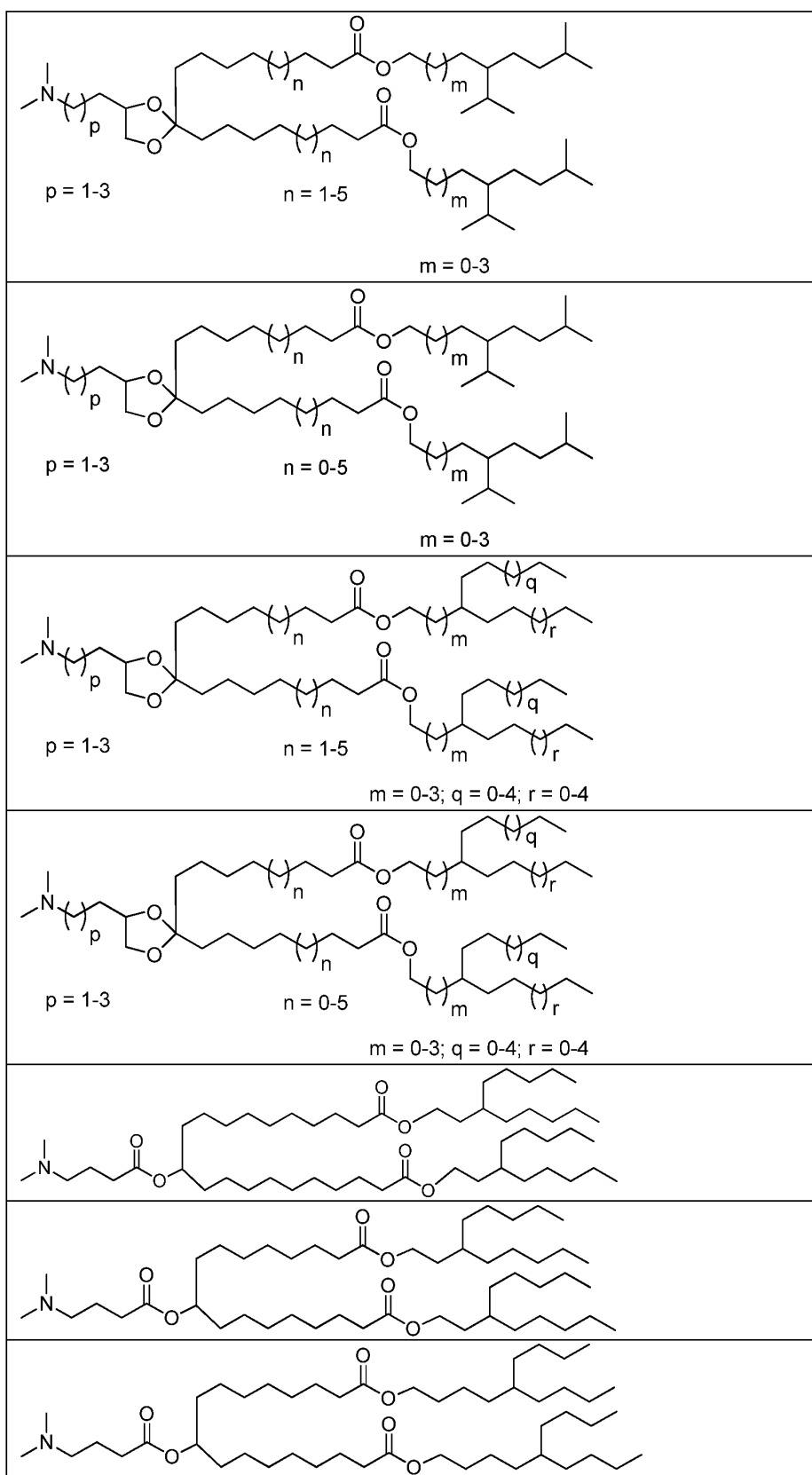
- In another embodiment, a cationic lipid of any of the embodiments described herein containing a biodegradable group or groups has an in vivo half life ($t_{1/2}$) (e.g., in the liver, spleen or plasma) of less than about 10% (e.g., less than about 7.5%, less than about 5%, less than about 2.5%) of that for the same cationic lipid without the biodegradable group or groups.

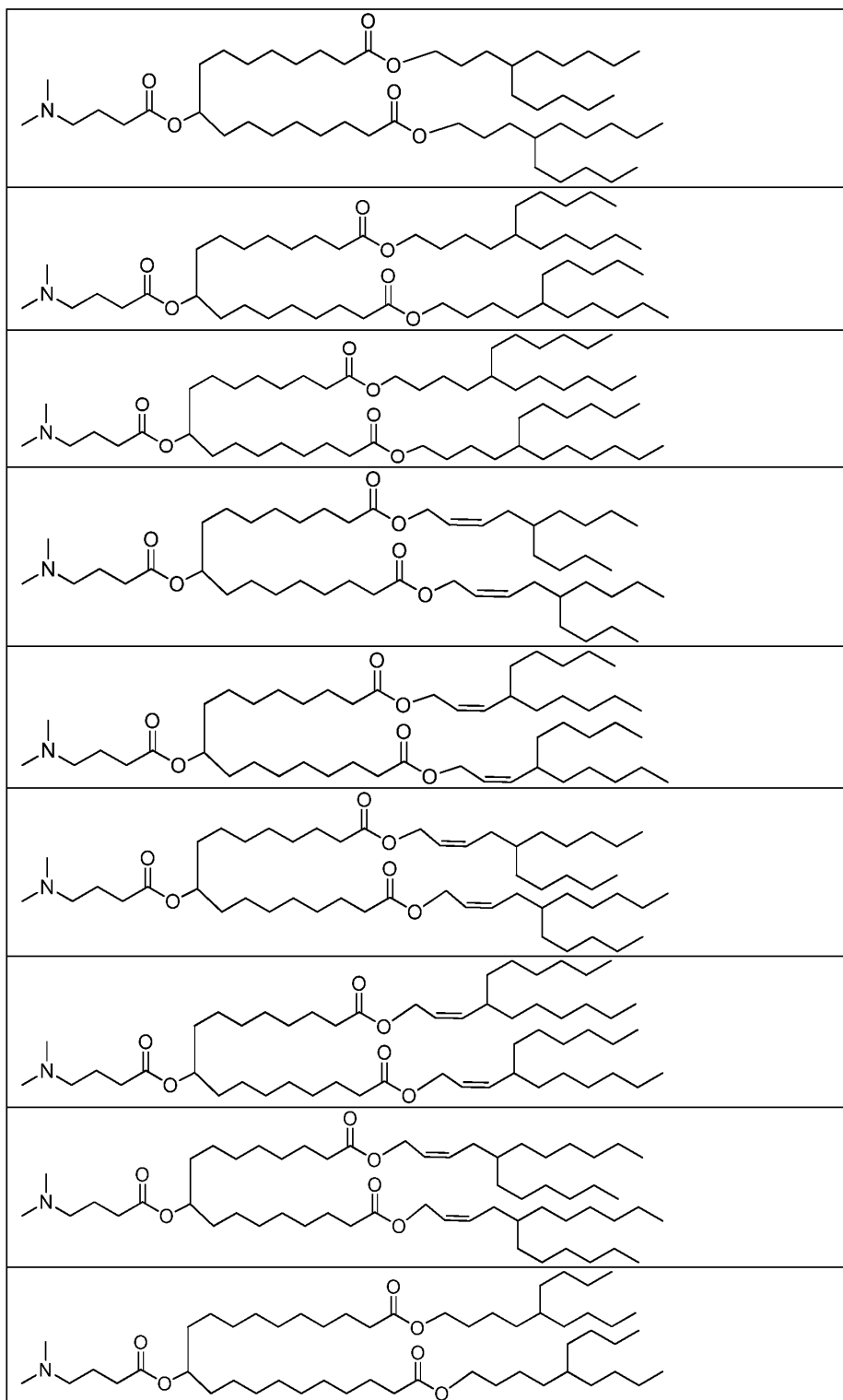
Representative cationic lipids include, but are not limited to:

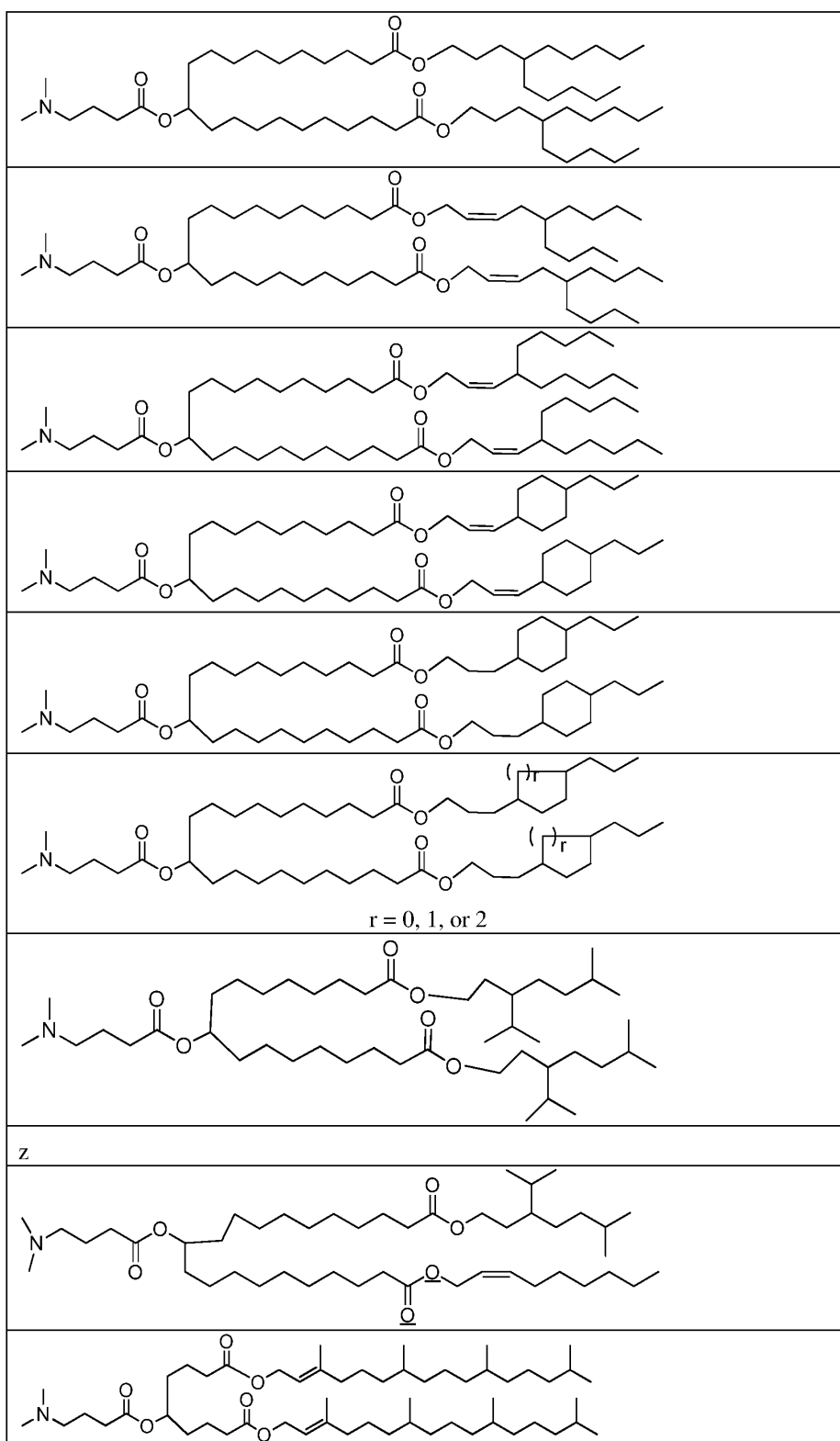


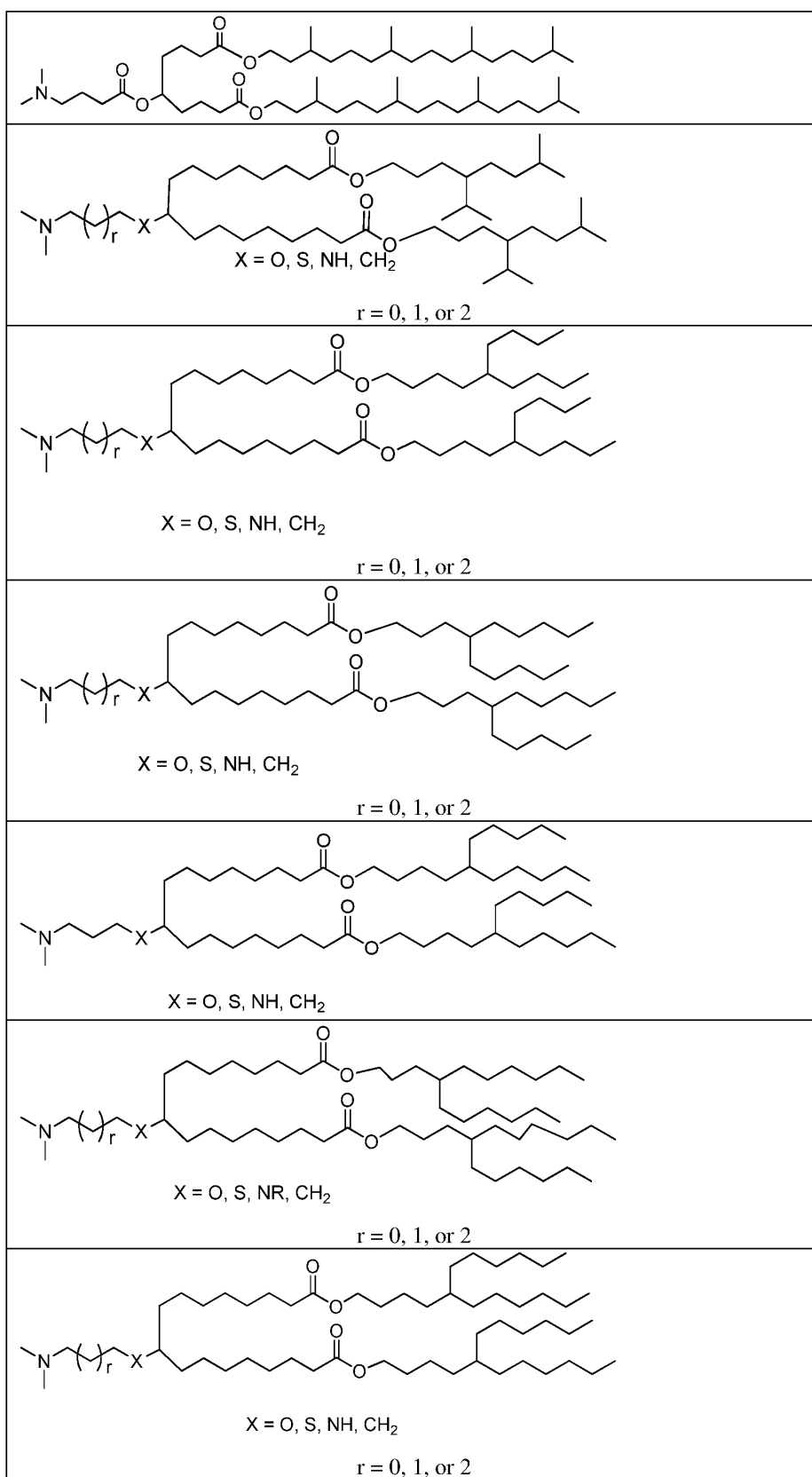


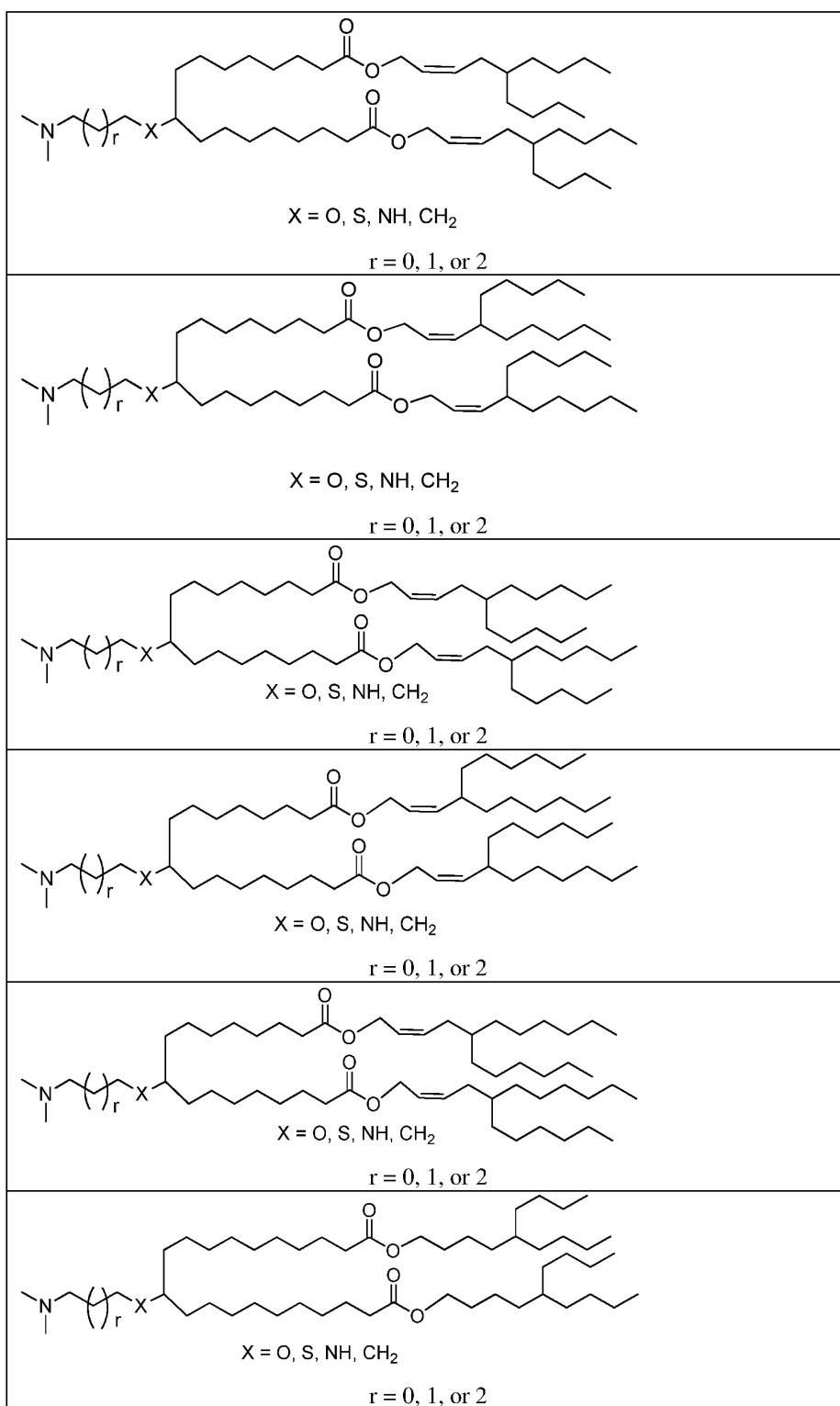


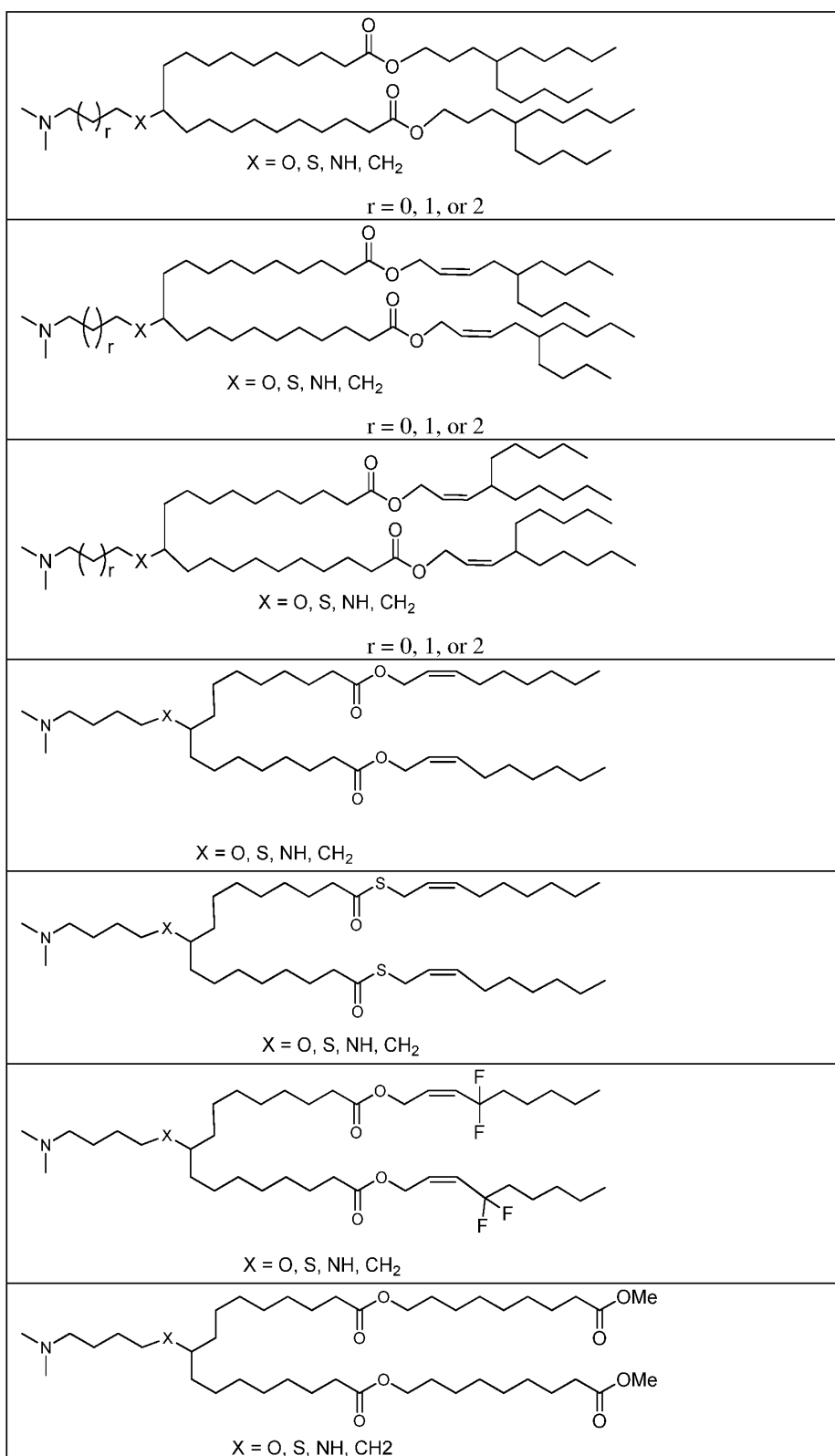


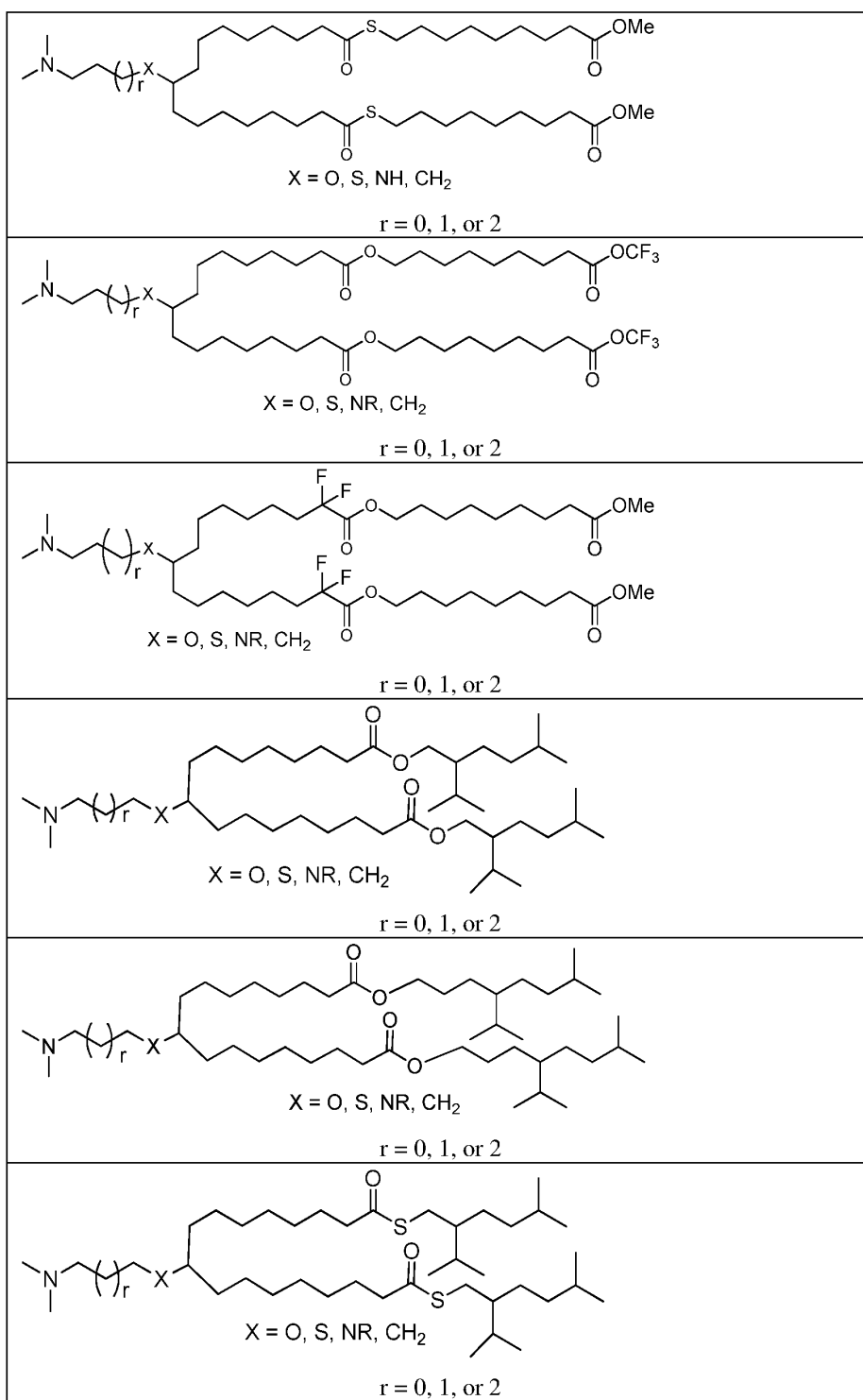


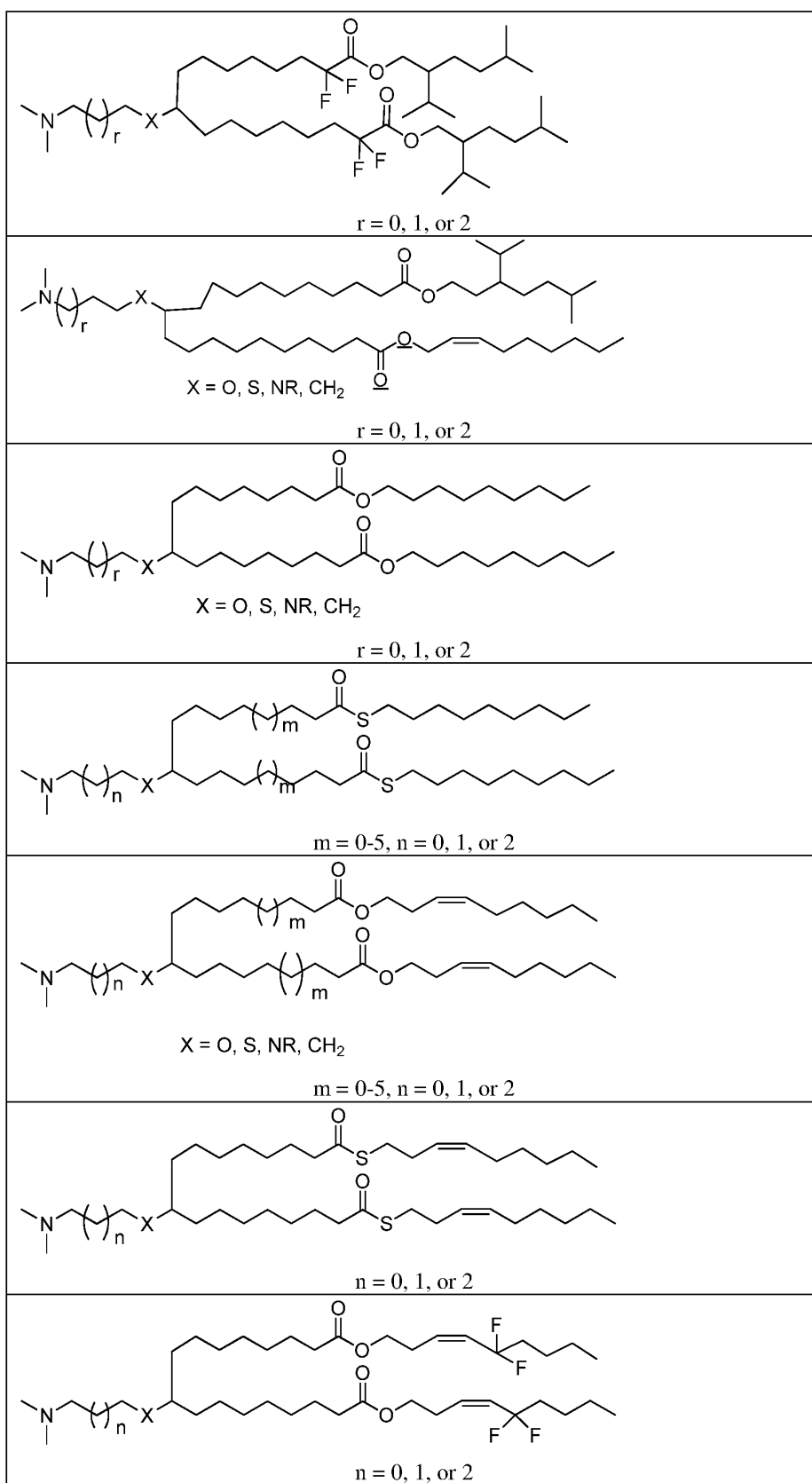


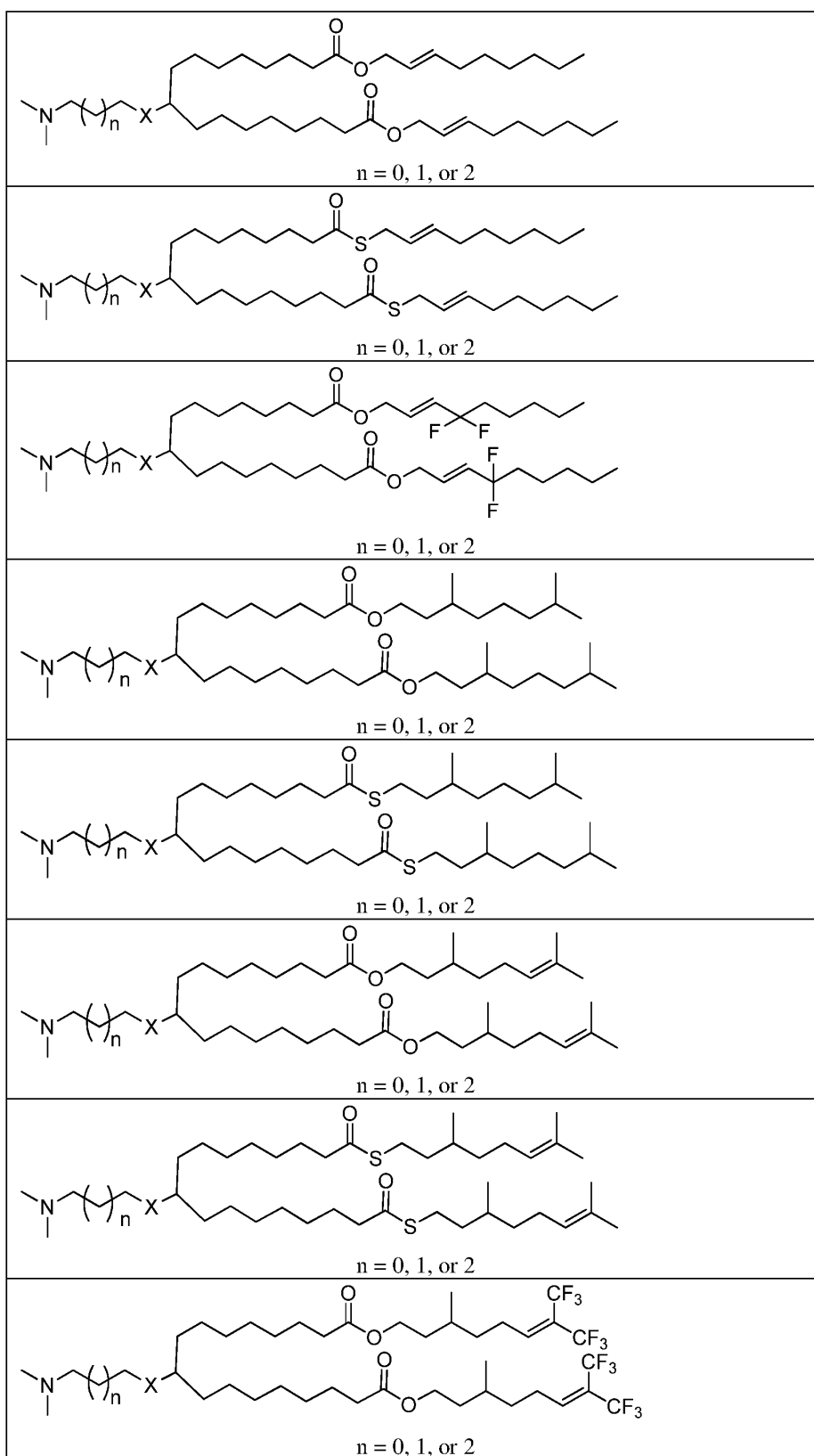




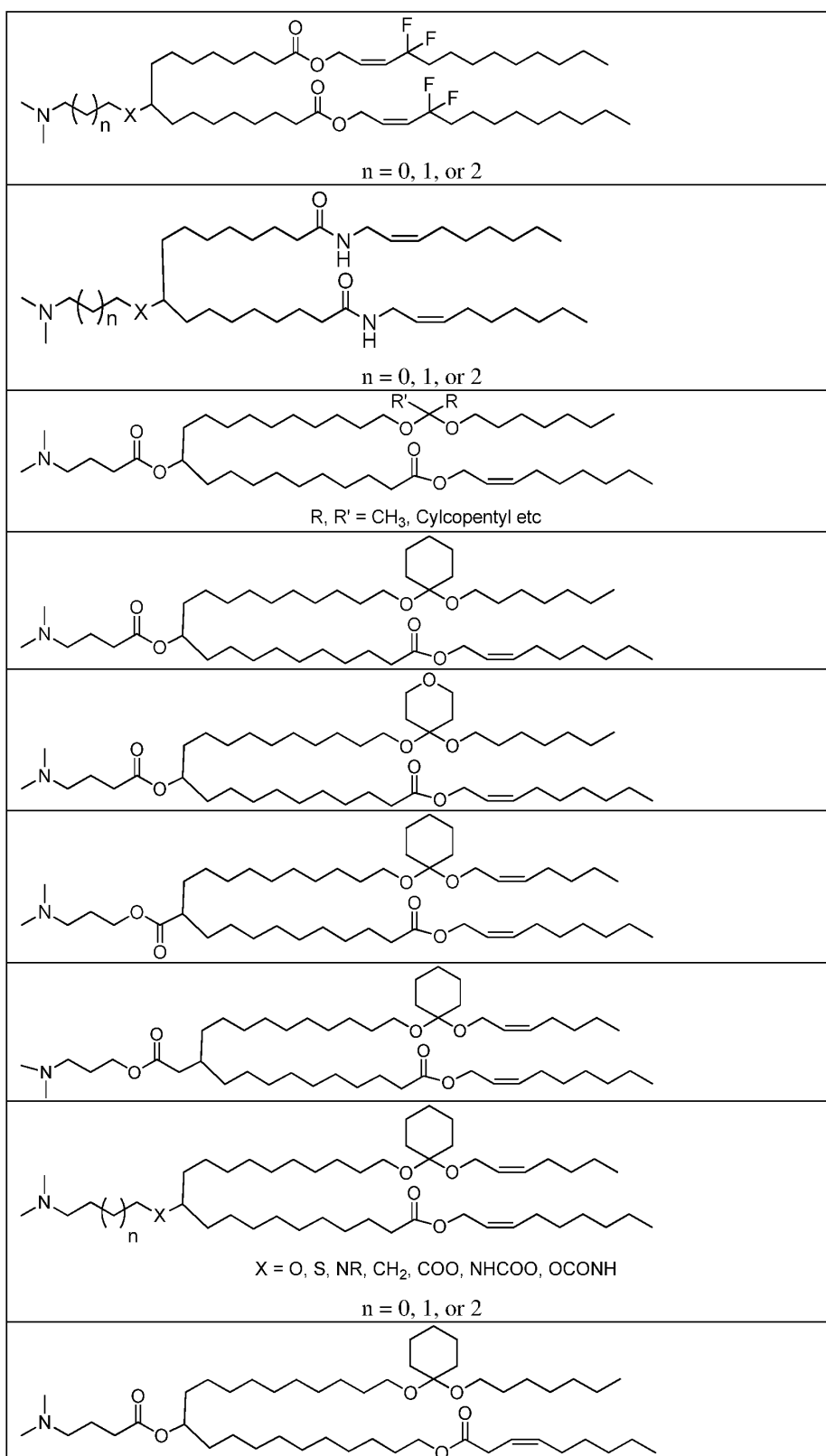




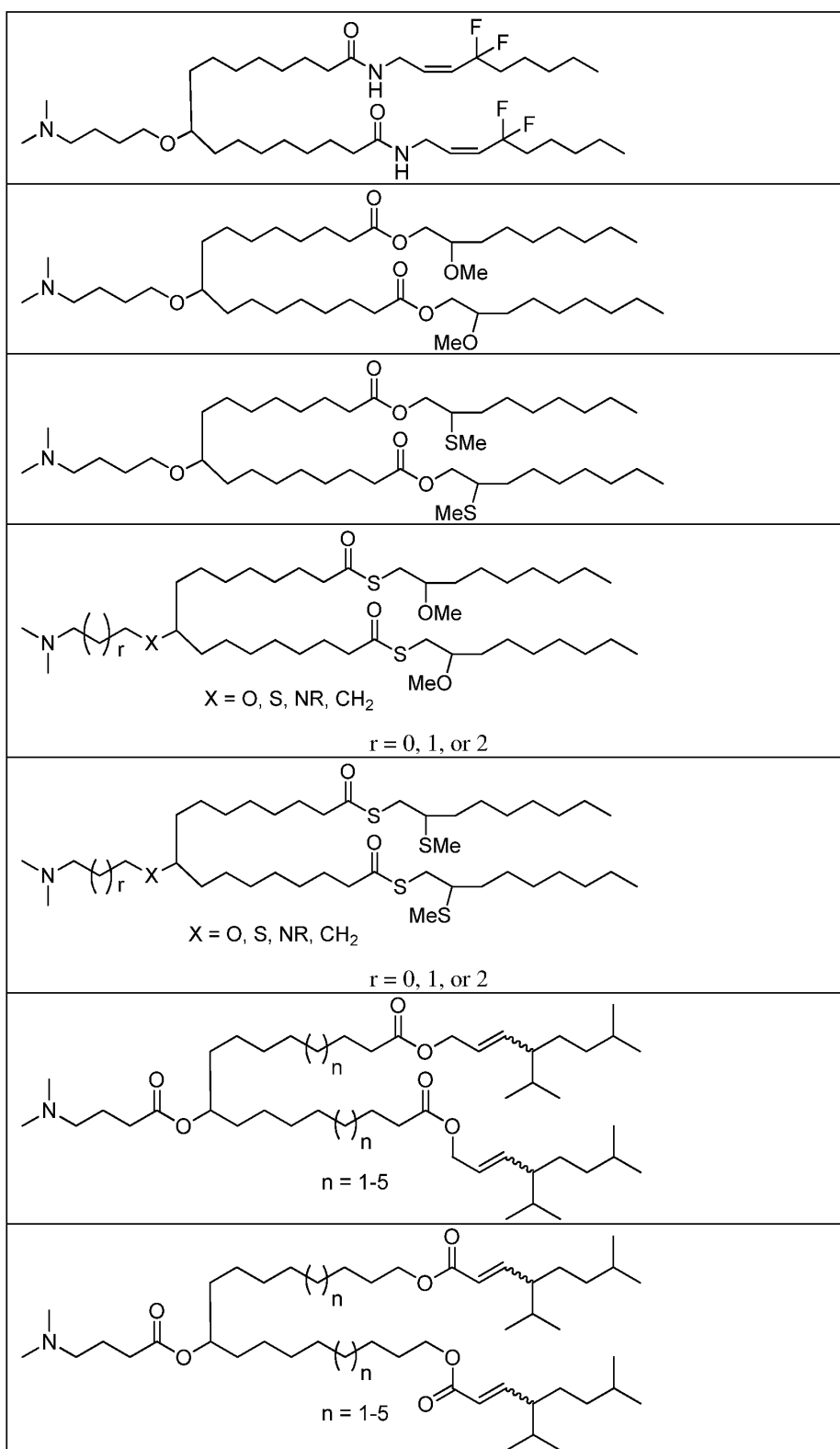


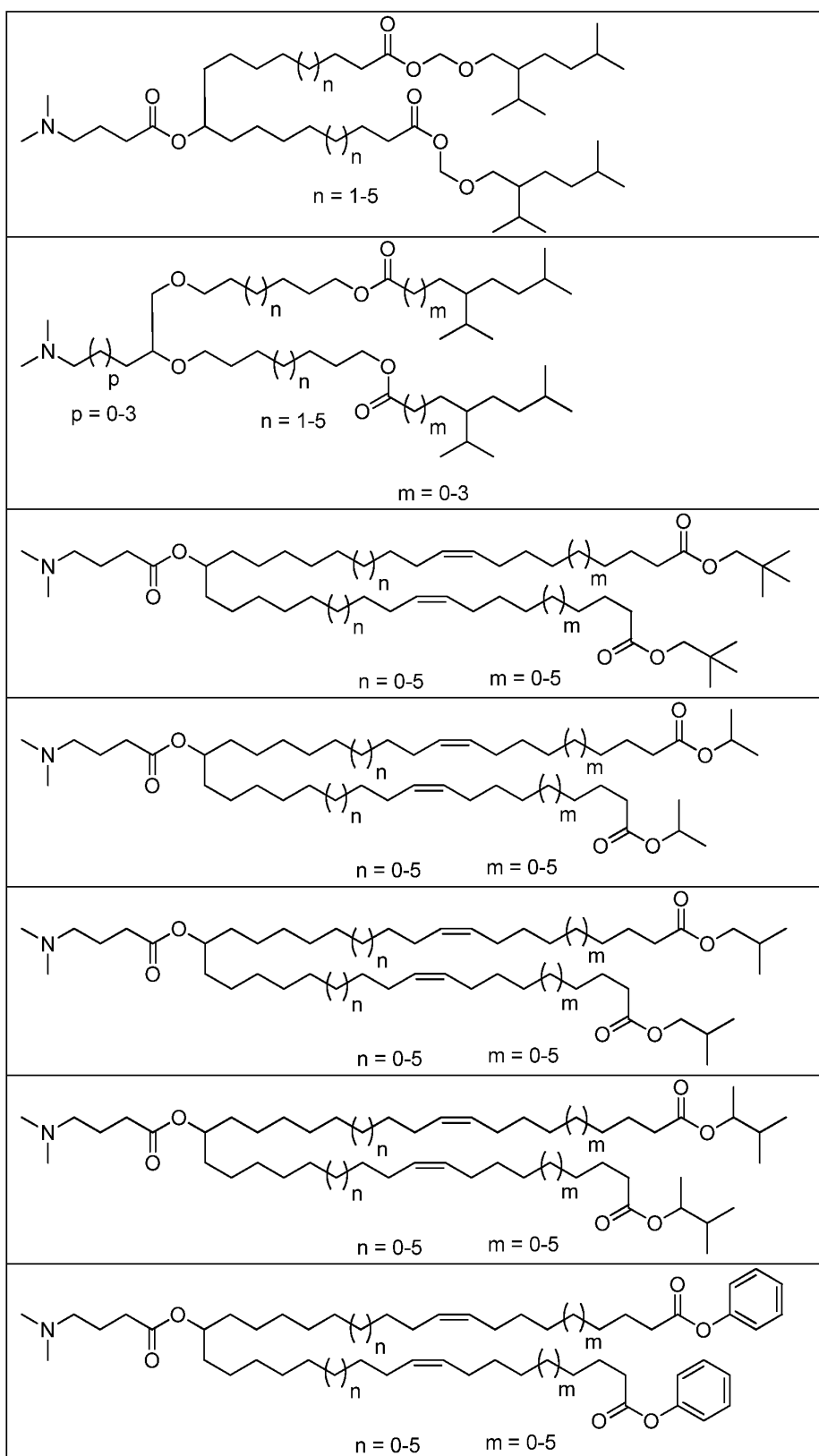


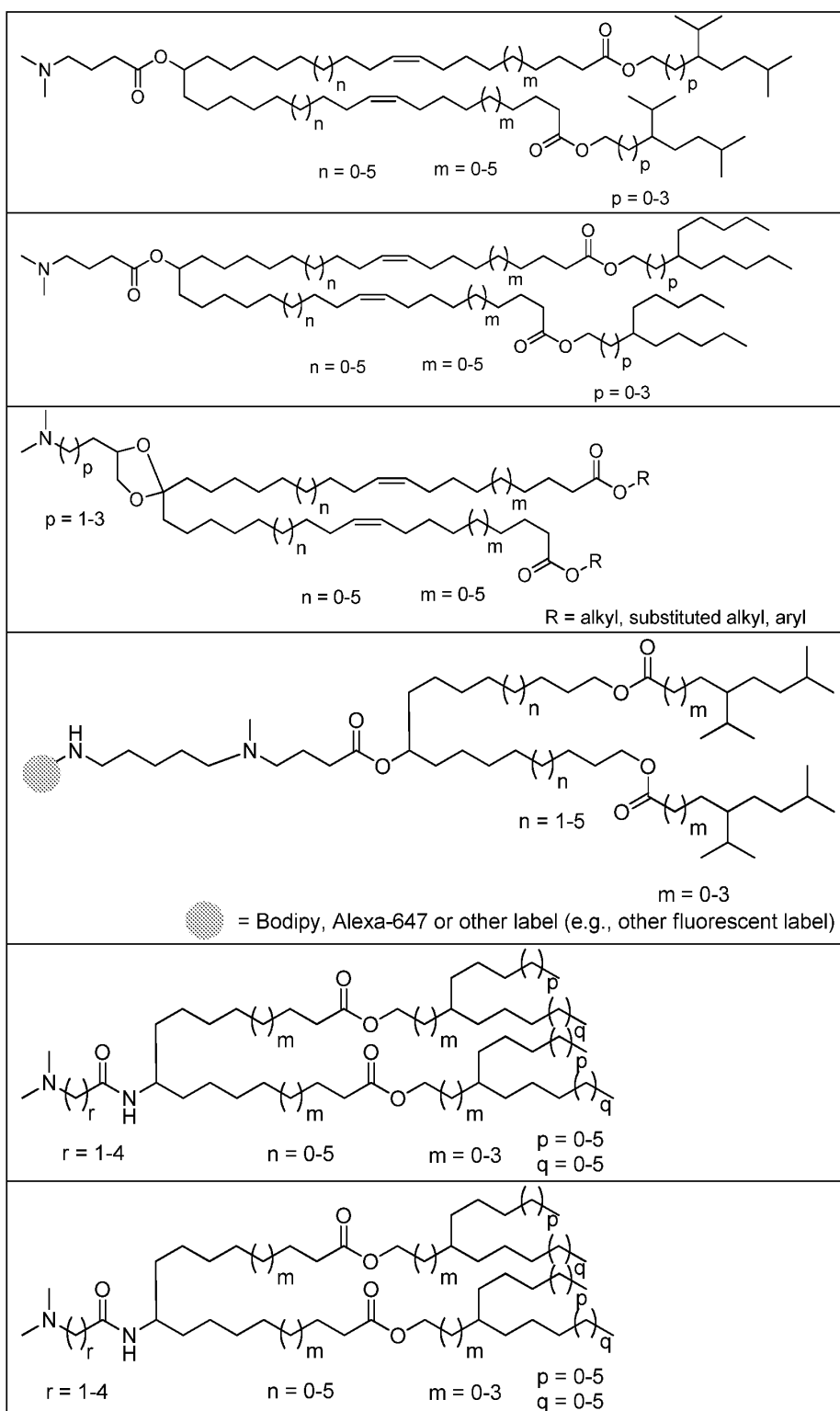


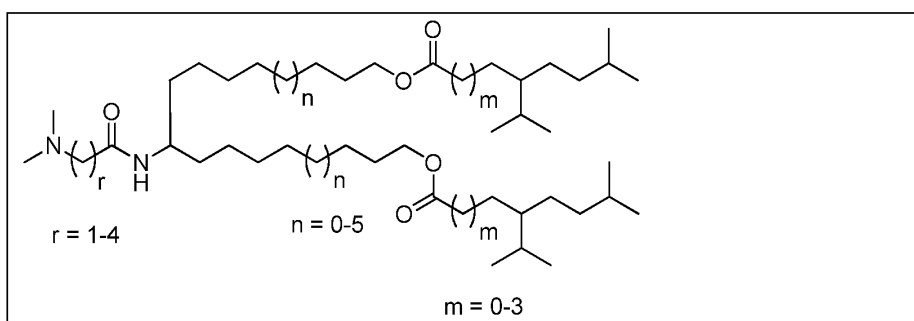




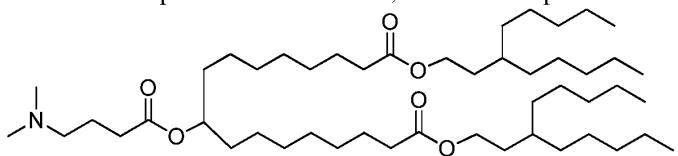








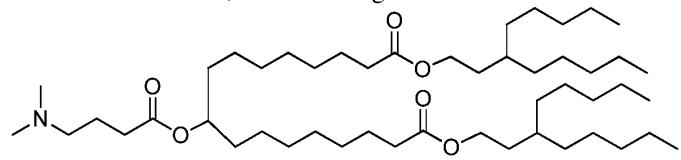
In one preferred embodiment, the cationic lipid is



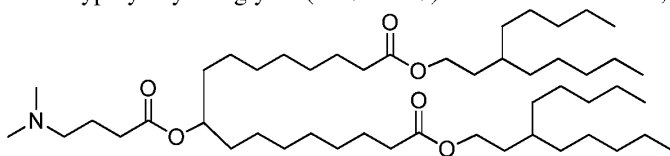
In certain embodiments, the dsRNA agents of the invention are formulated with a cationic

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lipid, *e.g.*,



distearoylphosphatidylcholine (DSPC), cholesterol (Chol), and 1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol (PEG-DMG). In one embodiment, the ratio of



:DSPC:Chol:PEG-DMG is about 50:12:36:2, respectively.

10

Included in the present invention is the free form of the cationic lipids described herein, as well as pharmaceutically acceptable salts and stereoisomers thereof. The cationic lipid can be a protonated salt of the amine cationic lipid. The term "free form" refers to the amine cationic lipids in non-salt form. The free form may be regenerated by treating the salt with a suitable dilute aqueous base solution such as dilute aqueous NaOH, potassium carbonate, ammonia and sodium bicarbonate. The pharmaceutically acceptable salts of the instant cationic lipids can be synthesized from the cationic lipids of this invention which contain a basic or acidic moiety by conventional chemical methods. Generally, the salts of the basic cationic lipids are prepared either by ion exchange chromatography or by reacting the free base with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents. Similarly, the salts of the acidic compounds are formed by reactions with the appropriate inorganic or organic base.

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Thus, pharmaceutically acceptable salts of the cationic lipids of this invention include non-toxic salts of the cationic lipids of this invention as formed by reacting a basic instant cationic lipids with an inorganic or organic acid. For example, non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like, as well as salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and trifluoroacetic (TFA).

When the cationic lipids of the present invention are acidic, suitable "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases including inorganic bases and organic bases. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, and zinc. In one embodiment, the base is selected from ammonium, calcium, magnesium, potassium and sodium. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, and tromethamine.

It will also be noted that the cationic lipids of the present invention may potentially be internal salts or zwitterions, since under physiological conditions a deprotonated acidic moiety in the compound, such as a carboxyl group, may be anionic, and this electronic charge might then be balanced off internally against the cationic charge of a protonated or alkylated basic moiety, such as a quaternary nitrogen atom.

C. 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, L.V., Popovich N.G., and Ansel H.C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al.*, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions

are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution 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.

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- 10 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
- 15 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
- 20 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.,
- 25 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
- 30 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
- 35 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.

- 5 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
- 10 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
- 15 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.

20 *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
- 25 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,
- 30 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
- 35 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 CTNNB13-associated disorder, *e.g.*, a cancer.

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, such as, 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 a CTNNB1-associated disorder, *e.g.*, cancer. 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 CTNNB1 Expression

The present invention also provides methods of inhibiting expression of a CTNNB1 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 CTNNB1 in the cell, thereby inhibiting expression of CTNNB1 in the cell. In some embodiments of the disclosure, expression of a CTNNB1 gene is inhibited preferentially in the liver (*e.g.*, hepatocytes).

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 some embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a GalNAc₃ 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 CTNNB1” is intended to refer to inhibition of expression of any CTNNB1 gene (such as, *e.g.*, a mouse CTNNB1 gene, a rat CTNNB1 gene, a monkey CTNNB1 gene, or a human CTNNB1 gene) as well as variants or mutants of a CTNNB1 gene. Thus, the CTNNB1 gene may be a wild-type CTNNB1 gene, a mutant CTNNB1 gene, or a transgenic CTNNB1 gene in the context of a genetically manipulated cell, group of cells, or organism.

“Inhibiting expression of a CTNNB1 gene” includes any level of inhibition of a CTNNB1 gene, *e.g.*, at least partial suppression of the expression of a CTNNB1 gene. The expression of the CTNNB1 gene may be assessed based on the level, or the change in the level, of any variable associated with CTNNB1 gene expression, *e.g.*, CTNNB1 mRNA level or CTNNB1 protein level. It is understood that CTNNB1 is expressed predominantly in the liver.

The expression of a CTNNB1 may also be assessed indirectly based on other variables associated with CTNNB1 gene expression, *e.g.*, level of beta-catenin expression in the cytoplasm, nuclear localization of beta-catenin, or expression of certain target genes such as Jun, c-Myc and CyclinD-1 or other oncogenes under transcription control of beta-catenin.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with CTNNB1 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 CTNNB1 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 some embodiments, expression of a CTNNB1 gene is inhibited by at least 70%. It is further understood that inhibition of CTNNB1 expression in certain tissues, *e.g.*, in liver, without a significant inhibition of expression in other tissues, *e.g.*, brain, may be desirable. In some 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.*, CTNNB1), *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 CTNNB1 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 CTNNB1 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 CTNNB1 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 some 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 CTNNB1 gene may be assessed in terms of a reduction of a parameter that is functionally linked to CTNNB1 gene expression, *e.g.*, CTNNB1 protein level in blood or serum from a subject. CTNNB1 gene silencing may be determined in any cell expressing CTNNB1, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of a CTNNB1 protein may be manifested by a reduction in the level of the CTNNB1 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 CTNNB1 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 CTNNB1 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 CTNNB1 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.*, mRNA of the CTNNB1 gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasyTM RNA preparation kits (Qiagen®) or PAXgeneTM (PreAnalytixTM, 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 CTNNB1 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 CTNNB1. 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 CTNNB1 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 CTNNB1 mRNA.

An alternative method for determining the level of expression of CTNNB1 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 CTNNB1 is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System). In some embodiments, expression level is determined by the method provided in Example 2 using, *e.g.*, a 10 nM siRNA concentration, in the species matched cell line.

The expression levels of CTNNB1 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 CTNNB1 expression level may also comprise using nucleic acid probes in solution.

In some 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 some 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 CTNNB1 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 CTNNB1 mRNA or protein level (*e.g.*, in a liver biopsy).

In some embodiments, the efficacy of the methods of the invention can be monitored by detecting or monitoring a reduction in tumor formation. Reducing tumor, as used herein, includes any decrease in the size, number, or severity of tumor, or to a prevention or reduction in the formation of tumor, within a tissue of a subject, as may be assessed *in vitro* or *in vivo* using any method known in the art.

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 CTNNB1 may be assessed using measurements of the level or change in the level of CTNNB1 mRNA or CTNNB1 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 CTNNB1, thereby preventing or treating a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma. 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 a CTNNB1 gene, *e.g.*, 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, CTNNB1 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 CTNNB1 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, intraocular (*e.g.*, periocular, conjunctival, subtenon, intracameral, intravitreal, 5 intraocular, anterior or posterior juxtасcleral, subretinal, subconjunctival, retrobulbar, or intracanalicular injection), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), 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 10 certain embodiments, the compositions are administered by intramuscular injection.

The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

In one aspect, the present invention also provides methods for inhibiting the expression of a 15 CTNNB1 gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets a CTNNB1 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the CTNNB1 gene, thereby inhibiting expression of the CTNNB1 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 20 Example 2. Reduction in protein production can be assessed by any methods known in the art, *e.g.* ELISA. In certain embodiments, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in the CTNNB1 gene or protein expression. In other embodiments, a blood sample serves as the subject sample for monitoring the reduction in the CTNNB1 protein expression.

The present invention further provides methods of treatment in a subject in need thereof, *e.g.*, 25 a subject diagnosed with a CTNNB1-associated disorder, such as cancer, *e.g.*, hepatocellular carcinoma.

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 CTNNB1 expression, in a prophylactically 30 effective amount of a dsRNA targeting a CTNNB1 gene or a pharmaceutical composition comprising a dsRNA targeting a CTNNB1 gene.

In one aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in CTNNB1 expression, *e.g.*, a CTNNB1-associated disorder, such as cancer, *e.g.*, hepatocellular carcinoma.

35 Treatment of a subject that would benefit from a reduction and/or inhibition of CTNNB1 gene expression includes therapeutic treatment (*e.g.*, a subject is having a cancer) and prophylactic treatment (*e.g.*, the subject is not having a cancer or a subject may be at risk of developing a cancer).

In some embodiments, the CTNNB1-associated disorder is cancer. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, myeloma and leukemia. In some

embodiments, the cancer comprises a solid tumor cancer. In other embodiments, the cancer comprises a blood based cancer, e.g., leukemia, lymphoma or myeloma. More particular nonlimiting examples of such cancers include squamous cell cancer, small-cell lung cancer, pituitary cancer, esophageal cancer, astrocytoma, soft tissue sarcoma, non-small cell lung cancer (including squamous cell non-small cell lung cancer), adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cell carcinoma, hepatocellular carcinoma, hepatoblastomas, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, brain cancer, endometrial cancer, testis cancer, cholangiocarcinoma, gallbladder carcinoma, gastric cancer, melanoma, and various types of head and neck cancer (including squamous cell carcinoma of the head and neck).

In some embodiments, the CTNNB1-associated disorder is hepatocellular carcinoma.

In some embodiments, the RNAi agent is administered to a subject in an amount effective to inhibit CTNNB1 expression in a cell within the subject. The amount effective to inhibit CTNNB1 expression in a cell within a subject may be assessed using methods discussed above, including methods that involve assessment of the inhibition of CTNNB1 mRNA, CTNNB1 protein, or related variables, such as tumor formation.

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 CTNNB1 gene expression are subjects susceptible to or diagnosed with a CTNNB1-associated disorder, such as cancer, e.g., hepatocellular carcinoma. In an embodiment, the method includes administering a composition featured herein such that expression of the target a CTNNB1 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.

In one embodiment, the iRNAs useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target CTNNB1 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 CTNNB1-associated disorder, e.g., cancer, e.g., hepatocellular carcinoma. Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 mg/kg.

In one embodiment, the iRNA is 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
5 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
10 composition thereof for treating a subject that would benefit from reduction and/or inhibition of CTNNB1 gene expression, *e.g.*, a subject having a CTNNB1-associated disorder, 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.

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

For example, in certain embodiments, an iRNA targeting CTNNB1 is administered in combination with, *e.g.*, an agent useful in treating a CTNNB1-associated disorder. Exemplary
20 additional therapeutics and treatments for treating a CTNNB1-associated disorder, *e.g.*, cancer, may include surgery, chemotherapy, radiation therapy, or the administration of one or more additional anti-cancer agents, such as a chemotherapeutic agent, a growth inhibitory agent, an anti-angiogenesis agent and/or a anti-neoplastic composition. Nonlimiting examples of anti-cancer agents, chemotherapeutic agents, growth inhibitory agents, anti-angiogenesis agents, and anti-neoplastic compositions that can
25 be used in combination with the iRNA of the present invention are as follows.

A “chemotherapeutic agent” is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include, but are not limited to, alkylating agents such as thiotepa and Cytosan® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and
30 methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin;
35 nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne

antibiotics (e.g., calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin,

5 authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, Adriamycin® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin,

10 puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens

15 such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as aminogluthethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as

20 maytansine and ansamitocins; mitoguanzone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine;

25 mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., Taxol® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), Abraxane® Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumburg, Illinois), and Taxotere® doxetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; Gemzar® gemcitabine; 6-thioguanine;

30 mercaptopurine; methotrexate; platinum analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; Navelbine® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as

35 retinoic acid; capecitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); inhibitors of PKC-alpha, Raf, H-Ras, EGFR (e.g., erlotinib (Tarceva®)) and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Further nonlimiting exemplary chemotherapeutic agents include anti-hormonal agents that act to regulate or inhibit hormone action on cancers such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including Nolvadex® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and

5 Fareston® toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, Megase® megestrol acetate, Aromasin® exemestane, formestanie, fadrozole, Rivisor® vorozole, Femara® letrozole, and Arimidex® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine

10 analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; ribozymes such as a VEGF expression inhibitor (e.g., Angiozyme® ribozyme) and a HER2 expression inhibitor; vaccines such as gene therapy vaccines, for example, Allovectin® vaccine, Leuvectin® vaccine, and Vaxid® vaccine; Proleukin® rIL-2; Lurtotecan® topoisomerase 1 inhibitor;

15 Abarelix® rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

In some embodiments, the iRNA of the invention may be further administered with gemcitabine-based chemotherapy in which one or more chemotherapy agents including gemcitabine or including gemcitabine and nab-paclitaxel are administered. In some such embodiments, the iRNA of the invention may be administered with at least one chemotherapy agent selected from

20 gemcitabine, nab-paclitaxel, leukovorin (folinic acid), 5-fluorouracil (5-FU), irinotecan, and oxaliplatin. FOLFIRINOX is a chemotherapy regime comprising leukovorin, 5-FU, irinotecan (such as liposomal irinotecan injection), and oxaliplatin. In some embodiments, the iRNA of the invention may be further administered with gemcitabine-based chemotherapy. In some embodiments, the iRNA of the invention may be further administered with at least one agent selected from (a) gemcitabine; (b)

25 gemcitabine and nab-paclitaxel; and (c) FOLFIRINOX. In some embodiments, the at least one agent is gemcitabine. In some such embodiments, the cancer to be treated is pancreatic cancer.

An “anti-angiogenesis agent” or “angiogenesis inhibitor” refers to a small molecular weight substance, a polynucleotide (including, e.g., an inhibitory RNA (RNAi or siRNA)), a polypeptide, an isolated protein, a recombinant protein, an antibody, or conjugates or fusion proteins thereof, that

30 inhibits angiogenesis, vasculogenesis, or undesirable vascular permeability, either directly or indirectly. It should be understood that the anti-angiogenesis agent includes those agents that bind and block the angiogenic activity of the angiogenic factor or its receptor. For example, an anti-angiogenesis agent is an antibody or other antagonist to an angiogenic agent, e.g., antibodies to VEGF-A (e.g., bevacizumab (Avastin®)) or to the VEGF-A receptor (e.g., KDR receptor or Flt-1

35 receptor), anti-PDGFR inhibitors such as Gleevec® (Imatinib Mesylate), small molecules that block VEGF receptor signaling (e.g., PTK787/ZK2284, SU6668, Sutent®/SU11248 (sunitinib malate), AMG706, or those described in, e.g., international patent application WO 2004/113304). Anti-angiogenesis agents also include native angiogenesis inhibitors, e.g., angiostatin, endostatin, etc. See, e.g., Klagsbrun and D’Amore (1991) *Annu. Rev. Physiol.* 53:217-39; Streit and Detmar (2003)

Oncogene 22:3172-3179 (e.g., Table 3 listing anti-angiogenic therapy in malignant melanoma); Ferrara & Alitalo (1999) Nature Medicine 5(12):1359-1364; Tonini et al. (2003) Oncogene 22:6549-6556 (e.g., Table 2 listing known anti-angiogenic factors); and, Sato (2003) Int. J. Clin. Oncol. 8:200-206 (e.g., Table 1 listing anti-angiogenic agents used in clinical trials).

5 A “growth inhibitory agent” as used herein refers to a compound or composition that inhibits growth of a cell (such as a cell expressing VEGF) either in vitro or in vivo. Thus, the growth inhibitory agent may be one that significantly reduces the percentage of cells (such as a cell expressing VEGF) in S phase. Examples of growth inhibitory agents include, but are not limited to, agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1
10 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn
15 and Israel, eds., The Molecular Basis of Cancer, Chapter 1, entitled “Cell cycle regulation, oncogenes, and antineoplastic drugs” by Murakami et al. (W.B. Saunders, Philadelphia, 1995), e.g., p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (Taxotere®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (Taxol®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of
20 microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

 The term “anti-neoplastic composition” refers to a composition useful in treating cancer comprising at least one active therapeutic agent. Examples of therapeutic agents include, but are not limited to, e.g., chemotherapeutic agents, growth inhibitory agents, cytotoxic agents, agents used in
25 radiation therapy, anti-angiogenesis agents, cancer immunotherapeutic agents, apoptotic agents, anti-tubulin agents, and other-agents to treat cancer, such as anti-HER-2 antibodies, anti-CD20 antibodies, an epidermal growth factor receptor (EGFR) antagonist (e.g., a tyrosine kinase inhibitor), HER1/EGFR inhibitor (e.g., erlotinib (Tarceva®), platelet derived growth factor inhibitors (e.g., Gleevec® (Imatinib Mesylate)), a COX-2 inhibitor (e.g., celecoxib), interferons, cytokines,
30 antagonists (e.g., neutralizing antibodies) that bind to one or more of the following targets ErbB2, ErbB3, ErbB4, PDGFR-beta, BlyS, APRIL, BCMA, or VEGF receptor(s), and other bioactive and organic chemical agents, etc. Combinations thereof are also included in the invention.

 In some embodiments, the iRNA targeting CTNNB1 is administered in combination with, e.g., an agent useful in treating hepatocellular carcinoma (HCC), for example, but not limited to,
35 sorafenib.

 The iRNA and additional therapeutic agents 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.

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.

5

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 CTNNB1 (*e.g.*, means for measuring the inhibition of CTNNB1 mRNA, CTNNB1 protein, and/or CTNNB1 activity). Such means for measuring the inhibition of CTNNB1 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.

30

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.

35

EXAMPLES

Example 1. iRNA SynthesisSource of reagents

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

siRNA Design

siRNAs targeting the human beta-catenin (CTNNB1) gene (human: NCBI refseqID
10 NM_001904.4, NCBI GeneID: 1499) were designed using custom R and Python scripts. The human NM_001904.4 REFSEQ mRNA, has a length of 3661 bases.

Detailed lists of the unmodified CTNNB1 sense and antisense strand nucleotide sequences are shown in Table 2. Detailed lists of the modified CTNNB1 sense and antisense strand nucleotide sequences are shown in Table 3.

15 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

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

Briefly, siRNA sequences were synthesized on a 1 μ mol scale using a Mermade 192 synthesizer (BioAutomation) with phosphoramidite chemistry on solid supports. The solid support was controlled pore glass (500-1000 Å) loaded with a custom GalNAc ligand (3'-GalNAc conjugates), universal solid support (AM Chemicals), or the first nucleotide of interest. Ancillary
25 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
30 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").

35 Upon completion of the solid phase synthesis, solid-supported oligoribonucleotides were treated with 300 μ L of Methylamine (40% aqueous) at room temperature in 96 well plates for approximately 2 hours to afford cleavage from the solid support and subsequent removal of all additional base-labile protecting groups. For sequences containing any natural ribonucleotide linkages (2'-OH) protected with a tert-butyl 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 μ L of dimethyl sulfoxide (DMSO) and 300 μ L TEA.3HF and the solution was incubated for approximately 30 mins at 60 °C. After incubation, the plate was allowed to come to room temperature and crude oligonucleotides were precipitated by the addition of 1 mL of 9:1 acetonitrile: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 μ M 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

Cell culture and 384-well transfections

Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO₂ 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 7.5 μ L of Opti-MEM plus 0.1 μ L of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA, cat # 13778-150) to 2.5 μ L of each siRNA duplex to an individual well in a 384-well plate. The mixture was then incubated at room temperature for 15 minutes. Forty μ L of complete growth media without antibiotic containing $\sim 1.5 \times 10^4$ cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10 nM, 1 nM, and 0.1 nM final duplex concentration.

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

Cells were lysed in 75 μ L of Lysis/Binding Buffer containing 3 μ L 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 90 μ L) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 10 μ L 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 H₂O 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.

5

Real time PCR

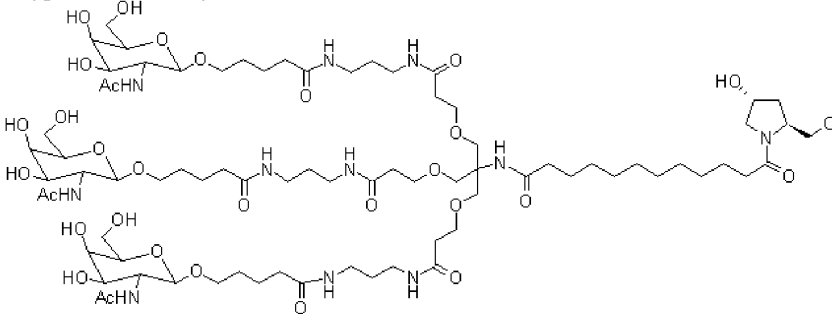
Two microlitre (µl) of cDNA were added to a master mix containing 0.5µl of human GAPDH TaqMan Probe (4326317E), 0.5µl human CTNNB1, 2µl nuclease-free water and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche).

To calculate relative fold change, data were analyzed using the $\Delta\Delta C_t$ method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC₅₀s 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 and antisense UCGAAGuACUcAGCGuAAGdTsdT.

The results of a single dose screen of the agents in Tables 2 and 3 in Hep3b cells are shown in Table 4.

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).

Abbreviation	Nucleotide(s)
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3'-phosphate
Abs	beta-L-adenosine-3'-phosphorothioate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
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
Gbs	beta-L-guanosine-3'-phosphorothioate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate

Abbreviation	Nucleotide(s)
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-tetrahydrofuran-4-methoxy-3-phosphate (abasic 2'-OMe furanose)
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofuran-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
dT	2'-deoxythymidine-3'-phosphate
dTs	2'-deoxythymidine-3'-phosphorothioate
dU	2'-deoxyuridine
dUs	2'-deoxyuridine-3'-phosphorothioate

Abbreviation	Nucleotide(s)
(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. Unmodified Sense and Antisense Strand Sequences of CTNNB1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1548365.1	GAGGGUAAUUUGAAGUAUACCA		164-184	UGGUAAUACUUCAAAUACCCUCAG		162-184
AD-1548366.1	GAGGGUAAUUUGAAGUAUACCA		164-184	UGGUAAUACUUCAAAUACCCUCAG		162-184
AD-1548367.1	GGGUAAUUUGAAGUAUACCAUA		166-186	UAUGGUAAUACUUCAAAUACCCUC		164-186
AD-1548368.1	GGUAAUUUGAAGUAUACCAUAA		167-187	UUAUGGTAAUACUUCAAAUACCCU		165-187
AD-1548369.1	UUUGAAGUAUACCAUACAACA		171-191	UGUUGUAUGGUAAUACUUCAAAUA		169-191
AD-1548370.1	UUGAAGUAUACCAUACAACUA		172-192	UAGUTGTAUGGUAAUACUUCAAAU		170-192
AD-1548371.1	UGGACAAUGGCUACUCAAGCA		209-229	UGCUTGAGUAGCCAUUGUCCACG		207-229
AD-1548372.1	GACAAUGGCUACUCAAGCUGA		211-231	UCAGCUTGAGUAGCCAUUGUCCA		209-231
AD-1548373.1	ACAAUGGCUACUCAAGCUGAA		212-232	UUCAGCTUGAGUAGCCAUUGUCC		210-232
AD-1548374.1	AAGCUGAAUUUGAUGGAGUUGA		225-245	UCAAACUCCAUCAAAUACAGCUUGA		223-245
AD-1548375.1	AGCUGAAUUUGAUGGAGUUGGA		226-246	UCCAACTCCAUCAAAUACAGCUUG		224-246
AD-1548376.1	CCUUCUCUGAGUGGUAAGGA		344-364	UCCUTUACCACTCAGAGAGGAG		342-364
AD-1548377.1	CAGUCCUUCACUCAAGAACA		428-448	UUGUTCTUGAGUGAAGGACUGAG		426-448
AD-1548378.1	CUUCACUCAAGAACAAGUAGA		433-453	UCUACUTGUUCTUGAGUGAAGGA		431-453
AD-1548379.1	CACUCAAGAACAAGUAGCUGA		436-456	UCAGCUACUUGTUCUUGAGUGAA		434-456
AD-1548380.1	GUAGCUGAAUUGAUGGACAA		449-469	UUGUCCAUCAAUACAGCUACUU		447-469
AD-1548381.1	AUUGAUGGACAGUAGUCAAUA		458-478	UAUUGCAUACUGUCCAUCAAUAU		456-478
AD-1548382.1	AGUAGCAAUGACUCGAGCUA		468-488	UAGCTCGAGUCAUUGCAUACUGU		466-488
AD-1548383.1	GGUACGAGCUGCUAUGUCCCA		493-513	UGGAACAUAGCAGCUCGUACCCU		491-513
AD-1548384.1	ACGAGCUGCUAUGUCCCUCA		496-516	UCAGGGAACAUAAGCAGCUCGUAC		494-516
AD-1548385.1	UGUCCCUUGAGACAUUAGUA		507-527	UAUCTAAUGUCTCAGGGAAACAUA		505-527
AD-1548386.1	UUCCCUUGAGACAUUAGUAGA		509-529	UUCATCTAAUGUCUCAGGGAAACA		507-529
AD-1548387.1	AGAUGAGGCGAUGCAGAUCCA		523-543	UGGATCTGCAUGCCCUCAUCUAA		521-543
AD-1548388.1	CUGCUCAUCCCAUAAUGUCA		561-581	UGACAUTAGUGGGAGUGAGCAGCA		559-581
AD-1548389.1	ACCAUCACAGAUGCUGAAACA		595-615	UGUUTCAGCAUCUGUGAUGGUUC		593-615
AD-1548390.1	CAUCACAGAUUGCUGAAACAUA		597-617	UAUGTUTCAGCAUCUGUGAUGGU		595-617
AD-1548391.1	CAUCACAGAUUGCUGAAACAUA		597-617	UAUGTUTCAGCAUCUGUGAUGGU		595-617
AD-1548392.1	AUGCUGAAACAUGCAGUUGUA		605-625	UACAACCTGCAUGUUUCAGCAUCU		603-625
AD-1548393.1	AUGCUGAAACAUGCAGUUGUA		605-625	UACAACCTGCAUGUUUCAGCAUCU		603-625
AD-1548394.1	ACAUGCAGUUGUAAACUUGAA		613-633	UUCAAGTUUACAACUCGCAUGUUU		611-633
AD-1548395.1	AUGCAGUUGUAAACUUGAUUA		615-635	UAAUCAAGUUUACAACUCGCAUGU		613-635

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1548396.1	AUGCAGUUGUAAACUUGAUUA		615-635	UAAUCAAGUUACAACUGCAUGU		613-635
AD-1548397.1	ACGUGCAAUCCUGAACUGAA		664-684	UUCAGUTCAGGGAUUGCACGUGU		662-684
AD-1548398.1	UGCAAUCCUGAACUGACAAA		667-687	UUUGTCAGUUCAGGGAUUGCACG		665-687
AD-1548399.1	GACCAGGUGGUGGUAAUAAA		704-724	UUUATUAAACCACCAUUGGUCCU		702-724
AD-1548400.1	GACCAGGUGGUGGUAAUAAA		704-724	UUUATUAAACCACCAUUGGUCCU		702-724
AD-1548401.1	CCAGGUGGUGGUAAUAAAGGA		706-726	UCCUTATUAAACCACCAUUGGUCCU		704-726
AD-1548402.1	GUUAAUAGGUCUGCAUUAUA		716-736	UAUAACAGCAGCCUUAUUAACCA		714-736
AD-1548403.1	CCUCAGAUUGGUGUCUGUAUA		788-808	UAUAGCAGACACCAUUGAGGAG		786-808
AD-1548404.1	UCAGAUUGGUGUCUGUAUUGA		790-810	UCAATAGCAGACACCAUUGAGG		788-810
AD-1548405.1	AGAUGGUGUCUGUAUUGUA		792-812	UUACAATAGCAGACACCAUUGA		790-812
AD-1548406.1	AGAUGGUGUCUGUAUUGUA		792-812	UTACAATAGCAGACACCAUUGA		790-812
AD-1548407.1	ACGUACCAUGCAGAAUACAAA		811-831	UUUGTATUCUGCAUUGGUACGUAC		809-831
AD-1548408.1	ACGUACCAUGCAGAAUACAAA		811-831	UTUGTATUCUGCAUUGGUACGUAC		809-831
AD-1548409.1	CGUACCAUGCAGAAUACAAA		812-832	UUUUGUAUUCUGCAUUGGUACGUAC		810-832
AD-1548410.1	GUACCAUGCAGAAUACAAAUA		813-833	UAUUTGTAUUCUGCAUUGGUACGU		811-833
AD-1548411.1	UACCAUGCAGAAUACAAAUGA		814-834	UCAUTUGUAUUCUGCAUUGGUACG		812-834
AD-1548412.1	ACCAUGCAGAAUACAAAUGAA		815-835	UTCATUTGUAUUCUGCAUUGGUAC		813-835
AD-1548413.1	CCAUGCAGAAUACAAAUGAUA		816-836	UAUCAUTUGUAUUCUGCAUUGGU		814-836
AD-1548414.1	CAUGCAGAAUACAAAUGAUA		817-837	UCAUCATUUGUAUUCUGCAUUGGU		815-837
AD-1548415.1	AUGCAGAAUACAAAUGAUGUA		818-838	UACATCAUUUGUAUUCUGCAUUGG		816-838
AD-1548416.1	GCAGAAUACAAUUGAUGUA		820-840	UCUACATCAUUTGUUUCUGCAU		818-840
AD-1548417.1	CAGAAUACAAUUGAUGAUA		821-841	UUCUACAUCAUUUGUAUUCUGCA		819-841
AD-1548418.1	UGAUGUAGAAACAGCUCGUUA		832-852	UAACGAGCUGUTUUCUACAUCAU		830-852
AD-1548419.1	GAUGUAGAAACAGCUCGUUGA		833-853	UCAACGAGCUGTUUUCUACAUCAU		831-853
AD-1548420.1	ACUGGCCAUUUUAAGUCUGA		898-918	UCAGACTUAAAAGAUUGGCCAGUAA		896-918
AD-1548421.1	CUGGCCAUUUUAAGUCUGGA		899-919	UCCAGACUUAAAAGAUUGGCCAGUA		897-919
AD-1548422.1	UGGCCAUUUUAAGUCUGGAA		900-920	UCCAGACUUAAAAGAUUGGCCAGU		898-920
AD-1548423.1	GGCCAUCUUUAAGUCUGGAGA		901-921	UCUCCAGACUUAAAAGAUUGGCCAG		899-921
AD-1548424.1	GCCAUCUUUAAGUCUGGAGGA		902-922	UCCUCCAGACUTAAAAGAUUGGCCA		900-922
AD-1548425.1	UGGUUACACAGUGGAUUCUGA		946-966	UCAGAATCCACTGGUGAACCAAG		944-966
AD-1548426.1	ACCAGUGGAUUCUGUGUUGUA		952-972	UACAACACAGAAUCCACUGGUGA		950-972
AD-1548427.1	AUGGCAGUGCGUUUAGCUGGA		1025-1045	UCCAGCTAAACGCACUGCCAUUU		1023-1045

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1548428.1	AUGGCAGUGGCUUAGCUGGA		1025-1045	UCCAGCTAAACGCACUGCCAUUU		1023-1045
AD-1548429.1	UUGCCUUGCUCACAAACAA		1062-1082	UUGUTUTGUUGAGCAAGGCAACC		1060-1082
AD-1548430.1	UGCCUUGCUCACAAACAA		1063-1083	UTUGTUTGUUGAGCAAGGCAAC		1061-1083
AD-1548431.1	UGCCUUGCUCACAAACAA		1063-1083	UUUGTUTGUUGAGCAAGGCAAC		1061-1083
AD-1548432.1	GCCUUGCUCACAAACAA		1064-1084	UUUGTUTGUUGAGCAAGGCAAC		1062-1084
AD-1548433.1	UUAAAUUCUUGGCUAUUACGA		1086-1106	UCGUAATAGCCAAAGAAUUUAA		1084-1106
AD-1548434.1	AUUCUUGGCUAUUACGACAGA		1090-1110	UCUGTCGUAAUAGCCAAAGAAUUU		1088-1110
AD-1548435.1	GGCUAUUACGACAGACUGCCA		1096-1116	UGGCAGTCUGUGCUAAUAGCCAA		1094-1116
AD-1548436.1	UACGACAGACUGCCUUCAAA		1102-1122	UUUUGAAGGCAGUCUGUCGUAUU		1100-1122
AD-1548437.1	CUUAGUAUAUAAUAGAGGA		1182-1202	UCCUCATUAUATUACUAAAGCU		1180-1202
AD-1548438.1	UAUAAUGAGGACCUAUACUUA		1192-1212	UAAGTATAGGUCCUCAAUUAUU		1190-1212
AD-1548439.1	AGCUGGUGGAAUGCAAGCUUA		1291-1311	UAAGCUTGCAUCCACCAGCUUC		1289-1311
AD-1548440.1	CAGAUCCAAGUCAACGUCUUA		1326-1346	UAAGACGUUGACUUGGAUCUGUC		1324-1346
AD-1548441.1	CCAAGUCAACGUCUUGUUCAA		1331-1351	UUGAACAAAGACGUUGACUUGGAU		1329-1351
AD-1548442.1	CCAAGUCAACGUCUUGUUCAA		1331-1351	UTGAACAAGAGCGUUGACUUGGAU		1329-1351
AD-1548443.1	AGAACUGUCUUGGACUCUCA		1350-1370	UGAGAGTCCAAAGACAGUUCUGA		1348-1370
AD-1548444.1	CUUUCAGAUUCUGCAACUAAA		1376-1396	UUUAGUTGCAGCAUCUGAAAGAU		1374-1396
AD-1548445.1	UCAGAUUCUGCAACUAAACAA		1379-1399	UUUGTUAGUUGCAGCAUCUGAAA		1377-1399
AD-1548446.1	GAUGCUGCAACUAAACAGGAA		1382-1402	UUCCTGTUUAAGUUGCAGCAUCUG		1380-1402
AD-1548447.1	CUGCAACUAAACAGGAAGGGA		1386-1406	UCCCTUCCUGUTUAGUUGCAGCA		1384-1406
AD-1548448.1	GAAGGGAUGGAAGGUCUCCUA		1400-1420	UAGGAGACCUUCCAUCCCUCCU		1398-1420
AD-1548449.1	CUUCUGGCUUCAGAUUAUA		1436-1456	UAUATCAUCUGAACCCAGAAGCU		1434-1456
AD-1548450.1	CCUGUGCAGCUGGAAUUCUUA		1467-1487	UAAGAATUCCAGCUGCACAGGUG		1465-1487
AD-1548451.1	UGUGCAGCUGGAAUUCUUA		1469-1489	UGAAAGAAUCCAGCUGCACAGG		1467-1489
AD-1548452.1	AUUCUUUUAACCCUCACUUA		1480-1500	UAAGTGAGGUUAGAAAGAAUCC		1478-1500
AD-1548453.1	UUCUUUAACCCUCACUUGCA		1482-1502	UGCAAGTGAGGTUAGAAAGAAU		1480-1502
AD-1548454.1	CUUUCUAAACCCUCACUUGCA		1484-1504	UUUGCAAGUGAGGUUAGAAAGAA		1482-1504
AD-1548455.1	UUUCUAAACCCUCACUUGCAUUA		1485-1505	UAUUGCAAGUGAGGUUAGAAAGA		1483-1505
AD-1548456.1	CUAACCUCACUUGCAUUAUA		1488-1508	UAUUAUTGCAAGUGAGGUUAGAA		1486-1508
AD-1548457.1	UAACCUCACUUGCAUUAUA		1489-1509	UAUTATUGCAAGUGAGGUUAGA		1487-1509
AD-1548458.1	AACCUCACUUGCAUUAUA		1490-1510	UTAATUAUUGCAAGUGAGGUUAG		1488-1510
AD-1548459.1	ACCUCACUUGCAUUAUA		1491-1511	UAUAAUTAUUGCAAGUGAGGUUA		1489-1511

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1548460.1	CCUCACUUGCAAUAAUUAUAA		1492-1512	UTAUAAUUAUUGCAAGUGAGGUU		1490-1512
AD-1548461.1	CUCACUUGCAAUAAUUAUAA		1493-1513	UUUATAUUUAUUGCAAGUGAGGU		1491-1513
AD-1548462.1	CUCACUUGCAAUAAUUAUAA		1493-1513	UTUATAUUUAUTGCAAGUGAGGU		1491-1513
AD-1548463.1	UCACUUGCAAUAAUUAUAA		1494-1514	UCUUAAUUUAUUGCAAGUGAGG		1492-1514
AD-1548464.1	CACUUGCAAUAAUUAUAA		1495-1515	UTCUTATAUUUAUUGCAAGUGAG		1493-1515
AD-1548465.1	CAAGUGGGUGUAUAGAGGCA		1532-1552	UGCCTCTAUACCCACCCAUUGGC		1530-1552
AD-1548466.1	GCUCUUGGCGUACUGUCCUA		1550-1570	UAGGACAGUACGCACAAGAGCCU		1548-1570
AD-1548467.1	CCUGCCAUUCUGUCUUCGUA		1601-1621	UCGAAGAGCACACAGUGGCAGGU		1599-1621
AD-1548468.1	CUGCCAUUCUGUCUUCGUA		1602-1622	UACGAAGAGCACAGUAGGCAGGC		1600-1622
AD-1548469.1	UGCCAUUCUGUCUUCGUA		1603-1623	UGACGAAGAGCACAGUAGGCAGG		1601-1623
AD-1548470.1	GCCAUUCUGUCUUCGUA		1604-1624	UTGACGAAGAGCACAGUAGGCAG		1602-1624
AD-1548471.1	CCAUUCUGUCUUCGUA		1605-1625	UAUGACGAAGAGCACAGUAGGCA		1603-1625
AD-1548472.1	AUCUGUCUCUUCGUAUCUA		1607-1627	UAGATGACGAAGAGCACAGAUUG		1605-1627
AD-1548473.1	UCUGUCUCUUCGUAUCUA		1608-1628	UCAGAUGACGAAGAGCACAGAU		1606-1628
AD-1548474.1	CUGUCUCUUCGUAUCUGAA		1609-1629	UUCAGATGACGAAGAGCACAGAU		1607-1629
AD-1548475.1	UGUGUCUUCGUAUCUGACA		1610-1630	UGUCAGUAGACGAAGAGCACAGA		1608-1630
AD-1548476.1	UGGACUACCAAGUUGGUUAA		1681-1701	UUAAACCACAACUGGUAGUCCAUA		1679-1701
AD-1548477.1	GACUACCAAGUUGGUUAA		1683-1703	UCUUAACCACAACUGGUAGUCCA		1681-1703
AD-1548478.1	UGUGGUUAAAGCUCUACACCA		1693-1713	UGGUGUAAAGAGCUUAAACCACAAC		1691-1713
AD-1548479.1	GAUAAAGGCUACUGUUGGAUA		1732-1752	UAUCCAACAGUAGCCUUUAUCAG		1730-1752
AD-1548480.1	AUAAAGGCUACUGUUGGAUUA		1733-1753	UAUCCAACAGUAGCCUUUAUCA		1731-1753
AD-1548481.1	AUAAAGGCUACUGUUGGAUUA		1733-1753	UAUCCAACAGTAGCCUUUAUCA		1731-1753
AD-1548482.1	AAGGCUACUGUUGGAUUGAUA		1736-1756	UAUCAATCCAACAGUAGCCUUUA		1734-1756
AD-1548483.1	AAGGCUACUGUUGGAUUGAUA		1736-1756	UAUCAATCCAACAGUAGCCUUUA		1734-1756
AD-1548484.1	AGGCUACUGUUGGAUUGAUA		1737-1757	UAUCAAUCCAACAGUAGCCUUU		1735-1757
AD-1548485.1	GGCUACUGUUGGAUUGAUA		1738-1758	UGAATCAAUCCAACAGUAGCCUU		1736-1758
AD-1548486.1	GCUACUGUUGGAUUGAUA		1739-1759	UCGAUCAAUCCAACAGUAGCCU		1737-1759
AD-1548487.1	CUACUGUUGGAUUGAUAUCGAA		1740-1760	UTCGAATCAAUCCAACAGUAGCC		1738-1760
AD-1548488.1	UACUGUUGGAUUGAUAUCGAA		1741-1761	UTUCGAUUAATCCAACAGUAGC		1739-1761
AD-1548489.1	ACUGUUGGAUUGAUAUCGAAA		1742-1762	UTUUCGAAUCAAUCCAACAGUAG		1740-1762
AD-1548490.1	CUGUUGGAUUGAUAUCGAAA		1743-1763	UAUUTCGAUUAUCAAUCCAACAGUA		1741-1763
AD-1548491.1	GUUGGAUUGAUAUCGAAAUCUA		1745-1765	UAGATUTCGAATCAAUCCAACAG		1743-1765

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1548492.1	GUUGGAUUUGAUUCGAAAUUCUA		1745-1765	UAGATUTCGAAUCAAUCCAAACAG		1743-1765
AD-1548493.1	UUGGAUUUGAUUCGAAAUUCUA		1746-1766	UAGAUAUUCGAAUCAAUCCAAACA		1744-1766
AD-1548494.1	GGAUUGAUUCGAAAUUCUUGCA		1748-1768	UGCAAGAUUUCGAAUCAAUCCAA		1746-1768
AD-1548495.1	GGAUUGAUUCGAAAUUCUUGCA		1748-1768	UGCAAGAUUUCGAAUCAAUCCAA		1746-1768
AD-1548496.1	UUGAUUCGAAAUUCUUGCCUA		1751-1771	UAGGCAAGAUUUUCGAAUCAAUC		1749-1771
AD-1548497.1	AUCCACGACUAGUUCAGUUA		1811-1831	UAACCTGAACUAGUCGUGGAAUGG		1809-1831
AD-1548498.1	CCACGACUAGUUCAGUUGCUA		1814-1834	UAGCAACUGAACUAGUCGUGGAA		1812-1834
AD-1548499.1	UUCAGUUGCUUUGUUCGUGCA		1824-1844	UTGCACGAAACAAACUACUGAACU		1822-1844
AD-1548500.1	GGACACAGCAGCAAUUUGUGA		1878-1898	UCACAAAUUGCTGUGUGUCCCA		1876-1898
AD-1548501.1	AUCCUAGCUCGGGAUUGUCAA		1949-1969	UUGAACAUCCCGAGCUAGGAUGU		1947-1969
AD-1548502.1	AGCUCGGGAUUGUUCACAACCA		1954-1974	UGGUTGTGAACAUCGCCGAGCUAG		1952-1974
AD-1548503.1	GAUGUUCACAACCCGAAUUGUA		1961-1981	UACAATCGGUTGTUGAACAUCC		1959-1981
AD-1548504.1	UAUCAGAGGACUAAAUACCAA		1981-2001	UUGGTATUUAAGUCCUCUGAUAA		1979-2001
AD-1548505.1	AUCAGAGGACUAAAUACCAA		1982-2002	UAUGGUUUUAAGUCCUCUGAUAA		1980-2002
AD-1548506.1	UCAGAGGACUAAAUACCAA		1983-2003	UAUUGTAUUUAAGUCCUCUGAU		1981-2003
AD-1548507.1	GAGGACUAAAUACCAUCCAA		1986-2006	UUGGAATGGUAUUUAAGUCCUCUG		1984-2006
AD-1548508.1	GAGGACUAAAUACCAUCCAA		1986-2006	UTGGAATGGUAUUUAAGUCCUCUG		1984-2006
AD-1548509.1	GGACUAAAUACCAUCCAA		1988-2008	UAUUGGAUUGGTAUUUAAGUCCUC		1986-2008
AD-1548510.1	ACUAAAUACCAUCCAAUUGUA		1990-2010	UACAAGGAAUGGUAUUUAAGUCC		1988-2010
AD-1548511.1	CUAAAUACCAUCCAAUUGUA		1991-2011	UAACAATGGAATGGUAUUUAAGUC		1989-2011
AD-1548512.1	UAAAUACCAUCCAAUUGUA		1992-2012	UAAACAUGGAAUGGUAUUUAAGU		1990-2012
AD-1548513.1	AAAUACCAUCCAAUUGUA		1993-2013	UCAAACAUGGAAUGGUAUUUAAG		1991-2013
AD-1548514.1	AAUACCAUCCAAUUGUA		1994-2014	UACAACAUGGAAUGGUAUUUA		1992-2014
AD-1548515.1	AUACCAUCCAAUUGUA		1995-2015	UCACAACAUGGAAUGGUAUUU		1993-2015
AD-1548515.2	AUACCAUCCAAUUGUA		1995-2015	UCACAACAUGGAAUGGUAUUU		1993-2015
AD-1548516.1	UACCAUCCAAUUGUA		1996-2016	UGCACAACAATGGAAUGGUAUU		1994-2016
AD-1548517.1	ACCAUCCAAUUGUA		1997-2017	UTGCACAACAUGGAAUGGUAU		1995-2017
AD-1548518.1	ACCAUCCAAUUGUA		1997-2017	UUGCACAACAUGGAAUGGUAU		1995-2017
AD-1548519.1	CCAUCCAAUUGUA		1998-2018	UCUGCACAACAUGGAAUGGUA		1996-2018
AD-1548520.1	CAUCCAAUUGUA		1999-2019	UGCUGCACAACAUGGAAUGGUA		1997-2019
AD-1548521.1	UCCAUGUAUUGUA		2002-2022	UGCAGCTGCACAACAUGGAAU		2000-2022
AD-1548522.1	GUUUGGCGAGCUGCUUUAUUA		2008-2028	UAUAAGGCGAGCUGCACAACA		2006-2028

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1548523.1	CUCCUCGACAGAGUUAUCUUA		2127-2147	UAGTAACUCUGACAGAGGAGCU		2125-2147
AD-1548524.1	GACAGAGUUACUUCACUCUAA		2134-2154	UUAGAGTGAAGUAAACUCUGUCAG		2132-2154
AD-1548525.1	AGUUACUUCACUCUAGGAUUA		2139-2159	UAUUCCTAGAGUGAAGUAAACUCU		2137-2159
AD-1548526.1	GAGGACAAAGCCACAAGAUUAA		2204-2224	UUAATCTUGUGGCUUGUCCUCAG		2202-2224
AD-1548527.1	CACAAGAUUACAAGAAACGGGA		2214-2234	UCCGTCTCUUGTAAUCUUGUGGC		2212-2234
AD-1548528.1	ACAAGAUUACAAGAAACGGCA		2215-2235	UGCCGTUCUUGUAAUCUUGUGG		2213-2235
AD-1548529.1	CAAGAUUACAAGAAACGGCUA		2216-2236	UAGCCGTUUCUTGUAAUCUUGUG		2214-2236
AD-1548530.1	AAGAUUACAAGAAACGGCUUA		2217-2237	UAGCCGUUUUCTUGUAAUCUUGU		2215-2237
AD-1548531.1	UUACAAGAAACGGCUUUCAGA		2221-2241	UCUGAAAGCCGTUUCUUGUAAUC		2219-2241
AD-1548532.1	UACAAGAAACGGCUUUCAGUA		2222-2242	UACUGAAAGCCGUUUCUUGUAAU		2220-2242
AD-1548533.1	AAACGGCUUUCAGUUGAGCUA		2228-2248	UAGTCAACUCUGAAAGCCGUUUCU		2226-2248
AD-1548534.1	GCUUUCAGUUGAGCUGACCAA		2233-2253	UUGGTCAGCUCAAACUGAAAGCCG		2231-2253
AD-1548535.1	CUUGGACUUGAUUUUGGUGCA		2300-2320	UGCACCAUAUCAAGUCCAAGAU		2298-2320
AD-1548536.1	UAUCGCCAGGAUGAUCCUAGA		2339-2359	UCUAGGAUCAUCCUGGCGAUUUC		2337-2359
AD-1548537.1	CACCACCCUGGUGGUGACUAA		2438-2458	UUAGTCAGCACCAGGGUGGUGGC		2436-2458
AD-1548538.1	AUCAGCUGGCCUGGUUUGAUA		2529-2549	UAUCAAAACAGGCCAGCUGAUUG		2527-2549
AD-1548539.1	UCAGCUGGCCUGGUUUGAUA		2530-2550	UUUCAAAACAGGCCAGCUGAUU		2528-2550
AD-1548540.1	UCAGCUGGCCUGGUUUGAUA		2530-2550	UTAUCAAAACAGGCCAGCUGAUU		2528-2550
AD-1548541.1	CUGGCCUGGUUUGAUACUGAA		2534-2554	UUCAGUAUCAAAACAGGCCAGCU		2532-2554
AD-1548542.1	CUGUAAAUCAUCCUUUAGGUA		2555-2575	UACCTAAAGGATGAUUUACAGGU		2553-2575
AD-1548543.1	UGUAAAUCAUCCUUUAGGUA		2556-2576	UTACCUAAAGGAUGAUUUACAGG		2554-2576
AD-1548544.1	AAAGACUUUGGUUGGAGGGUA		2634-2654	UACCCUACCAACCAAGUCUUUCU		2632-2654
AD-1548545.1	ACUUUGAAAGGAGAUUCUUA		2701-2721	UAAGACAUCUCCUUUCAAAAGUAU		2699-2721
AD-1548546.1	ACUUUGAAAGGAGAUUCUUA		2701-2721	UAAGACAUCUCCUUUCAAAAGUAU		2699-2721
AD-1548547.1	UUCUCAGAUUUCUGGUUGUUA		2735-2755	UACAACCCAGAAAUUCUGAGAAAC		2733-2755
AD-1548548.1	UCUCAGAUUUCUGGUUGUUA		2736-2756	UUAACAACCCAGAAAUUCUGAGAAC		2734-2756
AD-1548549.1	UCUCAGAUUUCUGGUUGUUA		2736-2756	UTAAACAACCCAGAAAUUCUGAGAAC		2734-2756
AD-1548550.1	CUCAGAUUUCUGGUUGUUAUA		2737-2757	UAUAACAACCCAGAAAUUCUGAGAA		2735-2757
AD-1548551.1	GUUGUUAUGUGAUCAUGUGUA		2749-2769	UACACATGAUCACAUAAACAACCA		2747-2769
AD-1548552.1	UUGUUAUGUGAUCAUGUGUGA		2750-2770	UCACACAUGAUCACAUAAACAACC		2748-2770
AD-1548553.1	GUGAUCAUGUGUGGAAGUUAA		2757-2777	UUAACUTCCACACAUGAUCACAU		2755-2777
AD-1548554.1	GAUCAUGUGUGGAAGUUUAUA		2759-2779	UAAUAACUUCCACACAUGAUCAC		2757-2779

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1548555.1	AUCAUGUGGGAAGUUAUUAA		2760-2780	UTAAATAACUCCACACAUGAUA		2758-2780
AD-1548556.1	UGCAACUUAUACUCAAUUGA		2806-2826	UCAUTUGAGUATUAGUUGCAAA		2804-2826
AD-1548557.1	CCUUUCUCUUUAUACAGCA		2859-2879	UGCUGUAUAAAGAGAGAAAGGCU		2857-2879
AD-1548558.1	UUUAUACAGCUGUAUUGUCUA		2869-2889	UAGACAUAACAGCUGUAUAAAGA		2867-2889
AD-1548559.1	UUUAUACAGCUGUAUUGUCUA		2869-2889	UAGACAUAACAGCUGUAUAAAGA		2867-2889
AD-1548560.1	AUUGUCUGAAAUUGCAUUGUA		2882-2902	UACAAUGCAAAGTUCAGACAAUAC		2880-2902
AD-1548561.1	UUGGCCUGUAGAGUUGCUGAA		2904-2924	UUCAGCAAACUCUACAGGCCAAUC		2902-2924
AD-1548562.1	AGUGCCUGACACACUAAACCAA		2956-2976	UUGGTUAGUGUGUCAGGCACUUU		2954-2976
AD-1548563.1	CUGACACACUAAACCAAGCUGA		2961-2981	UCAGCUTGGUUAAGUGUGUACGGC		2959-2981
AD-1548564.1	UAAACCAAGCUGAGUUUCCUAA		2970-2990	UTAGGAAACUCAGCUUGGUUAGU		2968-2990
AD-1548565.1	AGCUGAGUUUCCUUAUUGGAAA		2976-2996	UUUCCCAUAGGAAACUCAGCUUG		2974-2996
AD-1548566.1	UUUCCUUAUGGGAACAAUUGAA		2983-3003	UTCAAUTGUUCCCAUAGGAAACU		2981-3003
AD-1548567.1	GUCGAGGAGUAAACAAUACAAA		3031-3051	UUUGTATUGUUUACUCCUCGACCA		3029-3051
AD-1548568.1	GUCGAGGAGUAAACAAUACAAA		3031-3051	UTUGTATUGUUUACUCCUCGACCA		3029-3051
AD-1548569.1	AUCAAACCCUAGCCUUGCUUA		3114-3134	UAGCAAGGCUAGGUUUUGAUAA		3112-3134
AD-1548570.1	UUUGCUUGCUUUUGAAGUAGCA		3183-3203	UGCUACTUCAAAAGCAAGCAAAAGU		3181-3203
AD-1548571.1	UGCUUGCUUUUGAAGUAGCUCA		3185-3205	UGAGCUACUUCAAAGCAAGCAAA		3183-3205
AD-1548572.1	GCUUGCUUUUGAAGUAGCUCA		3186-3206	UAGAGCTACUUCAAAGCAAGCAA		3184-3206
AD-1548573.1	AAGUCUCUCGAGUGUUAAAGA		3246-3266	UCUUAACACUACGAGAGACUUA		3244-3266
AD-1548574.1	UCUCUCGUAAGUGUUAAAGUUA		3249-3269	UUAACUTAAACACUACGAGAGACU		3247-3269
AD-1548575.1	CUCUCGUAAGUGUUAAAGUUAUA		3250-3270	UAUAACTUAAACACUACGAGAGAC		3248-3270
AD-1548576.1	CUCUCGUAAGUGUUAAAGUUAUA		3250-3270	UAUAACTUAAACACUACGAGAGAC		3248-3270
AD-1548577.1	CUCGUAAGUGUUAAAGUUAUAGA		3252-3272	UCUATAACUUAACACUACGAGAG		3250-3272
AD-1548578.1	CGUAGUGUUAAAGUUAUAGUGA		3254-3274	UCACTATAACUTAAACUACGAG		3252-3274
AD-1548579.1	UAGUGUUAAGUUAUAGUGAAA		3256-3276	UUUCACTAUAACUUAACACUACG		3254-3276
AD-1548580.1	GUGAAUACUGCUACAGCAUUA		3271-3291	UAUUGCTGUAAGCAUUAUUCACUA		3269-3291
AD-1548581.1	CUGCUACAGCAAUUUCUAAUA		3278-3298	UAUUAGAAAUUUGCUGUAGCAGUA		3276-3298
AD-1548582.1	GAAUUGAGUUAUGGUGUAGAA		3304-3324	UUCUACACCAUUAUCUAAUUCUU		3302-3324
AD-1548583.1	UUGAGUUAUGGUGUAGAACAA		3307-3327	UUGUTCTACACCAUUAUCUAAU		3305-3327
AD-1548584.1	GAGUUAUGGUGUAGAACACUA		3309-3329	UAGUGUTCUACACCAUUAUCUAA		3307-3329
AD-1548585.1	AGUAAUGGUGUAGAACACUAA		3310-3330	UUAGTGTUCUACACCAUUAUCUA		3308-3330
AD-1548586.1	UAAUGGUGUAGAACACUAAUA		3312-3332	UAUUAGTGUUCUACACCAUUAUCU		3310-3332

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1548587.1	AUGGUGUAGAACACACUAAUUA		3314-3334	UGAATUAGUGUTCUACACCAUUA		3312-3334
AD-1548588.1	UGGUGUAGAACACACUAAUUA		3315-3335	UTGAAUTAGUGTUCUACACCAU		3313-3335
AD-1548589.1	GGUGUAGAACACACUAAUUA		3316-3336	UAUGAATUAGUGUUCUACACCAU		3314-3336
AD-1548590.1	GUGUAGAACACACUAAUUA		3317-3337	UUUGAAUAGUGUUCUACACCA		3315-3337
AD-1548591.1	UGUAGAACACACUAAUUA		3318-3338	UUATGAAUAGUGUUCUACACC		3316-3338
AD-1548592.1	GUAGAACACACUAAUUA		3319-3339	UAUUAGAAUAGUGUUCUACAC		3317-3339
AD-1548593.1	UAGAACACACUAAUUA		3320-3340	UGAUTATGAAUTAGUGUUCUACA		3318-3340
AD-1548594.1	AGAACACACUAAUUA		3321-3341	UTGATUAGAAUAGUGUUCUAC		3319-3341
AD-1548595.1	AGAACACACUAAUUA		3321-3341	UUGATUAGAAUAGUGUUCUAC		3319-3341
AD-1548596.1	GAACACUAAUUA		3322-3342	UGUGAUAUAGAAUAGUGUUCU		3320-3342
AD-1548597.1	AACACUAAUUA		3323-3343	UAGUGATUAGAAUAGUGUUCU		3321-3343
AD-1548598.1	AUGGUCCAAUAGUUCCUUA		3413-3433	UAAGGAAACUAAUUGGACCAUU		3411-3433
AD-1548599.1	UUUGGUAUAGUAGGGUAGA		3489-3509	UCUACCCAUACAUAUCCCAAAU		3487-3509
AD-1548600.1	UGUAGGGUAGGGUAAUUA		3498-3518	UUGATUTACCCUACCCAUACAUA		3496-3518
AD-1548601.1	AUGGUAGGGUAAUUA		3501-3521	UUACTGAUUUACCCUACCCAUAC		3499-3521
AD-1548602.1	GUAGGGUAAUUA		3505-3525	UCUCTUACUGATUUAUCCCUACCC		3503-3525
AD-1548603.1	AGGGUAAUUA		3507-3527	UACCTCTUACUGAUUAUCCCUAC		3505-3527
AD-1548604.1	GGGUAAUUA		3508-3528	UCACCCUUAUUAUUAUCCCUA		3506-3528
AD-1548605.1	GUAAUUA		3510-3530	UAACACCCUUAUUAUUAUCCCUA		3508-3530
AD-1548606.1	AAUUA		3512-3532	UAUAACACCCUUAUUAUUAUUA		3510-3532
AD-1548607.1	AUUA		3513-3533	UAUAACACCCUUAUUAUUAUUA		3511-3533
AD-1548608.1	CAGUAGAGGUGUUAUUA		3516-3536	UCCAAATAACACCCUUAUUAUUA		3514-3536
AD-1548609.1	UUGGACUAGGCCAUGGA		242-262	UGGUTCCAUGGCCAUGGA		240-262
AD-1548610.1	UGGACUUGGAUUAUUA		306-326	UAGAAUGGAUUAUUAUUAUUA		304-326
AD-1548611.1	CUCUGAGUGGUAUUA		348-368	UAUUGCCUUAUUAUUAUUAUUA		346-368
AD-1548612.1	UCAAGAACAAUUAUUA		439-459	UUUAAGCAUUAUUAUUAUUAUUA		437-459
AD-1548613.1	UAGCUGAUUAUUAUUA		450-470	UCUGTCCAUAUUAUUAUUAUUA		448-470
AD-1548614.1	AGCUGAUUAUUAUUA		451-471	UACUGUCCAUAUUAUUAUUAUUA		449-471
AD-1548615.1	CUGAUUAUUAUUAUUA		453-473	UAUACUGUCCAUAUUAUUAUUA		451-473
AD-1548616.1	UGAUGGACAGUAUUA		460-480	UUCATUGCAUAUUAUUAUUAUUA		458-480
AD-1548617.1	AGUAUGCAUUAUUAUUA		468-488	UAGCTCGAGUCAUUAUUAUUAUUA		466-488
AD-1548618.1	AGCUCAGAGGGUACGAGCUGA		484-504	UCAGCUCGUAUUAUUAUUAUUAUUA		482-504

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1548619.1	CUCAGAGGUAACGAGCUGCUA		486-506	UAGCAGCUCGUACCCUCUGAGCU		484-506
AD-1548620.1	AGAGGGUACGAGCUCUAUGA		489-509	UCAUAGCAGCUCGUACCCUCUGA		487-509
AD-1548621.1	GCUAUGUUCUCCUGAGACAUUA		503-523	UAAUGUCACAGGGAACAUAAGCAG		501-523
AD-1548622.1	AGCGUUUGGCUGAACCAUCA		582-602	UUGATGGUUCAGCCAAACGCUGG		580-602
AD-1548623.1	UGCUGAAACAUGCAGUUGUAA		606-626	UUACAACUGCAUGUUUCAGCAUC		604-626
AD-1548624.1	CAUGCAGUUGUAAACUUGAU		614-634	UAUCAAGUUUACAACUGCAUGUU		612-634
AD-1548625.1	GCAGUUGUAAACUUGAUAAA		617-637	UUUAAUCAAGUUUACAACUGCAU		615-637
AD-1548626.1	UGAUUACAUAUCAAGAUUA		630-650	UAUCAUCUUGAUAGUUAAUCAAG		628-650
AD-1548627.1	CAGAACUUGCCACACGUGCAA		651-671	UUGCACGUGUGGCAAGUUCUGCA		649-671
AD-1548628.1	CACACGUGCAAUCCUUGAACA		661-681	UGUUCAGGGAUUGCACGUGUGGC		659-681
AD-1548629.1	GUGCAAUCCCUGAACUGACAA		666-686	UUGUCAGUUCAGGGAUUGCACGU		664-686
AD-1548630.1	GAGGACCAGGUGGUGGUAAA		701-721	UUUAAACCACCAACCGUGGUCUGU		699-721
AD-1548631.1	GUGGUGGUUAAUAAGGUGCA		710-730	UGCAGCCUUAUUAACCAACCAACCU		708-730
AD-1548632.1	UGCAGAAUACAUAUGAUUA		819-839	UUACAUAUUUGUAUUUCUGCAUG		817-839
AD-1548633.1	AGAAUACAAUUGAUAGAAA		822-842	UUUCTACAUCAUUUUGUAUUUCGC		820-842
AD-1548634.1	UGAUGUAGAAACAGCUCGUUA		832-852	UAACGAGCUGUUUCUACAUCAUU		830-852
AD-1548635.1	CUUGCAUAACCUUUCUCCAUA		865-885	UGAUGGGAAGGUUAUGCAAAGGU		863-885
AD-1548636.1	UUGCAUAACCUUUCUCCAUA		866-886	UUGATGGGAAAGGUUAUGCAAAGG		864-886
AD-1548637.1	CCAUUUUAAAGUCUGGAGGCA		903-923	UGCCTCCAGACUUAAAGAUUGGCC		901-923
AD-1548638.1	GCCAUUACAACUCUCCACAAA		977-997	UUUGTGGAGAGUUUGUAAUUGGCAU		975-997
AD-1548639.1	GUUGCCUUGCUCACAAAACA		1061-1081	UGUUTUGUUGAGCAAGGCAACCA		1059-1081
AD-1548640.1	AUUCUUGGCUAUUACGACAGA		1090-1110	UCUGTCGUAAUAGCCAAAGAAUUU		1088-1110
AD-1548641.1	AAAUUAUAUGAGGACCUAUA		1189-1209	UUUAAGGUCCUUAUUUAUUUAC		1187-1209
AD-1548642.1	AAUAUAUAUGAGGACCUAUA		1190-1210	UGUATAGGUCCUUAUUUAUUUA		1188-1210
AD-1548643.1	GCAGAGUGCUGAAGGUGCUAA		1236-1256	UUAGCACCUUCAGCACUCUCUUCU		1234-1256
AD-1548644.1	UUCUGGUUUCAGAUUAUA		1437-1457	UUUAUUAUCUUGAACCAGAAAGC		1435-1457
AD-1548645.1	GAUGAUUAUAUUGUGGUCACA		1448-1468	UGUGACCACAUUUUAUUAUCUCUG		1446-1468
AD-1548646.1	GGAAUUCUUUCUAACCUACACA		1478-1498	UGUGAGGUUAGAAAGAAUUCACAG		1476-1498
AD-1548647.1	UCUUUCUAACCUACUUGCAA		1483-1503	UUGCAAGUGAGGUUAGAAAGAAU		1481-1503
AD-1548648.1	UUCUAACCUACUUGCAAUA		1486-1506	UUAUTGCAAGUGAGGUUAGAAAG		1484-1506
AD-1548649.1	UCUAACCUACUUGCAAUA		1487-1507	UUUATUGCAAGUGAGGUUAGAAA		1485-1507
AD-1548650.1	AAGUGGGUGGUUAUAGAGGCUA		1533-1553	UAGCCUUAUAACCAACCCACUUGG		1531-1553

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1548651.1	UGGUGGUAUAGAGGCUCUUA		1536-1556	UAGAGCCUCUAUACCAACCCACU		1534-1556
AD-1548652.1	AGCCUGCCAUCUGUGCUCUUA		1599-1619	UAAGAGCACAGAGGCGAGGCUCA		1597-1619
AD-1548653.1	GCCUGCCAUCUGUGCUCUUA		1600-1620	UGAAGAGCACAGAGGCGAGGCUUC		1598-1620
AD-1548654.1	CAUCUGUGCUCUUCGUCUUA		1606-1626	UGAUGACGAAGAGCACAGAUUGC		1604-1626
AD-1548655.1	GCUCUCGUCUACUGACCGA		1613-1633	UCUGGUCAGAUAGACGAAGAGCAC		1611-1633
AD-1548656.1	AUGCAGUUCGCCUUCACUUA		1662-1682	UAUAGUGAAGGCCAACUGCAUUC		1660-1682
AD-1548657.1	UGGUUAGCUCUUAACCCAA		1695-1715	UUGGGUGUAAGAGCUUAACCCACA		1693-1715
AD-1548658.1	CUGUUGGAUUGAUUCGAAUA		1743-1763	UAUUTCGAUUAUACCAACAGUA		1741-1763
AD-1548659.1	UGUUGGAUUGAUUCGAAUA		1744-1764	UGAUTUCGAUUAUACCAACAGUA		1742-1764
AD-1548660.1	CGUGAGCAGGUGCCAUUCCA		1796-1816	UGGAAUGGCACCCUGCUCACGCA		1794-1816
AD-1548661.1	CCACGACUAGUUCAGUUCUA		1814-1834	UAGCAACUGAACUAGUCGUGGAA		1812-1834
AD-1548662.1	UUCAGUUGCUUGUUCGUGCAA		1824-1844	UUGCACGAACAAGCAACUGAAAU		1822-1844
AD-1548663.1	UCAGUUGCUUGUUCGUGCACA		1825-1845	UGUGCACGAACAAGCAACUGAAAC		1823-1845
AD-1548664.1	AGUUGCUUGUUCGUGCACAUA		1827-1847	UAUGTGACGAACAAGCAACUGA		1825-1847
AD-1548665.1	CAGAGGACUAAUACCAUUA		1984-2004	UGAATGGUAUUUAGUCCUCUGAU		1982-2004
AD-1548666.1	GACUAAUACCAUCCAUUGA		1989-2009	UCAATGGAAUGGUAUUUAGUCCU		1987-2009
AD-1548667.1	CCAUUCCAUGUUUGUGCAGA		1998-2018	UCUGCACAAACAUAUGGAAUGGUA		1996-2018
AD-1548668.1	AUCCAUUGUUUGUGCAGCUA		2000-2020	UAGCTGCACAAACAUAUGGAAUGG		1998-2020
AD-1548669.1	UCCAUUGUUUGUGCAGCUGA		2001-2021	UCAGCUGCACAAACAUAUGGAAUG		1999-2021
AD-1548670.1	CCAUUGUUUGUGCAGCUGCUA		2003-2023	UAGCAGCUGCACAAACAUAUGGAA		2001-2023
AD-1548671.1	GCCACAGCUCUUCUGACAGAA		2120-2140	UUCUGUCAGAGGAGCUGUGGCUC		2118-2140
AD-1548672.1	AAGCCACAAGAUUACAAGAA		2210-2230	UUUCTUGUAUUCUUGUGGCUUGU		2208-2230
AD-1548673.1	CCACAAGAUUACAAGAAACGA		2213-2233	UCGUTUCUUGUAUUCUUGUGGCU		2211-2233
AD-1548674.1	AAGAUUACAAGAAACGGCUUA		2217-2237	UAAGCCGUUUCUUGUAUUCUUGU		2215-2237
AD-1548675.1	AUUACAAGAAACGGCUUUCAA		2220-2240	UUGAAAGCCGUUUCUUGUAUUCU		2218-2240
AD-1548676.1	GGCUUUCAGUUGAGCUGACCA		2232-2252	UGGUCAGCUCAACUGAAAGCCGU		2230-2252
AD-1548677.1	UGAGCUGACCAAGCUCUCUA		2242-2262	UAGAGAGAGCUGGUCAGCUCUAC		2240-2262
AD-1548678.1	GAGCCAAUGGCUUGGAAUGAA		2270-2290	UUCATUCCAAGCCAUUGGCUCUG		2268-2290
AD-1548679.1	CUGAUCUUGGACUUGAUUUA		2295-2315	UAUAUCAAGUCCAAGAUCAGCA		2293-2315
AD-1548680.1	UGGUUUGAUACUGACCUGUA		2540-2560	UUACAGGUCAGUAUCAAAACAGG		2538-2560
AD-1548681.1	UUUGAUACUGACCUGUAAUA		2543-2563	UAUUTACAGGUCAGUAUCAAAAC		2541-2563
AD-1548682.1	GGCUAUUUUGUAAAUUCUGCCAA		2666-2686	UUGGCAGAUUUACAAAUAGCCUA		2664-2686

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1548683.1	UUUCUGUUUUGUUAUGUGAUCA		2743-2763	UGAUCACAUACAACACGAAAUC		2741-2763
AD-1548684.1	GAUCAUGUGUGGAAGUUUAUA		2759-2779	UAAUAACUCCACACAUAGUAC		2757-2779
AD-1548685.1	UAAUCACAAAGAGUAACAA		2813-2833	UUGUTACUAUUUGAGUAUUAAG		2811-2833
AD-1548686.1	AUUGUCUGAACUUGCAUUGUA		2882-2902	UACAAGCAAGUUCAGACAAUAC		2880-2902
AD-1548687.1	CAGAAAGUGCCUGACACACUA		2951-2971	UAGUGUGACGGCACUUUCUGAG		2949-2971
AD-1548688.1	GAAAGUGCCUGACACACUAAA		2953-2973	UUUAGUGUGACGGCACUUUCUG		2951-2973
AD-1548689.1	GCCUGACACACUAAACCAAGCA		2959-2979	UGCUTGGUUAGUGUGACGGCAC		2957-2979
AD-1548690.1	UGGGAACAAUUGAAGUAAACA		2990-3010	UGUUTACUACA AUUGUCCCAUA		2988-3010
AD-1548691.1	AUGGAUCACAAAGUAGAAUA		3093-3113	UAAUTCCAUCUUGUGAUCCAUA		3091-3113
AD-1548692.1	UUUAUCAACCCUAGCCUUGA		3111-3131	UCAAGGCUAGGUGUUUGAUAAUU		3109-3131
AD-1548693.1	UAUCUGUAAUGGUACUGACUA		3164-3184	UAGUCAGUACCAUACAGAUUU		3162-3184
AD-1548694.1	UUGCUGCUUUGAAGUAGCUA		3184-3204	UAGTACUCAAAGCAAGCAAAAG		3182-3204
AD-1548695.1	AGUGUAAAGUUUAUGUGAAUA		3257-3277	UAUUCACUAUAACUUAACACUAC		3255-3277
AD-1548696.1	UGUUAAGUUUAUGUGAAUACA		3259-3279	UGUATUCACUAUAACUUAACACU		3257-3279
AD-1548697.1	GUAAUGGUGUAGAACACUAAA		3311-3331	UUUAGUGUUUACACCAUUAACUC		3309-3331
AD-1548698.1	AAUGGUGUAGAACACUAAUA		3313-3333	UAAUTAGUGUUUACACCAUUAAC		3311-3333
AD-1548699.1	CUAAUUCUAAUACACUCUAAA		3327-3347	UUUAGAGUGAUUAUGAAUUAAGUG		3325-3347
AD-1548700.1	UUUGGGAUUGUAUGGGUAGA		3489-3509	UCUACCCAUACAUAUCCCAAAUA		3487-3509
AD-1548701.1	GGGUAUUCAGUAAGAGGUGA		3508-3528	UCACCCUUAACUGAUUUACCCUA		3506-3528
AD-1548702.1	GUAAUUCAGUAAGAGGUGUUA		3510-3530	UAAACCCUUAACUGAUUUACCC		3508-3530
AD-1548703.1	UAAUUCAGUAAGAGGUGUUA		3511-3531	UUAAACCCUUAACUGAUUUACC		3509-3531
AD-1548704.1	AUUCAGUAAGAGGUGUUAUA		3513-3533	UAAUAAACCCUUAACUGAUUUUA		3511-3533

Table 3. Modified Sense and Antisense Strand Sequences of CTNNB1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1548365.1	gsasggguAfuUfUfGfaaguuacscsa		VPuGfugdAu(Agn)cuucaaAfuAfccecsasg		CUGAGGGUAUUUGAAAGUAUACCA	
AD-1548366.1	gsasggguauUfUfGfaaguuacscsa		VPudGgudAudAcuuedAaAfuaccecsasg		CUGAGGGUAUUUGAAAGUAUACCA	
AD-	gssguanUfUfGfAfAfguanaccasusa		VPuAfuagdGu(Agn)uacuuuAfaAfuaccesuse		GAGGGUAUUUGAAAGUAUACCAUA	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
1548367.1						
AD-1548368.1	gsgsuauuUfgAfAfGfuauacaasasa		VPuUfauGg(Tgn)uauuuCfaAfuaacscsu		AGGGUAAUUUGAAGUAUACCAUAC	
AD-1548369.1	ususugaaGfuAfUfAfccauacaascsa		VPuGfuudGu(Agn)ugguauAfcUfuaaasusa		UAUUUGAAGUAUACCAUACAACU	
AD-1548370.1	ususgaagUfaUfAfCfcauacaacsusa		VPuAfgudTg(Tgn)augguaUfaCfuuaasasu		AUUUGAAGUAUACCAUACAACUG	
AD-1548371.1	usgsgacaAfuGfGfCfuacucaagscsa		VPuGfendTg(Agn)guagccAfuUfguaccscsg		CGUGGACAAUGGCUACUCAAGCU	
AD-1548372.1	gsascaaugCfUfAfcaucaagcugsga		VPudCagdTg(Tgn)augudAgCfaauugcscsa		UGGACAAUGGCUACUCAAGCUGA	
AD-1548373.1	ascsaangGfUfAfCfcaucaagcugsga		VPuUfcadGc(Tgn)ugaguaGfcCfaauugscsc		GGACAAUGGCUACUCAAGCUGAU	
AD-1548374.1	asascgugauUfUfGfauaggauugsga		VPudCaadCudCcaudAaAfuaagcuugsga		UCAAGCUGAUUUUGAUGGAGUUGG	
AD-1548375.1	asgscugauuUfGfAfugagauugsgsa		VPudCeadAcdTccaudCaAfuaagcucusg		CAAGCUGAUUUUGAUGGAGUUGGA	
AD-1548376.1	cscsuucucuGfAfGfugguuaagsgsa		VPudCcuTudAccadTcAfgagaagsgsag		CUCCUUCUCUGAGUGGUAAAGGC	
AD-1548377.1	csasguccUfuCfAfCfcaucaagaacsasa		VPuUfgudTc(Tgn)ugagugAfaGfagucgsag		CUCAGUCCUUCACUCUCAAGAACAA	
AD-1548378.1	csusucacucAfAfGfcaucaaguasgsa		VPudCuadCudTguncdTgGfagugaagsgsa		UCCUUCACUCUCAAGAACAAAGUAGC	
AD-1548379.1	csascucaagAfAfCfcaucaagcugsgsa		VPudCagdTudAcuugdTgCfuugaguggsasa		UUCACUCAAGAACAAAGUAGCUGA	
AD-1548380.1	gsusagcuGfaUfAfUfugauggacsasa		VPuUfgudCc(Agn)ucaauaUfcAfgeuacsusu		AAGUAGCUGAUUUUGAUGGACAG	
AD-1548381.1	asusugauGfGfAfCfAfguauucaasusa		VPuAfuudGc(Agn)uacugcCfcAfuaaasusu		AUAUUGAUGGACAGUAUGCAAUG	
AD-1548382.1	asgsuauucaAfUfGfcaucgagcsusa		VPudAgedTcdGaguedAuUfgcauacugsgsu		ACAGUAUGCAAUGACUCGAGCUC	
AD-1548383.1	gsgsuacgAfGfCfUfGfcauauugnuccsa		VPuGfgadAc(Agn)uagcagCfuCfuaacscsu		AGGGUACGAGCUGCUAUGUUCCC	
AD-1548384.1	ascsagcugCfUfAfugnuccugsgsa		VPudCagdTgdAacandAgCfagcucgusasc		GUACGAGCUGCUAUGUUCCCUGA	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1548385.1	usgsuuccuGfAfGfacaauuagasa		VPudAuedTadAugudTcAfgggaacasa		UAUGUCCCUGAGACAUUAGAUG	
AD-1548386.1	ususeccuGfAfGfafaauuagagsasa		VPuUfcauTc(Tgn)aaugucUfcAfgggaacasa		UGUCCCCUGAGACAUUAGAUGAG	
AD-1548387.1	asgsaagaGfgGfCfAfugcagaucscsa		VPuGfgadTc(Tgn)gcaugcCfcUfcaucusa		UUAGAUGAGGGCAUGCAGAUCC	
AD-1548388.1	csusgcueuCfCfCfacaauuaguscscsa		VPudGacdAudTagudGgAfugagcagscsa		UGCUGCUCAUCCCCACUAAUGUCC	
AD-1548389.1	ascscuacAfcAfGfAfugcugaauascsa		VPuGfuudTc(Agn)gcaucUfGfugaguscscsa		GAACCAUCACAGAUUGCUGAAACA	
AD-1548390.1	csasucacAfgAfUfGfugagaacasa		VPuAfugdTc(Tgn)cagcauUfcGfugaguscscsa		ACCAUCACAGAUUGCUGAAACAUG	
AD-1548391.1	csasucacagAfUfGfugagaacasa		VPudAugdTudTcagadAuCfugaguscscsa		ACCAUCACAGAUUGCUGAAACAUG	
AD-1548392.1	asusgcugAfaAfCfAfugcaguugscsa		VPuAfcauAc(Tgn)gcauUfUfCfagcuscscsa		AGAUGCUGAAACAUGCAGUUGUA	
AD-1548393.1	asusgcugaaAfCfAfugcaguugscsa		VPudAcadAcdTgcandGuUfucagcuscscsa		AGAUGCUGAAACAUGCAGUUGUA	
AD-1548394.1	ascsaugcAfgUfUfGfuaaauugscsa		VPuUfcauAg(Tgn)uuuacCfUfCfagcuscscsa		AAACAUGCAGUUGUAAAACUUGAU	
AD-1548395.1	asusgcagUfuGfUfAfaacuugausasa		VPuAfaudCa(Agn)uuuacAfaCfugcuscscsa		ACAUGCAGUUGUAAAACUUGAUUA	
AD-1548396.1	asusgcaguuGfUfAfaacuugausasa		VPudAaudCadAguuudAcAfucagcuscscsa		ACAUGCAGUUGUAAAACUUGAUUA	
AD-1548397.1	ascsgugcAfaUfCfCfugagaucscsa		VPuUfcauGu(Tgn)cagggaUfuGfagcuscscsa		ACACGUGCAUCCCCUGAACUGAC	
AD-1548398.1	usgscauUfcCfUfGfagcagacasa		VPuUfcauTc(Agn)uuuacGfUfUfagcuscscsa		CGUGCAUCCCCUGAACUGACAAA	
AD-1548399.1	gsasccagGfUfGfUfUfguuuauasasa		VPuUfuadTu(Agn)uacacAfcCfugcuscscsa		AGGACCAGGUGGUGGUUAAUUAAG	
AD-1548400.1	gsasccagguGfUfUfguuuauasasa		VPudTuadTudAaccadCcAfucgucscscsa		AGGACCAGGUGGUGGUUAAUUAAG	
AD-1548401.1	csasagaggUfUfGfUfuaauaagsgsa		VPudCendTadTuaadCaCfagcuscscsa		GACCAGGUGGUGGUUAAUUAAGGC	
AD-	gsusuaauAfaGfGfCfugcaguuaasasa		VPuAfuadAc(Tgn)gcaugcUfuAfuuaacscsa		UGGUUAAUUAAGGUGCAGUUAUG	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
1548402.1						
AD-1548403.1	cscsucagAfuGfGfUfgucugcuasusa		VPuAfuadGc(Agn)gacaccAfuCfugaggsag		CUCCUCAGAUUGGUGUCUGCUAUU	
AD-1548404.1	uscsgauggUfgUfUfgucuauggsa		VPudCaadTadGcagadCaCfcaucugagsg		CCUCAGAUUGGUGUCUGCUAUUGU	
AD-1548405.1	asgsauggUfgUfCfUfgcuauugusasa		VPuUfadcAa(Tgn)agcagaCfaCfcaucgsa		UCAGAUUGGUGUCUGCUAUUGUAC	
AD-1548406.1	asgsauggUfgUfCfUfgcuauugusasa		VPudTadAadTagcadGaCfcaucuggsa		UCAGAUUGGUGUCUGCUAUUGUAC	
AD-1548407.1	ascsguacCfaUfGfCfagaauacasasa		VPuUfadcTa(Tgn)ucugcaUfgGfuauggsa		GUACGUACCAUGCAGAAUACAAA	
AD-1548408.1	ascsguaccaUfgGfCfagaauacasasa		VPudTugdTadTucugdCaUfgguaggsa		GUACGUACCAUGCAGAAUACAAA	
AD-1548409.1	csgsuaccAfuGfCfAfagaauacaasasa		VPuUfudGu(Agn)uucugcAfuGfuauggsa		UACGUACCAUGCAGAAUACAAA	
AD-1548410.1	gsusaccaUfgCfAfGfagaauacaasusa		VPuAfuadTg(Tgn)auucugCfaUfgguaggsu		ACGUACCAUGCAGAAUACAAAUG	
AD-1548411.1	usascuagcAfgfAfaaacaauuggsa		VPudCaadTudGuaudCuGfcaugguaggsa		CGUACCAUGCAGAAUACAAAUGA	
AD-1548412.1	ascscuagcaGfAfAfaaacaauuggsa		VPudTcadTudTgnaudTcUfgcauggsa		GUACCAUGCAGAAUACAAAUGAU	
AD-1548413.1	cscsaugcagAfaUfUfacaauuggsa		VPudAuedAudTugudTuCfugcauggsa		UACCAUGCAGAAUACAAAUGAU	
AD-1548414.1	csasugcagaAfuAfaaacaauuggsa		VPudCaadCadTuugudAuUfugcauggsa		ACCAUGCAGAAUACAAAUGAU	
AD-1548415.1	asusgcagAfaUfAfaaacaauuggsa		VPuAfcadTc(Agn)uuuguaUfuCfugcauggsa		CCAUGCAGAAUACAAAUGAU	
AD-1548416.1	gscsagaauaCfAfAfaaacaauuggsa		VPudCuadCadTcaudTgUfaucuggsa		AUGCAGAAUACAAAUGAU	
AD-1548417.1	csasgaauAfcAfaAfaaacaauuggsa		VPuUfadcAc(Agn)ucauuuGfuAfuucuggsa		UGCAGAAUACAAAUGAU	
AD-1548418.1	usgsauguagAfaAfaaacaauuggsa		VPudAacdGadGcugdTuCfuaucuggsa		AAUGAUGAAGAAACAGCUCGUUG	
AD-1548419.1	gsasuguagaAfaAfaaacaauuggsa		VPudCaadCgdAgcugdTuUfuaucuggsa		AUGAUGAAGAAACAGCUCGUUGU	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1548420.1	ascsggccaUfcUfuuaagucsgsa		VPudCagdAcdTuaadGaUfggccagusa		UUACUGGCCAUCUUUAAGUCUGG	
AD-1548421.1	csusggccauCfUfUfuuaagucsgsa		VPudCeadGadCuuaadAgAfuggccagusa		UACUGGCCAUCUUUAAGUCUGGA	
AD-1548422.1	usgsgccaUfcUfUfUfaagucsgsa		VPuUfcdAg(Agn)cuuaaaGfaUfggccagsu		ACUGGCCAUCUUUAAGUCUGGAG	
AD-1548423.1	gsgsccaucUfUfAfagucggagsgsa		VPudCuedCadGacuudAaAfgaugccsag		CUGGCCAUCUUUAAGUCUGGAGG	
AD-1548424.1	gscscaucuuUfAfAfugucggagsgsa		VPudCcuCdAgacudTaAfagaugcscsa		UGGCCAUCUUUAAGUCUGGAGGC	
AD-1548425.1	usgsguucacCfAfGfugauucsgsa		VPudCagdAadTccacdTgGfugaaccasag		CUUGGUUACACAGUGGAUUCUGU	
AD-1548426.1	ascscaguggAfUfUfcugugucsgsa		VPudAcadAcdAcagadAuCfcacugucsgsa		UCACCAGUGGAUUCUGUGUUUU	
AD-1548427.1	asusggcaGfuGfCfGfuuaagucsgsa		VPuUfcadGc(Tgn)aaacgcAfcUfgccausu		AAAUGGCAGUGCGUUUAAGUCUGU	
AD-1548428.1	asusggcaguGfCfGfuuaagucsgsa		VPudCcadGcdTaaacdGcAfcugccausu		AAAUGGCAGUGCGUUUAAGUCUGU	
AD-1548429.1	ususgccuUfgCfUfCfaacaaacsasa		VPuUfgudTu(Tgn)guugagCfaAfgcaasc		GGUUGCCUUGCUCACAAAAACAA	
AD-1548430.1	usgsecuugcUfCfAfacaacacasa		VPudTugdTu(Tgn)guugadGaGfcaaggcasasc		GUUGCCUUGCUCACAAAAACAA	
AD-1548431.1	usgscuuGfcUfCfAfacaacacasa		VPuUfgudTu(Tgn)guugagGfcAfgccasasc		GUUGCCUUGCUCACAAAAACAA	
AD-1548432.1	gscscuugCfuCfAfAfcaaaacacasa		VPuUfuudGu(Tgn)uugaugAfgCfaaggcsasa		UUGCCUUGCUCACAAAAACAAU	
AD-1548433.1	ususaaauucUfUfGfgcuuaucsgsa		VPudCgudAadTagccdAaGfaauuaacsasa		UGUUAAAUCUUUGGCUAUUACGA	
AD-1548434.1	asusucuuugCfuUfAfuuaacagacsgsa		VPudCugdTcdGuaandAgCfcaagaauusu		AAAUUCUUGGCUAUUACGACAGA	
AD-1548435.1	gsgscuauUfaCfGfAfcagacugcscsa		VPuUfgcdAg(Tgn)cuugucUfaAfuagccsasa		UUGGCUAUUACGACAGACUGCCU	
AD-1548436.1	usascgacAfgAfcUfUfgccuuaacsasa		VPuUfuudGa(Agn)ggcaguCfuGfucguasasu		AUUACGACAGACUGCCUCAAU	
AD-	csusuuaaguaAfAfUfauaagagsgsa		VPudCcuCdTuaadTuUfaucuaagcsu		AGCUUUAAGUAAAUAUAAUUGAGGA	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
1548437.1						
AD-1548438.1	usasuauagaGfGfAfccuauacususa		VPudAagdTadTaggudCcUfcauauasusu		AAUAUAAUGAGGACCUAUACUUA	
AD-1548439.1	asgscuggUfgGfAfAfugcaagcususa		VPuAfagdCu(Tgn)gcauncCfAfCfagcuscusc		GAAGCUGGUGGAUUGCAAGCUUU	
AD-1548440.1	csasgaucuaAfGfUfcaacgucuscusa		VPudAagdAcGgugadCuUfggaucguscusc		GACAGAUCCAAGUCAACGUCUUG	
AD-1548441.1	cscsaagnCfAfCfGfucugucuscasa		VPuUfgadAc(Agn)agacguUfgAfcuuggsasu		AUCCAAGUCAACGUCUUGUUCAG	
AD-1548442.1	cscsaagucaAfCfGfucugucuscasa		VPudTgadAcAdAagacdGnUfgacuggsasu		AUCCAAGUCAACGUCUUGUUCAG	
AD-1548443.1	asgsaacuGfuCfUfUfggacucuscasa		VPuGfagdAg(Tgn)ccaagAfcAfguucuscasa		UCAGAACUGUCUUGGACUCUCA	
AD-1548444.1	csusuucaGfAfUfGfCfugcaacuacasa		VPuUfuadGu(Tgn)gcagcaUfcUfgaaagsasu		AUCUUUCAGAUUGCUGCAACUAAA	
AD-1548445.1	uscsgauGfCfUfGfCfuaacuaacasa		VPuUfgudTu(Agn)guugcaGfCfueugcasasa		UUUCAGAUUGCUGCAACUAAAACAG	
AD-1548446.1	gsasugcuGfCfAfAfCfuaacaggsasa		VPuUfcdTg(Tgn)uuaguuGfCfAfgcaucuscg		CAGAUUGCUGCAACUAAAACAGGAA	
AD-1548447.1	csusgcaacuAfAfAfcaaggaggsasa		VPudCcdTudCcuadTuAfguugcaggsasa		UGCUGCAACUAAAACAGGAAGGGA	
AD-1548448.1	gsasagggAfUfGfAfAfagguccuscusa		VPuAfggdAg(Agn)ccuuccAfUfCfucucuscusu		AGGAAGGGAUGGAAGGUCUCUUCU	
AD-1548449.1	csusucugGfGfUfUfCfagauauasusa		VPuAfuaTc(Agn)ucugaaCfCfagaaggsusu		AGCUUCUGGGUUCAGAUUAUA	
AD-1548450.1	cscsugugcaGfCfUfUfggaauucuscusa		VPudAagdAadTuccadGcUfgcacaggsuscg		CACCUGUGCAGCUGGAAUUCUUCU	
AD-1548451.1	usgsugcagcUfGfGfaauucuuuscasa		VPudGaadAgdAauucdCaGfucgacaggsuscg		CCUGUGCAGCUGGAAUUCUUCU	
AD-1548452.1	asastuucUfuCfUfAfaccucacuscusa		VPuAfagdTg(Agn)gguuagAfaAfgaaucscsc		GGAAUUCUUUCUAACCCUCACUUG	
AD-1548453.1	ususcuuucUfAfCfucucacugscasa		VPudGcadAgdTgaggdTuAfgaaagaauscusu		AAUUCUUUCUAACCCUCACUUGCA	
AD-1548454.1	csusuucUfAfCfCfUfUfcaucugcasasa		VPuUfuadCa(Agn)guagguUfuAfgaaaggsasa		UUCUUUCUAACCCUCACUUGCAAU	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1548455.1	usususuaAfcCfUfCfacuugcaasusa		VPuAfuudGc(Agn)agugagGfuUfagaasgsa		UCUUUCUAACCCUCACUUGCAAUA	
AD-1548456.1	csusaaccucAfcUfUfgeaauaasusa		VPudAuudAudTgcaadGuGfagguaasasa		UUCUAACCCUCACUUGCAAUAUAU	
AD-1548457.1	usasaccucaCfUfUfgeaauaasusa		VPudAuudAudTadTugcadAgUfagguaasgsa		UCUAACCCUCACUUGCAAUAUAU	
AD-1548458.1	asascuccuacUfUfGfcaauaauasasa		VPudTaadTudAuungcdAaGfugagguaasgsa		CUAACCCUCACUUGCAAUAUAUAU	
AD-1548459.1	ascscuccuacUfGfCfaauaauaasusa		VPudAuudAudTaanugdCaAfgugagguaasgsa		UAACCCUCACUUGCAAUAUAUAUA	
AD-1548460.1	cscscuccuacUfGfCfaauaauaasusa		VPudTaudAadTuaudGcAfgugagguaasgsa		AACCCUCACUUGCAAUAUAUAUA	
AD-1548461.1	csuscacuUfGfCfAfAfuaauaauasasa		VPuUfuadTa(Agn)uuaugCfaAfgugagguaasgsa		ACCUCACUUGCAAUAUAUAUAAG	
AD-1548462.1	csuscacuUfGfCfAfAfuaauaauasasa		VPudTaudTadAuuaudTgCfaagugagguaasgsa		ACCUCACUUGCAAUAUAUAUAAG	
AD-1548463.1	uscscacuUfGfCfAfAfuaauaauasgsa		VPudCuudAudAauudTuGfcaagugagguaasgsa		CCUCACUUGCAAUAUAUAUAAGA	
AD-1548464.1	csascuugcaAfUfAfuaauaauasgsa		VPudTaudTadTaaudAuUfgeaagugagguaasgsa		CUCACUUGCAAUAUAUAUAAGAA	
AD-1548465.1	csasagugGfGfUfGfGfuaauaagaggsa		VPuGfecdTc(Tgn)auaccaCfcCfacuugagguaasgsa		GCCAAAGUGGGUGGUUAUAGAGGCU	
AD-1548466.1	gscscuUfGfGfGfGfuaauaagaggsa		VPuAfggdAc(Agn)guacgcAfcAfgagagguaasgsa		AGGCUCUUGUGCGUAUAGAGGCUU	
AD-1548467.1	cscsugccauCfUfGfGfGfuaauaagaggsa		VPudCgadAgdAgcagdAgAfgaggagguaasgsa		AGCCUGCCAUUCUGUGGCUUCUUCGU	
AD-1548468.1	csusgccaucUfGfUfGfGfuaauaagaggsa		VPudAcgdAadGagcadCaGfaggagguaasgsa		GCCUGCCAUUCUGUGGCUUCUUCGUC	
AD-1548469.1	usgscuUfGfUfGfGfuaauaagaggsa		VPudGacdGadAgagadAcAfgaggagguaasgsa		CCUGCCAUUCUGUGGCUUCUUCGUA	
AD-1548470.1	gscscuUfGfUfGfGfuaauaagaggsa		VPudTgadCgdAgagadCaCfaggagguaasgsa		CUGCCAUUCUGUGGCUUCUUCGUAU	
AD-1548471.1	cscsaucugGfCfUfGfGfuaauaagaggsa		VPudAugdAcdGaagadGcAfcaggagguaasgsa		UGCCAUUCUGUGGCUUCUUCGUAUC	
AD-	asuscuguGfCfUfGfGfuaauaagaggsa		VPuAfgadTg(Agn)cgagaGfcAfcaggagguaasgsa		CCAUCUGUGGCUUCUUCGUAUCUG	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
1548472.1						
AD-1548473.1	uscsugugcuCfuUfGfucuaucugsas		VPudCagadAudGacgadAgAfgcacagasisug		CAUCUGUGCUCUUCGUCUAUCUGA	
AD-1548474.1	csusgugeUfcUfUfCfGfucuaucugsasa		VPuUfcaadGa(Tgn)gacgaaGfaGfcaacagsasu		AUCUGUGCUCUUCGUCUAUCUGAC	
AD-1548475.1	usgsugcuCfuUfCfGfucuaucugascsa		VPuGfucdAg(Agn)ugacgaAfgAfgcacagsa		UCUGUGCUCUUCGUCUAUCUGACC	
AD-1548476.1	usgsuacuAfcCfAfGfugugugguusasa		VPuUfaadCc(Agn)caacuGfuaAfguccasusa		UAUGGACUACCAGUUGUGGUUAA	
AD-1548477.1	gsascuaccaGfuUfGfugugguuaagsa		VPudCuudAadCcacadAcUfgguagucscsa		UGGACUACCAGUUGUGGUUAAAGC	
AD-1548478.1	usgsugguUfaAfgCfGfucuaucacsca		VPuGfugudGu(Agn)agagcuUfaAfgcacasasc		GUUGUGGUUAAAGCUCUUAACACCC	
AD-1548479.1	gsasuaaaggCfuUfAfcuguguggasusa		VPudAucdCadAcagudAgCfcuuuaucscasg		CUGAUAAAGGCUACUGUUGGAUU	
AD-1548480.1	asusaaagCfUfAfcUfGfugugugaususa		VPuAfaudCc(Agn)acaguaGfcCfuuaucsa		UGAUAAAGGCUACUGUUGGAUUUG	
AD-1548481.1	asusaaaggCfUfAfcUfGfugugugaususa		VPudAaudCcdAacagdTaGfcccuaucsa		UGAUAAAGGCUACUGUUGGAUUUG	
AD-1548482.1	asasggcuAfcUfGfUfGfugugugaususa		VPuAfuadAa(Tgn)ccaacaGfuAfgccuucsa		UAAAGGCUACUGUUGGAUUUGAUU	
AD-1548483.1	asasggcuacUfGfUfGfugugugaususa		VPudAucdAadTccaadCaGfuaugccuucsa		UAAAGGCUACUGUUGGAUUUGAUU	
AD-1548484.1	asgsuacuGfuUfGfugugugaususa		VPudAaudCadAuccadAcAfguagccuucsu		AAAGGCUACUGUUGGAUUUGAUUC	
AD-1548485.1	gsuacuacUfgUfUfGfugugugauucsa		VPuGfaadTc(Agn)auccaaCfaGfuagccsu		AAGGCUACUGUUGGAUUUGAUUCG	
AD-1548486.1	gsuacuacuUfGfGfugugauucscgsa		VPudCgadAudCaauedCaAfcagugacscsu		AGGCUACUGUUGGAUUUGAUUCGA	
AD-1548487.1	csusacuguuGfGfAfuugauucgsasa		VPudTegdAadTcaudCcAfacuagcsc		GGCUACUGUUGGAUUUGAUUCGAA	
AD-1548488.1	usascugugGfAfuUfugauucgsasa		VPudTuedGadAucaadTcCfaacagucsc		GCUACUGUUGGAUUUGAUUCGAAA	
AD-1548489.1	ascstugugGfAfuUfGfugauucgsasa		VPudTuudCgdAucaadAuCfaacagucsc		CUACUGUUGGAUUUGAUUCGAAAU	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1548490.1	csusguuggaUfUfGfaucgaaasusa		VPudAaundTcdGaauCdAaUfccaacagsusa		UACUGUUGGAUUGAUUCGAAAUC	
AD-1548491.1	gsusuggaauGfAfUfucgaaauususa		VPudAgadTudTogaadTcAfaucacaacsag		CUGUUGGAUUGAUUCGAAAUCUU	
AD-1548492.1	gsusuggaUfuGfAfUfucgaaauususa		VPuAfgadTu(Tgn)cgauacAfaUfccaacsag		CUGUUGGAUUGAUUCGAAAUCUU	
AD-1548493.1	usuggaauGfAfUfucgaaauususa		VPudAagdAudTucgadAuCfaucacaacsusa		UGUUGGAUUGAUUCGAAAUCUUG	
AD-1548494.1	gsgsaauGfAfUfCfGfaaauuugscsa		VPuGfcadAg(Agn)uuuugaAfuCfaucacaacsusa		UUGGAUUGAUUCGAAAUCUUGGCC	
AD-1548495.1	gsgsaauGfAfUfCfGfaaauuugscsa		VPudGcadAgdAuuuCdGaAfuCfaucacaacsusa		UUGGAUUGAUUCGAAAUCUUGGCC	
AD-1548496.1	usuggaauCfGfAfAfAfucuuugcccsusa		VPuAfggdGc(Agn)agaauuCfGfAfaucacaacsusa		GAUUGAUUCGAAAUCUUGGCCUU	
AD-1548497.1	asusuccaCfGfAfCfUfaguuuugscsa		VPuAfacdTg(Agn)acuaguCfGfUfgaauusg		CCAUUCACGACUAGUUCAGUUG	
AD-1548498.1	cscsacagauAfGfUfucaguuugscsa		VPudAgcdAadCugaadCuAfgucguggsusa		UUCCACGACUAGUUCAGUUGCUU	
AD-1548499.1	ususcaguuGfAfUfUfucgugscsa		VPudTgcdAcdGaacadAgCfaucagaaacsusa		AGUUCAGUUGCUUUGUUCGUGCAC	
AD-1548500.1	gsgsacacagCfAfGfcaauuuugscsa		VPudCaedAadAuuCdTgCfuguguccsusa		UGGGACACAGCAGCAAUUUGUGG	
AD-1548501.1	asusuccaGfCfUfCfGfaguuuugscsa		VPuUfgadAc(Agn)ucccgGfCfUfgaauusg		ACAUCCUAGCUCGGGAUUGUUCAC	
AD-1548502.1	asgscueGfGfAfUfGfucacaacsusa		VPuGfgdTg(Tgn)gaacauCfCfGfagucusa		CUAGCUCGGGAUUGUUCACAACCG	
AD-1548503.1	gsasuguucaCfAfAfCfcaauuugscsa		VPudAcadAudTcggudTgUfgaacaucsc		GGGAUGUUCACAAACCGAAUUGUU	
AD-1548504.1	usasucagAfGfGfAfCfuaauuacsa		VPuUfgdTg(Tgn)uuuagCfCfUfgaauasac		GUUAUCAGAGGACUAAAUAACCAU	
AD-1548505.1	asuscagaGfGfAfCfUfuaauuacsa		VPuAfgdTg(Agn)uuuagCfCfUfgaauasusa		UUAUCAGAGGACUAAAUAACCAU	
AD-1548506.1	uscsagGfAfCfUfAfuaauuacsa		VPuAfaudGg(Tgn)uuuagUfCfucgagusa		UAUCAGAGGACUAAAUAACCAUUC	
AD-	gsasggacUfaAfAfUfuaauuacsa		VPuUfgdTg(Tgn)gguaauUfaGfuccucusa		CAGAGGACUAAAUAACCAUCCAU	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
1548507.1						
AD-1548508.1	gsasggacuaAfAfUfaceauccsasa		VPudTggdAadTgguadTgUfaguccucusc		CAGAGGACUAAAUACCAUUCUCCAU	
AD-1548509.1	gsgsacuaaaUfAfCfcauuccaususa		VPudAandGgdAaugdTgUfuauguccusc		GAGGACUAAAUACCAUUCUCCAUUG	
AD-1548510.1	ascsuuaaauaCfCfAfuccauugcsusa		VPudAcadAadGgaandGgUfaunuaugusc		GGACUAAAUACCAUUCUCCAUUGUU	
AD-1548511.1	csusaauaacCfAfUfuccauugcsusa		VPudAacdAadTggadTgGfuaunuaugusc		GACUAAAUACCAUUCUCCAUUGUUU	
AD-1548512.1	usasaauaccAfUfUfuccauugcsusa		VPudAaadCadAuggadAuGfguaunuaugsu		ACUAAAUACCAUUCUCCAUUGUUUG	
AD-1548513.1	asasaauaccaUfUfCfcauuguuugsgsa		VPudCaadAcdAaugdAaUfgguauuuasag		CUAAAUACCAUUCUCCAUUGUUUGU	
AD-1548514.1	asasuaccanUfCfCfcauuguuugsgsa		VPudAcadAadCaauadGaAfugguauuuusa		UAAAUACCAUUCUCCAUUGUUUGUG	
AD-1548515.1	asusaccauuCfCfAfuguuuuugsgsa		VPudCaadAadAcaandGgAfauuguaususu		AAUACCAUUCUCCAUUGUUUGUGC	
AD-1548515.2	asusaccauuCfCfAfuguuuuugsgsa		VPudCaadAadAcaandGgAfauuguaususu		AAUACCAUUCUCCAUUGUUUGUGC	
AD-1548516.1	usascacuuCfAfUfuguuuuugsgcsa		VPudGcadCadAacaadTgGfauuguaususu		AAUACCAUUCUCCAUUGUUUGUGCA	
AD-1548517.1	ascscacuuCfAfUfuguuuuugsgcsa		VPudTgcdAcdAaacadAuGfgaauuguisasu		AUACCAUUCUCCAUUGUUUGUGCAG	
AD-1548518.1	ascscacuuCfAfUfUfuguuuuugsgcsa		VPuUfgedAc(Agn)aacaauGfgAfauuguisasu		AUACCAUUCUCCAUUGUUUGUGCAG	
AD-1548519.1	csascauuCfAfUfGfuuuuugcagsa		VPudCugdCadCaaacdAaUfggaauuggsusa		UACCAUUCUCCAUUGUUUGUGCAGC	
AD-1548520.1	csasuuCfAfUfGfUfuuuuugcagscsa		VPuGfcdGc(Agn)caacaAfUfGfgaauuggsu		ACCAUUCUCCAUUGUUUGUGCAGCU	
AD-1548521.1	uscscacuuGfUfUfGfugcagcugcsa		VPuGfcdGc(Tgn)gcacaaAfCfauuggasasu		AUUCUCCAUUGUUUGUGCAGCUGCU	
AD-1548522.1	gssuuuuugcAfGfCfugcuaauusasa		VPudAandAadAgcagdCuGfcaacaacsasa		UUGUUUGUGCAGCUGCUUUAUUC	
AD-1548523.1	csuucueugAfCfAfaguuuacusa		VPudAagdTadAcuendGuCfagagagagcsu		AGCUCCUCUGACAGAGAGUUAUUC	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1548524.1	gsascagaGfuUfAfCfucacucusasa		VPuUfagdAg(Tgn)gaaguaAfcUfcugucsasg		CUGACAGAGUUACUUCACUCUAG	
AD-1548525.1	asgsuuacUfuCfAfCfucuaaggaasusa		VPuAfuudCc(Tgn)agagugAfaGfuacusesu		AGAGUUACUUCACUCUAGGAAUG	
AD-1548526.1	gsasggacAfaGfCfCfacaagaunusasa		VPuUfaadTc(Tgn)uguggcUfuGfuccucsasg		CUGAGGACAAGCCACAAGAUUAC	
AD-1548527.1	csascaagauUfAfCfagaagaacgsgsa		VPudCegdTudTcuugdTaAfucuuugsgsc		GCCACAAAGAUUACAAGAAAACGGC	
AD-1548528.1	ascsaagauUfAfCfagaagaacgsgsa		VPudGcedGudTucudGuAfaucuuugsgg		CCACAAGAUUACAAGAAAACGGCU	
AD-1548529.1	csasagaauaCfAfAfgaagaacggsusa		VPudAgedCgdTucudTgUfaucuuugsgg		CACAAGAUUACAAGAAAACGGCUU	
AD-1548530.1	asasgaauacAfAfGfaaacggcususa		VPudAagdCcdGuuudTuGfuauucuuugsu		ACAAGAUUACAAGAAAACGGCUUU	
AD-1548531.1	ususacaagaAfAfCfggcuuucaggsa		VPudCuqdAadAgccgdTuUfcuuuuaasusc		GAUACAAGAAAACGGCUUUCAGU	
AD-1548532.1	usascaagaaAfCfGfgcuuucaggsusa		VPudAcudGadAagcdGuUfucuuuuaasasu		AUUACAAGAAAACGGCUUUCAGUU	
AD-1548533.1	asasacggCfuUfUfCfaguuagagcsusa		VPuAfgcdTc(Agn)acugaaAfcCfcguuucsu		AGAAAACGGCUUUCAGUUAGCUG	
AD-1548534.1	gscsuuucAfgUfUfGfagcuagaccsasa		VPuUfagdTc(Agn)gcucaaCfuGfaaagcsesg		CGGCUUUCAGUUAGCUGAGCCAG	
AD-1548535.1	csusuggacuUfGfAfuaauugugsgsa		VPudGcadCcdAauudCaAfguccaagsasu		AUCUUGGACUUGAUUUGGUGCC	
AD-1548536.1	usasucgcCfaGfGfAfugaucuaasgsa		VPuChuadGg(Agn)ucauceUfgGfcgaauasusc		GAUUCGCCAGGAUGAUUCCUAGC	
AD-1548537.1	csasccacCfcUfGfGfugcuagacusa		VPuUfagdTc(Agn)gcaccaGfgCfugugsgsc		GCCACCACCCUGGUGCUGACUUAU	
AD-1548538.1	asuscagcuGfCfCfugguuugausasa		VPudAuedAadAccagdGcCfagcuagaunusg		CAAUCAGCUGGCCUGGUUUUGAUUA	
AD-1548539.1	uscsagcuGfGfCfUfugguuugausasa		VPuUfaudCa(Agn)accaggCfcAfgcugaususu		AAUCAGCUGGCCUGGUUUUGAUUAC	
AD-1548540.1	uscsageuGfCfUfugguuugausasa		VPudTaudCadAaccadGgCfagcuagaususu		AAUCAGCUGGCCUGGUUUUGAUUAC	
AD-	csusggccUfgGfUfUfugaucuggsasa		VPuUfcaudGu(Agn)ucaaacCfaGfgccagcsesu		AGCUGGCCUGGUUUUGAUUACUGAC	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
1548541.1						
AD-1548542.1	csusguaaaCfAfUfcuuuaggsusa		VPuDAccdTadAaggadTgAfuuuacagsgsu		ACCUGUAAAUCAUCCUUUAGGUA	
AD-1548543.1	usgsuaaauCfUfcuuuaggsusa		VPuDTacdCudAaaggdAuGfauuuacagsgg		CCUGUAAAUCAUCCUUUAGGUA	
AD-1548544.1	asasagacuGfGfUfugguagggsusa		VPuDAccdCudAccaadCcAfagucuuuscsu		AGAAAGACUUGGUUGGUAGGGUG	
AD-1548545.1	asesuuuGfAfAfGfGfagagucususa		VPuAFagdAc(Agn)uuccuUfuCfaaagusasu		AUACUUUGAAAGGAGAUGUCUUG	
AD-1548546.1	asesuuuGaaAfGfGfagagucususa		VPuDAagdAcdAuctcdCuUfucaagusasu		AUACUUUGAAAGGAGAUGUCUUG	
AD-1548547.1	ususcucagAUfUfUfcugguugususa		VPuDAacdAadCcagadAaUfcugagaacsca		UGUUCUCAGAUUUCUGGUUGUUA	
AD-1548548.1	ususcagAfUfUfUfcugguugususa		VPuUfaadCa(Agn)ccagaaAfUcFugagasasc		GUUCUCAGAUUUCUGGUUGUUAU	
AD-1548549.1	ususcagauUfUfUfcugguugususa		VPuDTaadCadAccagdAaAfucugagasasc		GUUCUCAGAUUUCUGGUUGUUAU	
AD-1548550.1	csuscagauUfUfUfcugguuuaususa		VPuDAuadAcdAaccadGaAfaucugagsasa		UUCUCAGAUUUCUGGUUGUUAUG	
AD-1548551.1	gsusuguuauGfUfGfaucauggsusa		VPuDAcadCadTgaucdAcAfuaacaacsca		UGGUUGUUAUGUGAUCAUUGUGUG	
AD-1548552.1	ususcuuauGfGfAfucauguggsa		VPuDCacdAcdAugaudCaCfauaacaacscc		GGUUGUUAUGUGAUCAUUGUGUGG	
AD-1548553.1	gsusgaucAfUfUfUfGfuggauguusasa		VPuUfaadCu(Tgn)ccacacAfUgfaucacsasu		AUGUGAUCAUUGUGUGGAAGUUAU	
AD-1548554.1	gsasucauguGfUfGfagauuaususa		VPuDAudAadCuuccdAcAfcaugaucasasc		GUGAUCAUUGUGUGGAAGUUAUUA	
AD-1548555.1	asuscAugUfGfGfagauuausasa		VPuDTaadTadAcuuccdCaCfacaugauscsc		UGAUCAUUGUGUGGAAGUUAUUA	
AD-1548556.1	usgscaacuAfAfUfacucaaugsusa		VPuDCaudTudGaguadTuAfaguugcasasa		UUUGCAACUUAUACUCAAAUGA	
AD-1548557.1	csesuuuUfUfUfUfuaucaggsca		VPuGfendGu(Agn)uaaagaGfGfaaaggscsu		AGCCUUUCUCUCUUUAUACAGCU	
AD-1548558.1	ususuuaaCfAGfCfUfugaauugcsusa		VPuAFagdCa(Agn)uacageUfgUfuaaagsca		UCUUUAUACAGCUGUAUUGUCUG	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1548559.1	usuuuauacaGfCfUfguaauugncusua		VPudAgadCadAuacadGcUfguaauaasgsa		UCUUUAUACAGCUGUAUUUGUCUG	
AD-1548560.1	asusugueugAfAfCfugueaungusua		VPudAcadAudGcaagdTuCfagacaasasc		GUAUUGUCUGAACUUUGCAUUUGUG	
AD-1548561.1	usuggcccUfgUfAfGfaguuugcuggsasa		VPuUfcadGc(Agn)acucuaCfaGfgecaasusc		GAUUGGCCUGUAGAGUUUGCUGAG	
AD-1548562.1	asgsugcccUfgAfCfAfcaacuaccsasa		VPuUfagdTu(Agn)guguguCfaGfgecacususu		AAAGUGCCCUGACACACUAACCAA	
AD-1548563.1	csusgacacaCfUfAfacaagcuggsa		VPudCagdCudTggundAgUfgugucagsgsc		GCCUGACACACUAACCAAGCUGA	
AD-1548564.1	usasaccaggCfUfGfaguuuccusasa		VPudTagdGadAcucudAgCfuguguuasgsu		ACUAAACCAAGCUGAGUUUCCUAU	
AD-1548565.1	asgsucugaGfuUfUfCfcauagggasasa		VPuUfuedCc(Agn)uaggaaAfcUfcagcususg		CAAGCUGAGUUUCCUAUGGGAAC	
AD-1548566.1	usuuuccuauGfGfGfaacaauugsasa		VPudTcadAudTguudCcAfuaaggaaascsu		AGUUUCCUAUGGGAAACAAUUGAA	
AD-1548567.1	gsusegagGfaGfuUfAfacaauacasasa		VPuUfugdTa(Tgn)uguuacUfcCfucgacscsa		UGGUCGAGGAGUAACAAUACAAA	
AD-1548568.1	gsusecgaggGfuUfAfacaauacasasa		VPudTugdTadTguudAcUfcucgacscsa		UGGUCGAGGAGUAACAAUACAAA	
AD-1548569.1	asusecaaaCfcCfUfAfgecuugcuscusa		VPuAfagdCa(Agn)ggcuagGfGfUfuugausasa		UUAUCAAAACCCUAGCCUUGCUUG	
AD-1548570.1	usuuugcuUfgCfUfUfugaaguagscsa		VPuGfcudAc(Tgn)ucaaaGfAfgecaasgsu		ACUUUGCUUGCUUUUGAAAGUAGCU	
AD-1548571.1	usgsucuuGfuUfUfGfaaguagcuscusa		VPuGfagdCu(Agn)cuucaaAfgCfaagcasasa		UUUGCUUGCUUUUGAAAGUAGCUU	
AD-1548572.1	gscsuugcUfuUfGfAfaguagcuscusa		VPuAfagdGc(Tgn)acuucaAfaGfcaagcsasa		UUUGCUUGCUUUUGAAAGUAGCUU	
AD-1548573.1	asasguencuGfGfUfaguguuuaasgsa		VPudCundAadCacuadCgAfagacuuisasa		UUAAGUCUCUCUGUAGUUAAGU	
AD-1548574.1	uscsucueGfuAfGfUfguuaaguusasa		VPuUfaadCu(Tgn)aacacuAfcGfagagascu		AGUCUCUCUGUAGUGUUAAGUUAU	
AD-1548575.1	csusueugUfaGfuUfGfuuaaguuasasa		VPuAfuaadAc(Tgn)uaacacUfaCfagagascu		GUCUCUCUGUAGUGUUAAGUUAU	
AD-	csusueguagGfuUfGfuuaaguuasasa		VPudAuaadAcdTuaadAcUfaagagagascu		GUCUCUCUGUAGUGUUAAGUUAU	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
1548576.1						
AD-1548577.1	csuscguaGuGfUfaaGuuaaagsa		VPudCuadTadAaauadAcAfcuacgagsag		CUCUCGUAGUGUUAAGUUUAAGU	
AD-1548578.1	csusguaguguUfaAfAfGuuaaagsgsa		VPudCaedTadTaaudTfaAfcuacgagsag		CUCGUAGUGUUAAGUUUAAGUGA	
AD-1548579.1	usasguguUfaAfGfUfaaagugasasa		VPuUfudAc(Tgn)auacuUfaAfcuacsg		CGUAGUGUUAAGUUUAAGUGAAU	
AD-1548580.1	gsusgaauAfcUfGfCfuacagcaasasa		VPuAfuudGc(Tgn)guagcaGfuAfuacsa		UAGUGAAUACUGCUACAGCAAUU	
AD-1548581.1	csusgcuacaGfCfAfaaaucaasasa		VPudAuudAgdAaaudGcUfguacgagsa		UACUGCUACAGCAAUUUCUAAUU	
AD-1548582.1	gsasaungAfgUfaAfAfugguaggsasa		VPuUfudAc(Agn)ccauuaCfuCfaauesusu		AAGAAUUGAGUAAUGGUGUAGAA	
AD-1548583.1	ususgaguAfaUfGfGfuguagaacsasa		VPuUfgudTc(Tgn)acaccaUfuAfcuacsa		AAUUGAGUAAUGGUGUAGAACAC	
AD-1548584.1	gsasguaaUfgGfUfGfuguagaacsasa		VPuAfgudGu(Tgn)cuacacCfaUfuacsa		UUGAGUAAUGGUGUAGAACACUA	
AD-1548585.1	asgsuaauGfgUfGfUfagaacacusa		VPuUfagdTg(Tgn)ucuaaCfcAfuacsa		UGAGUAAUGGUGUAGAACACUAA	
AD-1548586.1	usasauugUfgUfaAfGfaacuaaasasa		VPuAfuudAg(Tgn)guucuaCfaCfaauesusu		AGUAAUGGUGUAGAACACUAAUU	
AD-1548587.1	asusgguguaGfAfcuacuaauesasa		VPudGaadTudAgugdTcUfacaccausasa		UAAUGGUGUAGAACACUAAUUCA	
AD-1548588.1	usgsuguaGfAfcuacuaauesasa		VPudTgadAudTagudTuCfuacaccasusu		AAUGGUGUAGAACACUAAUUCAU	
AD-1548589.1	gsusguagaAfCfAfcuacuaauesasa		VPudAugdAadTuagudGuUfcuacaccasusu		AUGGUGUAGAACACUAAUUCAUA	
AD-1548590.1	gsusguagAfaCfAfcuacuaauesasa		VPuUfaudGa(Agn)uuagugUfuCfuacaccsa		UGGUGUAGAACACUAAUUCAUAA	
AD-1548591.1	usgsuagaAfcAfcUfaaaucauasasa		VPuUfuadTg(Agn)auuaguGfuUfcuacaccsc		GGUGUAGAACACUAAUUCAUAAU	
AD-1548592.1	gsusagaacaCfuUfaaaucauasasa		VPudAuudAudGaaudAgUfguacaccsc		GUGUAGAACACUAAUUCAUAAUC	
AD-1548593.1	usasgaacacUfaAfAfuacuaauesasa		VPudGaudTadTgaudTaGfuguacaccsa		UGUAGAACACUAAUUCAUAAUCA	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1548594.1	asgsaacuAfaUfUfuaaauacsasa		VPudTgadTudAugaadTuAfguguuacsase		GUAGAACACUAAUUCAUAAUUCAC	
AD-1548595.1	asgsaacaCfuAfaUfUfuaaauacsasa		VPuUfgadTu(Agn)ugaaUafgUfguuacsase		GUAGAACACUAAUUCAUAAUUCAC	
AD-1548596.1	gsasacuaAfuUfUfuaaauacsasa		VPudGugdAudTaugadAuUfaguguuacsasa		UAGAACACUAAUUCAUAAUUCACU	
AD-1548597.1	asascacuAfaUfUfCfaaauacsasa		VPuAfgudGa(Tgn)uugaaUfuAfguuacsase		AGAACACUAAUUCAUAAUUCACUC	
AD-1548598.1	asusgguecaAfuUfUfaguuuacsasa		VPudAagdGadAacuadAuUfggaccuacsasa		AAUUGGUCCAAUUAUUAUUCUUU	
AD-1548599.1	usugggauAfuUfGfuaggguaagsa		VPudCuadCcdCauacdAuAfuuccaacsasa		UAUUUGGGAUAUGUAUUGGGUAGG	
AD-1548600.1	usgsuagGfgUfaUfGfguaaacsasa		VPuUfgadTu(Tgn)accuaCfcCfaaacsasa		UAUGUAUGGGUAGGGUAAUUCAG	
AD-1548601.1	asusggguAfgGfgUfaaauacsasa		VPuUfacdTg(Agn)uuuaccCfuAfcuacsase		GU AUGGGUAGGGUAAAUCAGUAA	
AD-1548602.1	gsusaggguaAfaUfUfaguaagagsa		VPudCuadTudAcugadTuUfacecuacsase		GGGUAGGGUAAAUCAGUAAGAGG	
AD-1548603.1	asgsagguaAfaUfCfAfguaagagsasa		VPuAfcdTg(Tgn)uacugaUfuUfacecuacsase		GUAGGGUAAAUCAGUAAGAGGUG	
AD-1548604.1	gsusguaaauCfaUfGfuagagagsasa		VPudCaedCudCuuaedTgAfuuuacsase		UAGGGUAAAUCAGUAAGAGGUGU	
AD-1548605.1	gsusaaaucaGfuUfAfgagaggusasa		VPudAacdAcdCucudAcUfguuuacsase		GGGUAAAUCAGUAAGAGGUGUUA	
AD-1548606.1	asasaucGfuAfaUfGfagguguuacsasa		VPuAfuadAc(Agn)ccuuAfcUfguuuacsase		GUAAAUCAGUAAGAGGUGUUAU	
AD-1548607.1	asacucaguaAfgUfAfguguuuacsasa		VPudAaudAadCaccudCuUfaceuacsase		UAAAUCAGUAAGAGGUGUUAU	
AD-1548608.1	csasguaaagaGfgUfUfguuuuuagsa		VPudCeadAadTaacadCeUfuuuacsase		AUCAGUAAGAGGUGUUAUUGGA	
AD-1548609.1	usuggacAfuGfgCfcAuggaacsasa		VPuGfgdTg(C2p)auggccAfuGfuacsase		AGUUGGACAUGGGCAUGGAACCA	
AD-1548610.1	usgsacuCfuGfgAfaucuuacsasa		VPuAfgadAu(G2p)uauuaccAfgAfguacsase		CCUGGACUCUGGAAUCCAUUCUG	
AD-	csuscugaGfuGfgUfaaaggcaacsasa		VPuAfuudGc(C2p)uuuaccAfcUfcagagsasa		UUCUCUGAGUGGUAAAGGCAUUC	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
1548611.1						
AD-1548612.1	uscsaagaAfcAfaGfugcugausasa		VPuUfaudCa(G2p)ucauuGfuUfcuagsgsu		ACUCAAGAACAAAGUAGCUGAUUAU	
AD-1548613.1	usasgcugAfuAfuUfUfgauggacagsa		VPuCfugdTe(C2p)aucaauAfuCfagcuascsu		AGUAGCUGAUUAUUGAUGGACAGU	
AD-1548614.1	asgscugaUfaUfuUfGfauggacagsusa		VPuAfcudGu(C2p)caucaaUfaUfcagcuscsc		GUAGCUGAUUAUUGAUGGACAGUA	
AD-1548615.1	csusgaauUfuGfAfuUfgacagauasusa		VPuAfuadCu(G2p)uccaueAfaUfaucagcsesu		AGCUGAUUAUUGAUGGACAGUAUG	
AD-1548616.1	usgsauggAfcAfgfUfaugcaaugasasa		VPuUfcadTu(G2p)cauacuGfuCfcaucasasu		AUUGAUGGACAGUAUGCAAUGAC	
AD-1548617.1	asgsuaugCfaAfuUfGfaucgagcsusa		VPuAfgcdTe(C2p)agucuuUfgCfaucucsgsu		ACAGUAUGCAAUGACUCGAGCUC	
AD-1548618.1	asgsucaGfaGfGfGfuaugagcugsa		VPuCfagdCu(C2p)guacccUfcUfgagcuscsg		CGAGCUCAGAGGGUACGAGCUGC	
AD-1548619.1	csuscagaGfgGfuUfAfcgagcugcsusa		VPuAfgcdAg(C2p)ueguacCfcUfcuagcsesu		AGCUCAGAGGGUACGAGCUGCUA	
AD-1548620.1	asgsaggUfaCfGfAfgcugcuauusgsa		VPuCfaudAg(C2p)agucuuUfaCfcucucsgsa		UCAGAGGGUACGAGCUGCUAUGU	
AD-1548621.1	gscsuauUfuCfCfCfugagacausasa		VPuAfaudGu(C2p)ueaggGfAfaCfaucagesag		CUGCUAUGUUCUCCUGAGACAUUA	
AD-1548622.1	asgscguuUfgGfCfUfgaaccacsa		VPuUfgadTg(G2p)uucageCfaAfacucsgsg		CCAGCGUUUGGCUGAACCACAC	
AD-1548623.1	usgscugaAfaCfaUfUfgcaguuusasa		VPuUfacedAa(C2p)ugcaugUfuUfcagcuscsc		GAUGCUGAAACAUAGCAGUUGUAA	
AD-1548624.1	csasugcaGfuUfgfUfaaacuugasusa		VPuAfuadAa(G2p)uuuacaAfcUfgcagcsusu		AACAUGCAGUUUGUAAACUUGAUU	
AD-1548625.1	gscsaguuGfuAfaAfcuugauuasasa		VPuUfuadAu(C2p)aguuuuAfcAfacucgsasu		AUGCAGUUUGUAAACUUGAUUAAC	
AD-1548626.1	usgsauuaAfcUfaUfUfcaagauasusa		VPuAfuadAu(C2p)uugauaGfuUfaucasag		CUUGAUUAACUUAUCAAGAUGAUG	
AD-1548627.1	csasgaacUfuGfCfCfacagucgsasa		VPuUfgedAc(G2p)uugugcAfaGfuucugscsa		UGCAGAAACUUGCCACACGUGCAA	
AD-1548628.1	csascagUfgCfAfaUfueccuagcsa		VPuGfuadCa(G2p)ggauugCfaCfugugugsgsc		GCCACACGUGCAAUCCCCUGAACU	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1548629.1	gsusgcaaUfcCfcUfugaacugacsasa		VPuUfgudCa(G2p)uucaggGfaUfugcacsusu		ACGUGCAAUCCCCUGAACUGACAA	
AD-1548630.1	gsasggacCfaGfGfUfgugguuasasa		VPuUfuadAc(C2p)accaccUfgGfuccucsusu		ACGAGGACCAGGUGGUGGUAAU	
AD-1548631.1	gsusggugGfuUfAfAfuaaggcugscsa		VPuGfcadGc(C2p)uuuuuaAfcCfaccacsusu		AGGUGGUGGUUAAUAAAGGUGCA	
AD-1548632.1	usgscagaAfuAfcAfafaugaugusasa		VPuUfacedAu(C2p)uuuuuuAfuUfugcacsusu		CAUGCAGAAUACAAAUGAUGUAG	
AD-1548633.1	asgsaauaCfaAfAfUfgauguagacsasa		VPuUfuedTa(C2p)aucauuUfgUfauuucsgc		GCAGAAUACAAAUGAUGUAGAAA	
AD-1548634.1	usgsauguAfgAfAfafagcugcugusasa		VPuAfacdGa(G2p)cuguuuUfuAfaucacsusu		AAUGAUGUAGAAACAGCUCGUUG	
AD-1548635.1	csusugcaUfaAfCfCfuuuuccaacsasa		VPuGfandGg(C2p)aaagguUfaUfgcaagsusu		ACCUUGCAUAAACUUUCCCCAUCA	
AD-1548636.1	ususgcauAfaCfCfUfuucccaacsasa		VPuUfgadTg(G2p)gaagguUfuAfucaasgsg		CCUUGCAUAAACUUUCCCCAUCAU	
AD-1548637.1	cscsaucUfuAfAfGfugcugggagscsa		VPuGfecdTc(C2p)agacuuAfaAfgaugsgsc		GGCCAUCUUUAAAGUCUGGAGGCA	
AD-1548638.1	gscsaauAfcAfAfCfuccaccacsasa		VPuUfgudTg(G2p)agaguuGfuAfaugcgsasu		AUGCCAUUACAACUCUCCACAAC	
AD-1548639.1	gsusugceUfuGfCfUfaacaaaaacsasa		VPuGfuudTu(G2p)uugagcAfaGfugcaacsasa		UGGUUGCCUUGCUCACAAACAA	
AD-1548640.1	asusucuuGfgCfUfAfuaagcagacsasa		VPuCfugdTc(G2p)uuuuuagCfcAfagaacsusu		AAAUUCUUGGCUAUUACGACAGA	
AD-1548641.1	asasaauAfaUfGfAfaggaccuauasasa		VPuUfandAg(G2p)uccucaUfuAfuaauusasc		GUAAAUAUAUGAGGACCUAUAC	
AD-1548642.1	asasuuaAfuGfAfGfaccuauasasa		VPuGfuadTa(G2p)guccucAfuUfaauuusasa		UAAAUAUAUGAGGACCUAUACU	
AD-1548643.1	gscsagagUfgCfUfGfaaggugcuscasa		VPuUfagdCa(C2p)uuuagCfCfucugcuscusu		AAGCAGAGUGCUGAAGGUGCUAU	
AD-1548644.1	ususcugGfuUfCfAfagaugauasasa		VPuUfandAu(C2p)aucugaAfcCfagaagsgc		GCUUCUGGGUUCAGAUUAUAA	
AD-1548645.1	gsasugauAfuAfAfAfugugcuscasa		VPuGfugdAc(C2p)acuuuAfuAfucacsusu		CAGAUGAUUAAAUGUGGUCACC	
AD-	ggsaauuUfuUfCfCfuaaccucacsasa		VPuGfugdAg(G2p)uuuagaaAfgAfauuccsag		CUGGAUUUUUUUUUAACCUACU	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
1548646.1						
AD-1548647.1	uscsmucUfaAfcCfucacucgcsasa		VPuUfgcdAa(G2p)ugagguUfaGfaagagasu		AUUCUUUCUAACCUCACUUGCAA	
AD-1548648.1	ususcuaaCfcUfCfAfcuugcauasasa		VPuUfandTg(C2p)aagugaGfgUfuagaasag		CUUUCUAACCUCACUUGCAAUA	
AD-1548649.1	uscuaacCfuCfAfcfuagcauasasa		VPuUfuadTu(G2p)caagugAfgGfuagagasasa		UUUCUAACCUCACUUGCAAUAU	
AD-1548650.1	asagugGfuGfGfUfaugagagcsusa		VPuAfgcdCu(C2p)uauaccAfcCfcauugsg		CCAAGUGGGUGGUUAAGAGGGCUC	
AD-1548651.1	usgsgugGfuAfuAfgagcucucusa		VPuAfgadAg(C2p)cucuaAfcCfaccascsu		AGUGGGUGGUUAAGAGGCUCUUG	
AD-1548652.1	asgscugCfcAfUfCfugugcucusa		VPuAfgadAg(C2p)acagauGfgCfaggcuscasa		UGAGCCUGCCAUUCUGUGCUCUUC	
AD-1548653.1	gscscugCfaUfCfUfgugcucucusa		VPuGfaadGa(G2p)cacagaUfgGfcaggcsusc		GAGCCUGCCAUUCUGUGCUCUUCG	
AD-1548654.1	csasucugUfgCfuCfCfuugcuaucusa		VPuGfaudGa(C2p)gagagCfaCfagaugsgsc		GCCAUCUGUGCUCUUGCUCUUCU	
AD-1548655.1	gscsucuuCfcUfCfAfucugaccagasa		VPuChugdGu(C2p)agaugaCfcAfaagagcsasc		GUGCUCUUCGUCUUCUGACCCAGC	
AD-1548656.1	asusgcagUfuCfGfCfucucacuasasa		VPuAfuadGu(G2p)aaggggAfaCfugcaususc		GAAUGCAGUUCGCCUUCACUUAUG	
AD-1548657.1	usgsguuaAfgCfuCfCfuacaccsasa		VPuUfggdGu(G2p)uagagCfuUfaaccascasa		UGUGGUUAAGCUCUUAACACCCAC	
AD-1548658.1	csusguugGfaUfuGfauugagaasasa		VPuAfuudTe(G2p)aucaaaUfcCfaacagsusa		UACUGUUGGAUUGAUUCGAAAUC	
AD-1548659.1	usgsuugAfuUfGfAfuugcaaaucasa		VPuGfaudTu(C2p)gaaucaAfuCfaacagsu		ACUGUUGGAUUGAUUCGAAAUCU	
AD-1548660.1	csugsugagCfaGfGfGfugccauucscsa		VPuGfgadAu(G2p)gcaccUfgCfucacgsasa		UGCGUGAGCAGGGUGCCAUUCCA	
AD-1548661.1	csacsagCfuAfgUfUfucaguugcsusa		VPuAfgcdAa(C2p)ugaacuAfgUfcguggsasa		UUCCACGACUAGUUCAGUUGCUU	
AD-1548662.1	ususcaguUfgCfuUfUfugucugcsasa		VPuUfgcdAc(G2p)aaacagCfaAfcuagascsu		AGUUCAGUUGCUUUGUUCGUGCAC	
AD-1548663.1	uscsaguuGfcUfuUfGfugugcascasa		VPuGfuadCa(C2p)gaacaaGfcAfacugasasc		GUUCAGUUGCUUUGUUCGUGCACA	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1548664.1	asgsuugcUfuGfUfUfcgugcacasusa		VPuAfuadTg(C2p)acgaacAfaGfcaacugsa		UCAGUUGCUUGUUCGUGCACAUC	
AD-1548665.1	csasgaggAfcUfAfAfaucacuuuscsa		VPuGfcaadTg(G2p)uauuaaGfuCfcaucugsasu		AUCAGAGGACUAAAUACCAUUC	
AD-1548666.1	gsascuaaAfuAfcCfcauuccauusgsa		VPuGfcaadTg(G2p)aaugguAfuUfuagucscsu		AGGACUAAAUACCAUUCCAUUGU	
AD-1548667.1	cscsauncCfaUfUfGfuungugcagsa		VPuGfcaadCa(C2p)aaacaaUfgGfaaunggsusa		UACCAUUCCAUUGUUGUUGCAGC	
AD-1548668.1	asusuccaUfuGfUfUfugugcagcsusa		VPuAfgcdTg(C2p)acaacAfaUfggaungsg		CCAUUCCAUUGUUGUUGCAGCUG	
AD-1548669.1	ususcacUfUfUfUfugcagcagcsusa		VPuAfgcdCu(G2p)cacaaaCfaAfuugaasug		CAUUCCAUUGUUGUUGCAGCUGC	
AD-1548670.1	cscsaungUfuUfGfUfUfgcagcugcsusa		VPuAfgcdAg(C2p)ugcacaAfaCfaaunggsasa		UUCCAUUGUUGUUGCAGCUGCUU	
AD-1548671.1	gscscacaGfcUfCfCfucugacagsasa		VPuUfcudGu(C2p)agaggaGfcUfugugcsusc		GAGCCACAGCUCCUCUCUGACAGAG	
AD-1548672.1	asasgccaCfaAfcGfAfuuaaagsasa		VPuUfcdTu(G2p)uaaucuUfgUfggcuungsu		ACAAGCCACAAGAUUACAAGAAA	
AD-1548673.1	cscsacaaGfaUfUfAfcagaacagsa		VPuUfcudTu(C2p)uunguaaUfcUfugugcsusc		AGCCACAAGAUUACAAGAAAACGG	
AD-1548674.1	asasgaauAfcAfaGfcaacggcuscusa		VPuAfgcdCc(G2p)uuuucuGfuAfaucuuugsu		ACAAGAUUACAAGAAAACGGCUUU	
AD-1548675.1	asusuaaAfgAfaAfcggcuuucsasa		VPuUfgadAa(G2p)ccguuuChuUfguaauuscu		AGAUAUACAAGAAAACGGCUUUACAG	
AD-1548676.1	gsgscuunCfaGfUfUfugcugacscsa		VPuGfcdCa(C2p)cuaacUfgAfaagccsgsu		ACGGCUUUCAGUUGAGCUGACCA	
AD-1548677.1	usgsagcuGfaCfCfAfcgucucucusa		VPuAfgadGa(G2p)agcuggUfcAfgcucasasc		GUUGAGCUGACCAAGCUCUCUCUU	
AD-1548678.1	gsasgccaAfuGfGfCfuggauggsasa		VPuUfcadTu(C2p)caagccAfuUfggcuungsg		CAGAGCCAAUGGCUUGGAAUAGAG	
AD-1548679.1	csusgaucUfuGfGfAfcungauaususa		VPuAfaudAu(C2p)aaguccAfaGfaucagsasa		UGCUGAUCUUGGACUUGAUUUG	
AD-1548680.1	usgsuuuGfaUfAfcfugaccugusasa		VPuUfcadAg(G2p)ueaguaUfcAfaaccasgsg		CCUGGUUUGAUACUGACCUGUAA	
AD-	ususugauAfcUfGfAfcuguaaasusa		VPuAfuudTa(C2p)agguaCfuAfucaaaesc		GGUUGAUACUGACCUGUAAAUC	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
1548681.1						
AD-1548682.1	gsgscuaUfuGfuAfauucgucscsa		VPuUfggdCa(G2p)auuuacAfaAfugccsusa		UAGGCUAUUUUUAUUGCCAC	
AD-1548683.1	ususucugGfuUfgfUfuugugauscsa		VPuGfandCa(C2p)auaacaAfcCfagaasusc		GAUUCUGGUUGUUAUGUGAUCA	
AD-1548684.1	gsasucanGfuGfuGfagaaguuauusasa		VPuAfaudAa(C2p)uuccacAfcAfugaesasc		GUGAUC AUGUGUGGAAGUUAUUA	
AD-1548685.1	usasauacUfcAfAfAfugaguuacsasa		VPuUfgudTa(C2p)ucauuuGfaGfuauuasasc		CUUAAUACUCAA AUGAGUAAACAU	
AD-1548686.1	asusugucUfgAfAfCfugcauugusasa		VPuAfcadAu(G2p)caaguuCfaGfacausasc		GU AUUGUCUGAACUUGCAUUGUG	
AD-1548687.1	csasgaagGfuGfCfCfugacacacsasa		VPuAfgudGu(G2p)ucaggeAfcUfuucugsasc		CUCAGAAAGUGCCUGACACACUA	
AD-1548688.1	gsasaaguGfcCfuUfGfacaacuasasa		VPuUfuadGu(G2p)ugucagGfcAfcuuucusc		CAGAAAGUGCCUGACACACU AAC	
AD-1548689.1	gscscugaCfaCfAfCfuacacacsasa		VPuGfendTg(G2p)uuagugUfgUfcaggesasc		GUGCCUGACACACUAACCAAGCU	
AD-1548690.1	usgsggaCfaAfUfUfgaaguuascsa		VPuGfuudTa(C2p)uucaauUfgUfuccasusa		UAUGGGAACAAUUGAAGUAAACU	
AD-1548691.1	asusggauCfaCfAfAfgauggaauusasa		VPuAfaudTe(C2p)aucuugUfgAfuccaususc		GAAUGGAUCACAAAGUUGGAAUUU	
AD-1548692.1	ususuaucAfAfCfCfuaagccuugsa		VPuGfaadGg(C2p)uaggguUfuGfauaaususu		AAUUUAUCAAACCCUAGCCUUGC	
AD-1548693.1	usasucugUfaAfUfGfuaucugacsusa		VPuAfgudCa(G2p)uaccanUfaCfagaauasusu		AAUAUCUGUAAUGGUACUGACUU	
AD-1548694.1	ususgcuuGfcUfuUfUfgaagugacsusa		VPuAfgcdTa(C2p)uucaaaGfcAfagcaasasc		CUUUGCUUGCUUUUGAAGUAGCUC	
AD-1548695.1	asgsuguuAfAfGfuUfaugugaausasa		VPuAfuudCa(C2p)uuuaacUfuAfacausasc		GUAGUGUUAAGUUAUAGUGAAUA	
AD-1548696.1	usgsuuuaGfuUfAfUfagugaausasa		VPuGfuudTu(C2p)acuuaaAfcUfuacascu		AGUGUUAAGUUAUAGUGAAUACU	
AD-1548697.1	gsusaagGfuGfuUfAfgaacacuasasa		VPuUfuadGu(G2p)uucuaacAfcCfauuaesusc		GAGUAAUGGUGUAGAACACUAAU	
AD-1548698.1	asasugguGfuAfGfAfacacuaausasa		VPuAfaudTa(G2p)ugnuuacAfcAfccausasc		GUAAUGGUGUAGAACACUAAUUC	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1548699.1	csusaauCfaUfAfAfucacucuasasa		VPuUfuadGa(G2p)ugaauaUfgAfauuagsug		CACUAAUUCAUAAUCACUCUAAU	
AD-1548700.1	ususugggAfuAfUfGfuauaggguasgsa		VPuUfuadCc(C2p)auacauAfuCfcaasasusa		UAUUUGGGAUUUGUAUGGGUAGG	
AD-1548701.1	gsgsguaaAfuCfAfGfuaagaggusgsa		VPuUfuadCu(C2p)uuacugAfuUfuacccesusa		UAGGGUAAAUCAGUAAAGAGGGUGU	
AD-1548702.1	gsusaauCfaGfUfAfagaggugususa		VPuUfuadAc(C2p)ucuuacUfgAfuuuacsesc		GGGUAAAUCAGUAAAGAGGGUGUUA	
AD-1548703.1	usasaauCfGfUfAfagagguguusasa		VPuUfuadCa(C2p)cucuuaCfuGfuuuacsesc		GGUAAAUCAGUAAAGAGGGUGUUAU	
AD-1548704.1	asasucagUfaAfGfAfagguguuaususa		VPuUfuadAa(C2p)accucuUfaCfugaauususa		UAAAUCAGUAAAGAGGGUGUUAUUU	

Table 4. Single Dose Screens in Hep3b Cells

	10 nM		1 nM		0.1 nM	
Duplex	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV
AD-1548396.1	6.38	1.03	8.14	0.85	7.67	0.28
AD-1548453.1	7.45	0.60	8.67	0.85	8.67	1.01
AD-1548515.1	6.52	0.20	8.04	1.05	13.41	1.52
AD-1548392.1	7.49	0.99	8.81	0.97	8.79	1.23
AD-1548503.1	5.63	0.33	6.74	0.42	9.09	1.34
AD-1548426.1	6.57	0.29	9.69	1.48	9.30	0.62
AD-1548440.1	9.93	1.75	10.55	0.90	9.50	0.33
AD-1548487.1	3.41	0.36	5.58	0.39	9.73	0.65
AD-1548513.1	6.68	0.55	10.00	1.36	13.85	1.46
AD-1548516.1	7.28	0.80	10.11	0.56	19.31	1.98
AD-1548404.1	10.39	0.55	11.08	1.12	10.02	0.97
AD-1548471.1	6.96	0.44	11.22	1.12	14.67	1.32
AD-1548512.1	8.02	0.18	10.54	1.63	16.98	1.85
AD-1548511.1	6.66	0.68	8.59	0.09	15.71	2.44
AD-1548414.1	14.20	1.19	14.68	1.56	13.32	1.68
AD-1548393.1	7.67	0.71	9.02	0.41	10.19	0.68
AD-1548672.1	6.39	0.31	7.62	0.80	10.46	1.71
AD-1548408.1	9.44	1.32	11.92	0.84	10.66	0.65
AD-1548372.1	9.55	1.53	12.01	0.64	10.04	1.96
AD-1548459.1	5.18	0.60	9.67	1.51	12.19	0.56
AD-1548488.1	3.62	0.46	8.29	1.49	10.67	1.06
AD-1548450.1	7.29	1.92	9.29	1.12	10.88	1.02
AD-1548419.1	9.67	1.28	10.25	1.16	10.88	1.07
AD-1548442.1	8.85	1.52	12.38	1.81	10.94	2.21
AD-1548397.1	7.99	0.70	11.90	1.06	10.98	0.95
AD-1548371.1	13.85	1.90	12.69	0.88	11.73	2.51
AD-1548416.1	10.39	0.46	10.76	0.71	13.19	1.85
AD-1548413.1	10.47	0.91	12.09	0.88	10.85	0.79
AD-1548458.1	6.58	0.54	9.68	0.55	12.90	2.99
AD-1548375.1	7.69	0.87	11.20	1.02	11.89	1.60
AD-1548612.1	11.49	1.89	15.86	0.60	11.90	4.52
AD-1548378.1	9.35	1.53	9.44	0.76	11.98	1.88
AD-1548438.1	11.23	0.85	12.12	1.18	12.01	1.26
AD-1548407.1	9.40	1.08	11.16	0.68	12.06	0.22
AD-1548627.1	9.40	1.33	12.63	2.23	12.18	4.01
AD-1548374.1	11.24	1.10	12.55	0.65	12.19	1.35
AD-1548384.1	12.70	1.74	13.02	0.75	12.21	1.36
AD-1548452.1	12.80	1.34	11.34	0.47	12.28	0.89
AD-1548456.1	5.17	0.70	7.42	2.27	11.28	2.45
AD-1548441.1	8.35	1.39	11.01	1.49	12.35	1.24
AD-1548457.1	5.68	0.27	6.23	1.71	11.24	3.32

Duplex	10 nM		1 nM		0.1 nM	
	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV
AD-1548510.1	6.38	0.21	7.57	0.58	11.50	0.23
AD-1548486.1	5.65	0.50	9.61	2.34	20.32	0.74
AD-1548379.1	15.88	2.14	14.66	1.39	12.56	1.61
AD-1548460.1	4.93	0.41	7.78	0.95	12.74	1.35
AD-1548420.1	10.96	1.03	12.19	0.77	12.82	0.92
AD-1548367.1	9.70	1.48	9.48	1.53	12.87	2.76
AD-1548385.1	13.75	0.39	12.75	1.83	12.88	2.32
AD-1548444.1	9.02	2.60	11.81	0.97	12.99	2.01
AD-1548649.1	4.50	0.55	8.05	1.03	13.24	1.43
AD-1548530.1	4.98	0.39	6.79	0.69	13.34	1.48
AD-1548412.1	11.99	2.06	12.72	0.64	13.36	0.53
AD-1548634.1	7.14	1.01	8.32	1.00	13.42	2.35
AD-1548489.1	4.83	0.68	8.46	1.23	13.56	2.12
AD-1548515.2	6.37	0.93	6.60	0.40	13.65	1.03
AD-1548647.1	4.83	0.46	5.58	0.60	13.72	1.92
AD-1548434.1	8.56	1.46	10.73	0.82	13.80	1.17
AD-1548636.1	8.56	0.90	7.90	0.63	14.00	2.14
AD-1548373.1	9.78	1.54	12.36	1.29	14.09	2.93
AD-1548462.1	6.93	0.64	7.82	0.54	14.19	1.42
AD-1548493.1	6.44	0.54	7.75	1.04	14.36	2.94
AD-1548380.1	12.76	2.64	12.82	3.47	14.70	1.39
AD-1548661.1	5.76	0.49	8.06	1.18	14.74	5.30
AD-1548646.1	6.74	0.66	8.04	0.56	15.03	2.10
AD-1548628.1	11.04	0.92	13.58	1.76	15.18	0.54
AD-1548568.1	9.90	0.86	13.13	0.54	15.29	1.19
AD-1548656.1	7.95	0.91	11.71	2.24	15.67	2.39
AD-1548389.1	11.48	0.95	13.50	2.69	15.77	1.34
AD-1548498.1	4.87	0.76	10.24	0.99	15.86	2.71
AD-1548654.1	14.94	0.85	14.24	1.57	16.04	3.97
AD-1548508.1	7.63	0.60	9.04	1.56	16.33	3.64
AD-1548479.1	5.68	0.36	13.48	1.31	16.35	0.88
AD-1548406.1	12.96	1.18	14.48	2.52	16.56	2.24
AD-1548623.1	9.27	0.86	15.72	2.21	16.81	2.08
AD-1548524.1	7.63	0.39	9.67	0.70	17.00	1.22
AD-1548667.1	5.29	0.39	6.76	1.52	17.09	1.86
AD-1548417.1	13.99	0.91	15.81	1.89	17.18	1.89
AD-1548463.1	6.24	0.58	10.25	1.35	17.21	0.78
AD-1548395.1	13.88	2.73	18.28	2.39	17.40	0.89
AD-1548531.1	6.98	0.83	9.32	0.72	17.42	2.09
AD-1548451.1	15.00	1.09	16.43	1.70	17.68	1.08
AD-1548472.1	10.59	1.11	11.66	0.63	17.75	1.96
AD-1548509.1	6.57	0.52	10.31	1.27	17.82	2.07

Duplex	10 nM		1 nM		0.1 nM	
	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV
AD-1548418.1	21.86	2.87	20.91	2.73	17.93	1.86
AD-1548673.1	6.19	1.10	9.52	1.04	17.97	4.26
AD-1548529.1	5.73	0.22	8.13	1.82	17.99	2.17
AD-1548491.1	7.40	0.70	10.89	0.54	18.26	1.46
AD-1548477.1	8.26	1.06	12.06	0.51	18.43	1.18
AD-1548522.1	6.47	0.54	9.15	0.28	18.48	1.71
AD-1548484.1	9.90	0.92	12.49	1.00	18.56	0.30
AD-1548644.1	6.13	1.28	8.70	0.18	19.07	3.28
AD-1548523.1	7.43	0.63	10.91	0.55	19.17	1.07
AD-1548492.1	5.55	0.40	10.03	1.65	19.35	1.98
AD-1548633.1	9.62	1.25	16.60	1.14	19.74	2.02
AD-1548635.1	9.48	1.36	11.04	1.83	19.75	4.14
AD-1548481.1	7.80	1.00	13.18	1.50	20.01	1.84
AD-1548443.1	19.56	1.55	21.60	0.56	20.22	2.30
AD-1548631.1	12.52	1.44	17.76	2.09	20.26	0.37
AD-1548526.1	7.39	0.42	10.58	0.86	20.33	3.06
AD-1548657.1	12.13	0.57	18.77	2.33	20.56	2.80
AD-1548587.1	9.33	1.31	17.28	0.88	20.68	1.12
AD-1548467.1	8.27	0.74	10.99	1.09	20.77	1.28
AD-1548629.1	15.55	0.86	16.64	2.21	20.78	1.95
AD-1548525.1	6.95	0.52	12.95	1.92	20.96	1.38
AD-1548376.1	11.36	2.27	14.99	2.74	21.14	2.87
AD-1548577.1	10.56	1.16	17.41	2.87	21.30	1.21
AD-1548527.1	6.72	0.85	11.13	1.04	21.31	2.80
AD-1548391.1	13.61	1.66	13.79	1.99	21.35	3.20
AD-1548598.1	15.53	1.28	18.39	1.40	21.47	2.29
AD-1548464.1	7.48	0.76	13.54	1.25	21.74	3.29
AD-1548495.1	5.64	0.78	12.72	1.19	21.85	0.68
AD-1548519.1	6.82	0.99	11.39	1.46	21.94	1.05
AD-1548674.1	10.13	0.44	11.33	0.76	22.04	2.25
AD-1548645.1	6.81	0.91	8.44	0.75	22.08	3.60
AD-1548368.1	19.56	1.04	14.07	6.45	22.43	4.40
AD-1548639.1	9.21	0.99	8.58	1.79	22.58	2.32
AD-1548500.1	9.39	1.45	13.35	1.85	22.70	4.33
AD-1548592.1	9.53	0.72	16.19	1.92	22.86	2.98
AD-1548663.1	11.04	1.16	15.07	2.45	23.09	7.78
AD-1548664.1	9.26	0.56	10.71	1.03	23.13	5.77
AD-1548437.1	10.58	0.73	15.25	2.29	23.28	4.19
AD-1548650.1	16.55	1.64	19.93	0.53	23.31	2.49
AD-1548470.1	8.86	0.30	11.95	1.73	23.40	1.85
AD-1548400.1	24.27	3.49	24.69	1.81	23.50	0.87
AD-1548447.1	15.34	1.63	17.24	2.43	23.54	1.51

Duplex	10 nM		1 nM		0.1 nM	
	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV
AD-1548490.1	6.08	0.39	10.68	1.64	23.58	3.59
AD-1548411.1	16.12	0.98	17.98	1.22	23.62	2.94
AD-1548478.1	10.55	0.36	16.18	1.05	23.64	1.08
AD-1548439.1	20.22	1.13	17.45	2.57	23.82	4.15
AD-1548528.1	8.01	0.84	12.14	1.29	24.05	1.11
AD-1548625.1	10.53	1.11	16.61	2.55	24.30	4.54
AD-1548651.1	10.00	1.04	14.27	0.50	24.40	2.83
AD-1548666.1	8.37	0.75	11.28	1.30	24.54	3.77
AD-1548473.1	8.07	1.74	16.17	1.87	24.56	1.26
AD-1548475.1	12.55	1.55	18.97	3.74	24.83	4.38
AD-1548507.1	9.05	0.82	11.94	0.61	24.86	1.23
AD-1548415.1	18.37	1.98	23.35	2.11	24.93	0.83
AD-1548432.1	15.29	1.47	17.18	3.00	24.98	2.37
AD-1548621.1	11.78	1.95	16.47	1.38	25.01	2.42
AD-1548421.1	14.29	1.36	20.69	1.98	25.33	2.94
AD-1548589.1	12.69	0.62	20.37	1.13	25.39	1.96
AD-1548542.1	8.92	0.54	14.23	1.21	25.50	1.82
AD-1548584.1	17.11	1.13	21.38	3.49	25.53	3.97
AD-1548662.1	14.97	2.02	18.28	1.63	25.80	6.01
AD-1548581.1	12.26	0.55	21.88	2.80	25.84	3.91
AD-1548640.1	8.81	1.67	8.58	2.43	25.87	5.07
AD-1548402.1	14.54	0.69	17.32	2.59	26.09	2.01
AD-1548632.1	13.51	1.05	21.62	1.40	26.72	6.06
AD-1548578.1	10.78	1.04	20.42	1.28	26.73	3.01
AD-1548593.1	11.74	1.09	19.67	1.47	26.78	3.58
AD-1548377.1	12.46	2.91	19.46	2.23	26.81	2.92
AD-1548496.1	11.31	1.64	14.83	1.74	26.95	3.35
AD-1548388.1	21.97	1.07	26.08	2.29	27.05	2.96
AD-1548590.1	12.25	1.59	21.74	1.53	27.21	2.34
AD-1548658.1	5.22	0.39	10.51	0.81	27.25	6.98
AD-1548605.1	20.18	0.67	29.82	1.60	27.41	3.01
AD-1548622.1	16.42	2.02	23.12	1.89	27.48	2.31
AD-1548538.1	8.58	1.48	12.92	1.65	27.62	3.04
AD-1548638.1	7.55	1.28	8.47	1.01	27.71	7.94
AD-1548585.1	15.28	1.54	21.00	3.11	27.97	3.47
AD-1548648.1	8.61	0.86	12.71	2.60	28.14	5.02
AD-1548571.1	17.57	2.16	23.78	3.76	28.29	3.82
AD-1548543.1	11.67	1.08	18.29	2.71	28.79	2.01
AD-1548680.1	7.08	0.83	7.68	0.62	28.83	3.59
AD-1548449.1	14.11	0.95	20.05	2.50	28.85	4.29
AD-1548660.1	13.84	1.14	20.22	0.80	28.94	7.75
AD-1548390.1	19.25	2.84	24.73	1.74	28.95	1.41

Duplex	10 nM		1 nM		0.1 nM	
	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV
AD-1548570.1	17.42	2.96	24.87	1.44	28.96	3.94
AD-1548588.1	14.55	1.40	26.35	2.36	28.97	3.87
AD-1548573.1	21.45	0.94	24.36	3.10	29.06	3.55
AD-1548483.1	9.85	0.27	16.80	1.43	29.46	3.36
AD-1548596.1	11.62	0.67	22.62	3.55	30.17	4.83
AD-1548607.1	17.82	0.85	26.01	3.58	30.36	5.10
AD-1548659.1	11.61	1.07	19.23	0.82	30.44	2.75
AD-1548668.1	9.82	1.11	13.03	1.92	30.49	4.08
AD-1548608.1	14.54	1.63	24.25	0.79	30.72	3.79
AD-1548675.1	7.84	1.33	10.33	0.68	30.92	12.04
AD-1548610.1	14.80	1.79	23.80	3.02	31.45	4.54
AD-1548366.1	26.87	5.12	21.19	5.66	31.64	4.05
AD-1548381.1	21.26	3.33	25.24	2.13	31.66	3.31
AD-1548572.1	13.12	1.54	22.46	0.38	31.93	5.76
AD-1548583.1	10.91	1.01	21.08	1.62	31.99	6.79
AD-1548536.1	12.87	0.64	15.24	2.42	32.44	2.87
AD-1548466.1	13.51	1.91	22.00	4.72	32.45	6.26
AD-1548403.1	16.94	2.15	22.97	1.86	32.70	2.72
AD-1548430.1	15.62	0.93	26.92	2.40	32.91	3.08
AD-1548665.1	11.39	1.00	16.60	0.86	32.98	3.84
AD-1548617.1	14.63	0.73	23.69	1.99	33.31	4.86
AD-1548695.1	10.55	1.10	15.38	1.12	33.33	3.42
AD-1548435.1	18.39	2.95	24.84	2.05	33.51	5.08
AD-1548678.1	11.29	1.58	20.13	1.17	33.88	12.74
AD-1548576.1	16.46	0.92	21.96	2.80	34.05	2.61
AD-1548405.1	43.97	2.86	30.82	1.28	34.28	5.50
AD-1548567.1	19.94	0.70	33.92	6.25	34.39	6.91
AD-1548398.1	40.94	2.30	34.52	3.56	34.48	4.35
AD-1548676.1	11.28	1.95	18.41	3.54	34.77	7.34
AD-1548703.1	13.68	1.10	16.41	1.30	35.02	3.94
AD-1548679.1	7.46	2.31	9.27	1.49	35.07	3.99
AD-1548653.1	11.97	1.36	19.94	1.39	35.27	11.85
AD-1548468.1	14.09	2.45	20.35	2.02	35.30	4.07
AD-1548424.1	21.68	0.33	25.41	1.54	35.35	1.98
AD-1548469.1	26.40	1.17	32.38	4.08	35.43	1.11
AD-1548559.1	27.67	2.64	34.28	4.55	35.58	4.63
AD-1548423.1	16.10	2.29	24.32	4.06	35.94	3.82
AD-1548582.1	17.06	1.23	28.34	3.52	36.25	7.23
AD-1548382.1	45.21	8.23	42.04	6.06	36.54	3.61
AD-1548580.1	14.06	0.70	26.71	0.83	36.58	3.69
AD-1548641.1	9.23	0.46	10.30	2.23	36.88	1.98
AD-1548575.1	18.56	1.19	26.60	2.59	37.22	5.34

Duplex	10 nM		1 nM		0.1 nM	
	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV
AD-1548604.1	19.01	1.57	27.01	1.26	37.24	1.15
AD-1548602.1	21.31	1.77	29.32	1.72	37.49	3.21
AD-1548698.1	14.96	0.60	17.71	1.02	37.57	7.68
AD-1548537.1	14.60	2.53	18.74	1.58	37.59	7.88
AD-1548532.1	9.71	0.88	18.89	1.45	37.64	0.60
AD-1548701.1	17.62	1.10	24.05	1.50	37.68	5.03
AD-1548560.1	27.12	2.64	30.64	3.75	37.86	2.07
AD-1548521.1	12.87	1.02	18.94	2.25	37.97	5.59
AD-1548586.1	28.93	3.53	38.43	4.62	37.99	3.03
AD-1548614.1	16.21	1.85	28.99	1.01	38.16	5.60
AD-1548579.1	20.14	3.26	30.17	5.02	39.14	4.64
AD-1548574.1	23.15	1.12	35.42	7.88	40.18	4.60
AD-1548594.1	12.07	1.69	26.96	1.21	40.43	2.06
AD-1548566.1	35.69	2.42	40.44	1.83	40.48	3.69
AD-1548669.1	12.29	0.81	23.07	2.86	41.45	4.39
AD-1548599.1	19.00	0.96	28.68	4.15	41.61	2.77
AD-1548702.1	13.33	3.44	19.36	1.43	41.68	7.97
AD-1548670.1	13.24	2.23	22.00	3.12	41.75	4.24
AD-1548603.1	21.91	1.14	31.21	0.39	42.02	4.96
AD-1548618.1	17.70	1.78	32.19	4.96	42.04	5.88
AD-1548454.1	48.70	7.62	35.11	8.17	42.08	3.08
AD-1548476.1	22.22	1.36	25.88	4.20	42.27	2.65
AD-1548642.1	13.28	2.72	20.28	0.72	42.79	3.65
AD-1548428.1	19.26	1.52	25.84	2.04	42.99	3.37
AD-1548591.1	28.95	1.33	41.38	6.83	43.15	2.82
AD-1548535.1	10.42	1.59	15.98	1.43	43.15	5.71
AD-1548497.1	21.94	1.09	31.64	6.08	43.54	3.70
AD-1548499.1	12.19	1.55	23.86	3.65	43.57	6.17
AD-1548485.1	20.98	1.67	25.49	1.00	43.78	7.35
AD-1548696.1	13.02	0.52	17.61	2.48	43.84	9.07
AD-1548595.1	18.63	1.33	28.11	2.17	43.93	5.75
AD-1548600.1	37.25	4.42	40.05	1.14	44.22	4.66
AD-1548562.1	30.58	4.74	38.90	6.24	44.29	4.01
AD-1548637.1	19.40	3.39	16.72	2.47	44.45	5.63
AD-1548461.1	14.88	2.05	23.14	5.89	44.89	6.85
AD-1548569.1	31.37	3.13	40.14	2.61	45.05	4.67
AD-1548643.1	12.27	2.88	20.13	2.05	45.16	6.86
AD-1548626.1	26.76	1.77	30.61	3.05	45.63	4.42
AD-1548564.1	39.03	6.65	48.94	6.30	46.42	5.03
AD-1548502.1	23.79	2.17	29.80	1.46	46.46	2.17
AD-1548624.1	17.45	1.21	27.91	3.87	46.60	4.51
AD-1548692.1	6.41	0.55	7.69	0.71	47.08	4.32

Duplex	10 nM		1 nM		0.1 nM	
	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV
AD-1548436.1	24.81	2.43	30.84	3.14	47.16	4.16
AD-1548704.1	23.91	3.34	24.84	1.62	47.72	11.64
AD-1548399.1	39.84	3.46	46.40	1.89	48.24	4.95
AD-1548480.1	16.30	1.74	30.98	4.33	48.56	4.19
AD-1548677.1	29.54	1.97	38.96	3.32	48.98	12.50
AD-1548700.1	28.81	3.39	35.89	1.73	49.29	8.86
AD-1548563.1	36.84	2.84	57.51	4.48	50.14	5.07
AD-1548409.1	36.18	2.63	36.45	4.31	50.63	4.52
AD-1548615.1	17.26	1.11	31.22	3.63	51.34	2.28
AD-1548655.1	21.30	1.03	36.46	4.30	52.54	6.51
AD-1548620.1	43.39	4.46	46.13	4.44	53.63	2.80
AD-1548561.1	44.16	3.11	51.68	11.02	54.08	7.09
AD-1548691.1	11.51	1.24	11.86	0.86	54.62	5.09
AD-1548630.1	33.06	4.42	48.73	4.25	54.71	1.58
AD-1548699.1	19.52	2.63	31.18	2.77	55.12	4.78
AD-1548565.1	50.21	1.79	56.31	4.17	55.24	4.41
AD-1548540.1	14.99	0.71	26.91	2.95	55.62	7.48
AD-1548547.1	42.53	6.12	43.33	9.30	55.94	8.82
AD-1548494.1	16.04	1.62	23.42	2.87	56.02	4.26
AD-1548427.1	20.30	1.80	39.62	2.79	56.31	5.48
AD-1548533.1	22.83	2.60	32.51	3.96	56.94	4.72
AD-1548551.1	64.67	5.99	70.86	8.34	59.94	3.46
AD-1548431.1	23.57	2.41	38.70	3.03	60.07	8.69
AD-1548546.1	58.04	4.95	55.65	14.18	61.06	4.60
AD-1548652.1	26.94	7.85	36.75	1.18	61.12	9.75
AD-1548365.1	42.82	5.73	47.35	8.81	61.23	6.99
AD-1548550.1	69.47	3.49	66.66	9.04	61.73	11.15
AD-1548597.1	77.13	7.94	85.82	6.84	62.23	1.92
AD-1548609.1	38.53	2.81	46.04	4.21	62.29	3.83
AD-1548611.1	27.84	4.41	44.97	3.59	62.77	8.22
AD-1548697.1	22.27	2.84	39.72	4.24	62.83	10.09
AD-1548448.1	32.75	11.47	41.01	2.67	62.97	11.53
AD-1548517.1	14.80	2.09	27.45	0.75	63.45	5.81
AD-1548554.1	45.81	3.39	61.81	12.03	64.22	3.38
AD-1548383.1	84.65	14.88	61.70	8.95	64.61	6.46
AD-1548401.1	25.14	2.36	40.57	4.28	64.77	4.53
AD-1548514.1	37.42	3.56	44.60	5.56	64.95	5.36
AD-1548606.1	60.53	5.55	72.68	7.18	64.98	12.46
AD-1548465.1	38.63	3.94	57.55	14.57	66.40	7.64
AD-1548394.1	33.78	4.96	41.43	5.79	66.49	9.12
AD-1548557.1	64.98	4.25	67.64	4.73	66.52	7.88
AD-1548558.1	75.14	6.26	81.46	9.27	66.78	8.61

Duplex	10 nM		1 nM		0.1 nM	
	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV
AD-1548548.1	52.05	10.64	55.97	11.61	67.41	3.48
AD-1548387.1	67.89	8.73	52.43	3.19	67.67	5.18
AD-1548474.1	50.99	6.78	65.89	7.44	67.88	7.82
AD-1548619.1	36.48	0.37	52.65	10.68	68.49	5.56
AD-1548601.1	51.77	0.64	56.93	4.47	68.83	5.62
AD-1548369.1	55.87	16.62	51.63	8.97	68.84	11.40
AD-1548613.1	35.52	1.70	41.91	2.62	69.40	12.19
AD-1548553.1	54.07	4.53	57.10	2.69	69.80	3.75
AD-1548693.1	15.16	0.34	15.48	1.04	69.92	5.84
AD-1548555.1	47.63	7.43	57.73	9.47	70.52	5.40
AD-1548520.1	16.86	0.88	32.98	2.06	70.81	3.77
AD-1548552.1	52.43	7.14	59.80	1.96	72.33	6.73
AD-1548370.1	46.33	12.84	53.84	6.50	72.52	8.10
AD-1548505.1	45.53	6.49	56.21	5.63	72.91	3.56
AD-1548545.1	58.24	11.98	58.99	8.49	74.12	16.72
AD-1548539.1	51.88	1.66	59.78	4.26	75.56	2.24
AD-1548549.1	61.61	2.48	64.92	10.07	75.70	5.95
AD-1548556.1	55.49	4.88	62.86	3.83	75.83	3.59
AD-1548616.1	45.80	7.83	55.40	7.94	79.15	2.99
AD-1548482.1	69.01	11.78	78.29	6.48	79.33	5.40
AD-1548501.1	60.77	2.61	73.05	6.25	79.67	7.90
AD-1548681.1	12.07	1.95	20.36	2.91	80.58	18.49
AD-1548504.1	77.48	6.09	84.06	5.28	81.64	7.97
AD-1548425.1	45.11	2.53	49.58	8.06	82.13	10.13
AD-1548671.1	51.86	8.58	72.37	5.01	84.11	14.72
AD-1548544.1	66.15	19.91	82.59	13.59	85.56	10.49
AD-1548534.1	41.26	0.92	57.38	2.99	86.89	4.85
AD-1548445.1	97.62	8.98	81.83	6.71	87.11	5.86
AD-1548433.1	94.39	5.60	83.20	3.63	88.16	2.91
AD-1548455.1	74.25	2.00	80.48	9.46	88.69	4.55
AD-1548429.1	72.90	5.96	74.01	4.26	89.02	9.93
AD-1548386.1	77.39	5.39	69.06	7.07	91.10	2.33
AD-1548690.1	28.19	5.69	30.38	4.70	91.93	10.63
AD-1548541.1	68.53	7.69	91.69	19.67	97.83	10.51
AD-1548506.1	68.16	9.18	78.19	5.42	102.21	9.47
AD-1548518.1	75.11	6.07	84.41	8.00	102.82	6.29
AD-1548410.1	87.37	18.16	95.77	9.50	102.99	5.91
AD-1548686.1	24.37	2.14	38.09	8.32	105.02	8.13
AD-1548694.1	14.47	0.55	21.59	1.41	106.25	12.84
AD-1548422.1	108.87	9.66	106.72	12.95	109.92	8.24
AD-1548689.1	35.02	4.55	32.82	2.77	120.89	23.72
AD-1548446.1	115.57	9.32	103.06	10.57	122.37	8.71

Duplex	10 nM		1 nM		0.1 nM	
	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV	% of Message Remaining	ST DEV
AD-1548688.1	44.04	2.29	54.21	2.64	141.92	15.39
AD-1548687.1	42.98	7.70	50.98	3.19	142.40	24.95
AD-1548683.1	55.32	5.04	59.79	5.28	156.44	36.70
AD-1548685.1	33.95	6.03	45.16	2.86	157.19	13.12
AD-1548682.1	69.60	5.53	56.71	2.73	161.28	8.92
AD-1548684.1	59.65	4.71	67.76	2.58	161.94	28.86

Example 3. Additional Duplexes Targeting Beta-Catenin (CTNNB1)

Additional siRNAs targeting the human beta-catenin (CTNNB1) gene (human: NCBI refseqID NM_001904.4, NCBI GeneID: 1499) were designed and synthesized as described above.

- 5 Single dose screens at 10 nM, 1 nM, and 01. nM were performed in Hep3B cells as described above.

Detailed lists of the additional unmodified CTNNB1 sense and antisense strand nucleotide sequences are shown in Table 5. Detailed lists of the additional modified CTNNB1 sense and antisense strand nucleotide sequences are shown in Table 6.

- 10 The results of a single dose screen of the agents in Tables 5 and 6 in Hep3B cells are shown in Table 7.

Table 5. Unmodified Sense and Antisense Strand Sequences of CTNNB1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_001904.4
AD-1210479	ACUCUGGAAUCCAUUCUGGUA		309-329	UACCAGAAUGGAUUCACAGAGUCC		307-329
AD-1210480	CUCUGGAAUCCAUUCUGGUA		310-330	UCACCAGAAUGGAUUCACAGAGUC		308-330
AD-1210732	UGCUCAUCCACUAAUGUCCA		562-582	UGGACAUUAGUGGGAUAGAGCAGC		560-582
AD-1210734	CUCAUCCACUAAUGUCCAGA		564-584	UCUGGACAUUAGUGGGAUAGAGCA		562-584
AD-1211064	CCAUGCAGAAUACAAUUGAUA		816-836	UAUCAUUUGAUUUCUGCAUGGUA		814-836
AD-1211065	CAUGCAGAAUACAAUUGAUA		817-837	UCAUCAUUUGAUUUCUGCAUGGU		815-837
AD-1211578	AGAGGAGCUAAAUGGCAGA		1012-1032	UCUGCCAUUUUAGCUCCUUCUUG		1010-1032
AD-1211582	GGAGCUAAAUGGCAGUGCGA		1016-1036	UCGCACUGCCAUUUUAGCUCCUU		1014-1036
AD-1211675	UACUGGCCAUUUUAAAGUCUA		897-917	UAGACUUAAAGAUGGCCAGUAAG		895-917
AD-1211676	ACUGGCCAUUUUAAAGUCUGA		898-918	UCAGACUUAAAGAUGGCCAGUAA		896-918
AD-1211677	CUGGCCAUUUUAAAGUCUGGA		899-919	UCCAGACUUAAAGAUGGCCAGUA		897-919
AD-1212720	UAACUCACUUGCAAUAUAUA		1489-1509	UAAUUUUGCAAAGUGAGGUUAGA		1487-1509
AD-1212721	AACCUCACUUGCAAUAUAUA		1490-1510	UUAUUUUGCAAAGUGAGGUUAG		1488-1510
AD-1213112	GCCAUUGUCUUCUGUCA		1604-1624	UUGACGAAGACACAGAGUGGCAG		1602-1624
AD-1213113	CCAUCUGUCUUCUGUCAUA		1605-1625	UAUGACGAAGACACAGAGUGGCA		1603-1625
AD-1213115	AUCUGUGCUUCUGUCAUA		1607-1627	UAGAUGACGAAGACACAGAUUG		1605-1627
AD-1213116	UCUGUGCUUCUGUCAUCUGA		1608-1628	UCAGAUGACGAAGACACAGAU		1606-1628
AD-1213117	CUGUGCUUCUGUCAUCUGAA		1609-1629	UUCAGAUGACGAAGACACAGAU		1607-1629
AD-1213118	UGUGCUUCUGUCAUCUGACA		1610-1630	UGUCAGAUGACGAAGACACAGA		1608-1630
AD-1213119	GUGCUUCUGUCAUCUGACCA		1611-1631	UGGUCAGAUGACGAAGACACAG		1609-1631
AD-1213749	ACUGUUGGAUUGAUUCGAAAA		1742-1762	UUUUCGAUCAAUCCAAACAGUAG		1740-1762
AD-1214765	AAAUACCAUUCUUAUUGUA		1993-2013	UCAACAUAUGGAUUGGUAUUUAG		1991-2013
AD-1214768	UACCAUCCAUUGUUAUUGCA		1996-2016	UGCACAAACAUAUGGAAUGGUAUU		1994-2016
AD-1214770	CCAUUCCAUUGUUAUUGCAGA		1998-2018	UCUGCACAACAUAUGGAAUGGUA		1996-2018
AD-1215017	GUCUCUGUGAACUUGUCUCAA		2063-2083	UUGAGCAAGUUCACAGAGGACCC		2061-2083
AD-1215018	UCCUCUGUGAACUUGUCUCAA		2064-2084	UCUGAGCAAGUUCACAGAGGACC		2062-2084
AD-1216131	AUCGUUUUUUACUUCUGGUA		2361-2381	UACCAGAGUGAAAAGAACGUAUAG		2359-2381
AD-1216132	UCGUUUUUUACUUCUGGUA		2362-2382	UACCCAGAGUGAAAAGAACGUAU		2360-2382
AD-1216345	UGGUGCUGACUUAUCCAGUUGA		2446-2466	UCAACUGGAUAGUCAGCACCAGG		2444-2466
AD-1216349	GCUGACUAUCCAGUUGAUGGA		2450-2470	UCCAUAACUGGAUAGUCAGCAC		2448-2470
AD-1216350	CUGACUAUCCAGUUGAUGGGA		2451-2471	UCCCAUCAACUGGAUAGUCAGCA		2449-2471

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM 001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM 001904.4
AD-1216352	GACUAUCCAGUUAUGGGGCUA		2453-2473	UAGCCCAUCAACUGGAUAGUCAG		2451-2473
AD-1216506	AUUGGCCUGUAGAGUUGCUGA		2903-2923	UCAGCAACUCUACAGGCCAAUCA		2901-2923
AD-1216525	GGCUGGUUUCUCAGAAAGUGA		2940-2960	UCACUUUCUGAGAUACCAAGCCCA		2938-2960
AD-1216526	GCUGGUUUCUCAGAAAGUGCA		2941-2961	UGCACUUUCUGAGAUACCAAGCCC		2939-2961
AD-1216528	UGGUUUCUCAGAAAGUGCCUA		2943-2963	UAGGCACUUUCUGAGAUACCAAGC		2941-2963
AD-1216546	CUGACACACUAAACCAAGCUGA		2961-2981	UCAGCUUGGUUAGUGUUCAGGGC		2959-2981
AD-1216556	AACCAAGCUGAGUUUCCUAUA		2971-2991	UAUAGGAAACUCAGCUUUGGUUAG		2969-2991
AD-1216558	CCAAGCUGAGUUUCCUAUGGA		2973-2993	UCCAUAGGAAACUCAGCUUUGGUU		2971-2993
AD-1216559	CAAGCUGAGUUUCCUAUGGGA		2974-2994	UCCCAUAGGAAACUCAGCUUUGGU		2972-2994
AD-1216560	AAGCUGAGUUUCCUAUGGGAA		2975-2995	UUCCCAUAGGAAACUCAGCUUUGG		2973-2995
AD-1216561	AGCUGAGUUUCCUAUGGGAAA		2976-2996	UUUCCCAUAGGAAACUCAGCUUUG		2974-2996
AD-1216592	UUUUCUGGUCCUUUUUGGUA		3013-3033	UACCAAAAAGGACCAGAAACAAA		3011-3033
AD-1216593	UGUUCUGGUCCUUUUUGGUCA		3014-3034	UGACCAAAAAGGACCAGAAACAAA		3012-3034
AD-1216594	GUUCUGGUCCUUUUUGGUCCA		3015-3035	UCGACCAAAAAGGACCAGAAACAA		3013-3035
AD-1216595	UUCUGGUCCUUUUUGGUCCGAA		3016-3036	UUCGACCAAAAAGGACCAGAAACA		3014-3036
AD-1216596	UCUGGUCCUUUUUGGUCCGAGA		3017-3037	UCUCGACCAAAAAGGACCAGAAAC		3015-3037
AD-1216597	CUGGUCCUUUUUGGUCCGAGGA		3018-3038	UCCUCGACCAAAAAGGACCAGAA		3016-3038
AD-1216613	GAGGAGUAAACAAUACAAUAGA		3034-3054	UCAUUUGUAUUUGUUAUCCUCGA		3032-3054
AD-1216784	AUGGUGUAGAACACUAAUUCUA		3314-3334	UGAAUUAGUUGUUAUACACCAUUA		3312-3334
AD-1216786	GGUGUAGAACACUAAUUCUAUA		3316-3336	UAUGAAUUAGUUGUUAUACACCAU		3314-3336
AD-1216787	GUGUAGAACACUAAUUCUAUA		3317-3337	UUAUGAAUUAGUUGUUAUACACCA		3315-3337
AD-1216790	UAGAACACUAAUUCUAUAUCA		3320-3340	UGAUUAUGAAUUAGUGUUAUCA		3318-3340
AD-1216792	GAACACUAAUUCUAUAUCACA		3322-3342	UGUGAUUAUGAAUUAGUGUUAUCA		3320-3342
AD-1216895	UGCUUAAAUAAGCAGGUGGA		3440-3460	UCCACCUUGCUGUUAUUUAAGCAUA		3438-3460
AD-1216904	UAAGCAGGUGGAUCUUAUUUCA		3449-3469	UGAAAUAAGAUCCACCUUGCUGUUAU		3447-3469
AD-1216906	AGCAGGUGGAUCUUAUUUCAUA		3451-3471	UAUGAAAUAAGAUCCACCUUGCUGUA		3449-3471
AD-1216907	GCAGGUGGAUCUUAUUUCAUGA		3452-3472	UCAUGAAAUAAGAUCCACCUUGCUGU		3450-3472
AD-1216910	GGUGGAUCUUAUUUCAUGUUUA		3455-3475	UAAACAUGAAAUAAGAUCCACCUUG		3453-3475
AD-1216911	GUGGAUCUUAUUUCAUGUUUUA		3456-3476	UAAAACAUGAAAUAAGAUCCACCU		3454-3476
AD-1216913	GGAUCUUAUUUCAUGUUUUUGA		3458-3478	UCAAAAACAUGAAAUAAGAUCCAC		3456-3478
AD-1216938	GGGUAGGGUAAAUCAGUAAAGA		3503-3523	UCUUUACUGAUUUACCUUACCCAU		3501-3523
AD-1216940	GUAGGGUAAAUCAGUAAAGAGA		3505-3525	UCUCUUACUGAUUUUACCUUACCC		3503-3525

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM 001904.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM 001904.4
AD-1216941	UAGGGUAAUCAGUAAGAGGA		3506-3526	UCCUCUUACUGAUUUACCCUACC		3504-3526
AD-1216942	AGGUAAUACAGUAAGAGGUA		3507-3527	UACCUCUUACUGAUUUACCCUAC		3505-3527
AD-1216944	GGUAAUACAGUAAGAGGUGUA		3509-3529	UACACCUCUUACUGAUUUACCCU		3507-3529
AD-1216945	GUAAUACAGUAAGAGGUGUUA		3510-3530	UACACCCUCUUACUGAUUUACCC		3508-3530
AD-1216989	UUACCAAGUUGCCUUUAUCCA		3554-3574	UGGAUAAAAGGCAACUGGUAAC		3552-3574
AD-1216990	UACCAAGUUGCCUUUAUCCCA		3555-3575	UGGGAUAAAAGGCAACUGGUAAC		3553-3575
AD-1217004	UAUCCCAAAGUUUGUUAACA		3569-3589	UGUUACAACAACUUUGGGAUAAA		3567-3589
AD-1217007	CCCAAAGUUUGUUAACCUGA		3572-3592	UCAGGUUACAACAACUUUGGGAU		3570-3592
AD-70947	UACUGUUGGAUUUGAUUCGAAA		1741-1761	UUUCGAAUCAAUCCAACAGUAGC		1739-1761

Table 6. Modified Sense and Antisense Strand Sequences of CTNNB1 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-1210479	ascstucugGfaAfUfCfcauucugguaL96		VPusAfsccaGfaAfUfggauUfcCfagaguscsc		GGACUCUGGAAUCCAUAUCUGGUG	
AD-1210480	csuscuggAfaUfCfcauucuggugaL96		VPusCfsaccAfgAfAfuggaUfuCfcaagagsusc		GACUCUGGAAUCCAUAUCUGGUGC	
AD-1210732	usgscucaUfcCfCfcauaanguccaL96		VPusGfsgacAfuUfAfugggGfaUfagagcagsc		GCUGCUCAUCCCCACUAUAUGUCCA	
AD-1210734	csuscaucCfcAfCfUfaauggccagaL96		VPusCfsuggAfcAfUfuaguGfgGfaugagscsa		UGCUCAUCCCCACUAUAUGUCCAAGC	
AD-1211064	cscsaugcAfgAfAfUfacaanguanaL96		VPusAfsucaUfuUfgfuaumCfuGfcaugggsusa		UACCAUGCAGAAUAACAAAUGAUG	
AD-1211065	csasugcaGfaAfUfAfaaanguaL96		VPusCfsaucAfuUfUfgnauUfcUfcaugggsu		ACCAUGCAGAAUAACAAAUGAUGU	PCT/US2022/037794
AD-1211578	asgsaaggAfgCfUfAfaauggccagaL96		VPusCfsugcCfaUfUfuaguCfuCfcauucususg		CAAGAAGGAGCUAAAAUUGGCAGU	
AD-1211582	gsgsagcuAfaAfAfUfggcagugcggaL96		VPusCfsgeaCfuGfCfcauuUfuAfgcucccsusu		AAGGAGCUAAAAUUGGCAGUGCGU	
AD-1211675	usascuggCfcAfUfCfuuaangucuaL96		VPusAfsagcUfuAfaagauGfgCfcaugasasg		CUUACUGGCCCAUCUUUAAGUCUG	

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-1211676	ascsuaggcCfaUfCfUfuaagucugaaL96		VPusCfsagaCfuUfAfaagaUfgGfccagusa		UUACUGGCCAUCUUUAAGUCUGG	WO 2023/003995
AD-1211677	csusggccAfuCfUfUfuaagucugaaL96		VPusCfsagAfcUfUfaaagAfuGfgccagsusa		UACUGGCCAUCUUUAAGUCUGGA	
AD-1212720	usasaccuCfaCfUfUfgcauaaauaL96		VPusAfsauuAfuUfgGfcaagUfgAfgguuasgsa		UCUAAACCUCACUUGCAAUAUUA	
AD-1212721	asascctucAfcUfUfGfcauaaauaL96		VPusUfisaauUfaUfUfgcaaGfuGfagguuasag		CUAACCCUCACUUGCAAUAUUAU	
AD-1213112	gscsaucUfgUfGfCfucucuguaaL96		VPusUfsgacGfaAfgGfagcaCfaGfauggcsasg		CUGCCAUCUGUGUCUUCGUCUCAU	
AD-1213113	cscsaucUfGfCfUfucucuguaaL96		VPusAfsugaCfGfAfgagcAfcAfgauggscsa		UGCCAUCUGUGUCUUCGUCUCAUC	
AD-1213115	asuscugUfGfCfUfucuguaaL96		VPusAfsagUfGfCfGfagaGfCfagcausgsg		CCAUCUGUGUCUUCGUCUCAUCUG	
AD-1213116	uscsugUfGfCfUfUfucuguaaL96		VPusCfsagaUfgAfcfGfagAfgCfagagasug		CAUCUGUGUCUUCGUCUCAUCUGA	
AD-1213117	csusgugUfCfUfCfGfucucugaaL96		VPusUfscagAfuGfAfgaaGfaGfcaagasa		AUCUGUGUCUUCGUCUCAUCUGAC	
AD-1213118	usgsugUfCfUfCfGfucugaaL96		VPusGfsucaGfaUfGfagcaAfgAfgcacasgsa		UCUGUGUCUUCGUCUCAUCUGACC	
AD-1213119	gsusgucUfCfGfUfcaucugaaL96		VPusGfsgucAfgAfuUfgagAfaGfagcacasgsa		CUGUGUCUUCGUCUCAUCUGACCA	
AD-1213749	ascsuuguGfGfUfUfGfauucgaaL96		VPusUfsuucGfaAfuUfcauUfcAfcagusaag		CUACUGUGGAUUGAUUCGAAAU	
AD-1214765	asasaucCfaUfUfCfcauuguaaL96		VPusCfsaaaCfaUfUfggaaUfgGfuaauuasag		CUAAAUAACCAUUCCAUUGUUUGU	PCT/US2022/037794
AD-1214768	usasccauUfCfAfuUfuguuguaaL96		VPusGfscacAfaAfcfaagGfaAfgguuasusu		AAUACCAUUCCAUUGUUUGUGCA	
AD-1214770	cscsaucCfaUfUfGfuuugucagaL96		VPusCfsugcAfcAfaaacaUfgGfauuggsusa		UACCAUUCCAUUGUUUGUGCAGC	
AD-1215017	gsusccucUfgUfGfAfaucuguaaL96		VPusUfsgagCfaAfgfuaCfaGfagagascsc		GGGUCCUCUGUGAACUUUGCUCAG	
AD-1215017	uscsencUfGfAfaucuguaaL96		VPusCfsugaGfcAfafguucAfcAfgagagascsc		GGUCCUCUGUGAACUUUGCUCAGG	

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-1216592	usugnuucUfgGfUfCfuuuuuuguaL96		VPusAfsccaAfaAfAfggacCfaGfaacaasasa		UUUUGUUUCUGGUCCUUUUUGGUC	
AD-1216593	usgsuucUfgUfCfUuuuuuguaL96		VPusGfsaccAfaAfAfaaggCfcAfgaacasasa		UUUGUUUCUGGUCCUUUUUGGUCG	
AD-1216594	gsusucUgGfUfCfUfuuuuguguaL96		VPusCfsgacCfaAfAfaaggAfcCfagaacsasa		UUGUUUCUGGUCCUUUUUGGUCGA	
AD-1216595	ususcugUfcCfUfUfuuuuguguaL96		VPusUfscgaCfcAfAfaaagGfaCfcagaacsasa		UGUUUCUGGUCCUUUUUGGUCGAG	
AD-1216596	uscsugUcCfcUfUfUfuuguguguaL96		VPusCfsuegAfcCfafaaaaGfgAfcagacsaasc		GUUCUGGUCCUUUUUGGUCGAGG	
AD-1216597	csusgucCfuUfUfUfuguguguaL96		VPusCfscucGfaCfcfaaaaAfgGfaccagsasa		UUCUGGUCCUUUUUGGUCGAGGA	
AD-1216613	gsasggagUfaAfCfAfauaacaauaL96		VPusCfsaunUfgUfafuugUfUfaCfuuccugsa		UCGAGGAGUAACAAUACAAUUGG	
AD-1216784	asusggUgUfaGfAfafcauaauaL96		VPusGfsaunUfaGfUfgnuUfaCfaccasusa		UAAUGGUGUAGAACACUAUUUCA	
AD-1216786	gsusguGfaAfCfAfcauaauaL96		VPusAfsugaAfuUfaAfgnuUfcUfacaccasua		AUGGUGUAGAACACUAUUUCAUA	
AD-1216787	gsusguagAfaCfAfcauaauaL96		VPusUfsaugAfaUfUfagugUfuCfuacacsasa		UGGUGUAGAACACUAUUUCAUAA	
AD-1216790	usasgaacAfcUfAfAfuaauaL96		VPusGfsaunAfuGfAfaunaGfuGfuacuacsasa		UGUAGAACACUAUUUCAUAAUCA	
AD-1216792	gsasacacUfaAfUfUfcauaauaL96		VPusGfsugaUfuAfUfgaauUfaGfugnuacsasa		UAGAACACUAUUUCAUAAUACACU	
AD-1216895	usgsuuaAfaAfUfAfagcagguguaL96		VPusCfscacCfuGfCfuuaUfuUfaagcasusa		UAUGCUUAAAAUAAAGCAGGUGGA	
AD-1216904	usasagcaGfgUfgGfaucauauaL96		VPusGfsaaaUfaGfAfucacCfcUfgcuuasusu		AAUAAAGCAGGUGGAUCUAUUUCA	
AD-1216906	asgsaggUfgGfAfUfcauauaL96		VPusAfsugaAfaUfafgaucCfaCfeguacsusa		UAAGCAGGUGGAUCUAUUUCAUG	
AD-1216907	gsasagguGfgAfUfCfuauuauaL96		VPusCfsaugAfaAfUfagauCfcAfcuacsusu		AAGCAGGUGGAUCUAUUUCAUGU	
AD-	gsusuggaUfcUfaUfUfuucauauaL96		VPusAfsaacAfuGfAfaauaGfaUfccaccusug		CAGGUGGAUCUAUUUCAUGUUUU	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
1216910						
AD-1216911	gsusggauCfuAfUfUfucauguuuuL96		VPusAfsaaaCfaUfGfaaauAfgAfuccacsu		AGGUGGAUCUAUUUCAUGUUUUU	
AD-1216913	gsgsaucUfUfUfCfauguuuuuL96		VPusCfsaaaAfaCfaugaaAfuAfgaucsasc		GUGGAUCUAUUUCAUGUUUUUGA	
AD-1216938	gsgsguagGfgUfAfAfaucaguuagaL96		VPusCfsuuaCfuGfAfuuaaCfcCfuaccsasu		AUGGGUAGGGUAAAUCAGUAAAGA	
AD-1216940	gsusagggUfaAfAfUfcaguuagagaL96		VPusCfsuenUfaCfuUfgauUfaCfcuacsasc		GGGUAGGGUAAAUCAGUAAAGAGG	
AD-1216941	usagsgguAfaAfUfCfaguuagaggaL96		VPusCfscucUfuAfCfugauUfuAfcuacsasc		GGUAGGGUAAAUCAGUAAAGAGGU	
AD-1216942	asgsgguAfaUfCfAfguaagagguaL96		VPusAfsccuCfuUfAfcugaUfuUfaccuacsasc		GUAGGGUAAAUCAGUAAAGAGGUG	
AD-1216944	gsgsuaaaUfcAfGfUfaagagguguaL96		VPusAfsaacCfuCfuUfuacuGfaUfuuaacsu		AGGGUAAAUCAGUAAAGAGGUGUU	
AD-1216945	gsusaaauCfaGfUfAfagagguguaL96		VPusAfsacaCfcUfCfuuaCfUfAfuuaacsasc		GGGUAAAUCAGUAAAGAGGUGUUA	
AD-1216989	ususaccaGfuUfGfCfcuuuuuacaaL96		VPusGfsgauAfaAfAfggcaAfcUfpguacsasc		GUUUACCAAGUUGCCUUUUUAUCCCC	
AD-1216990	usascagUfuGfCfcuuuuuacccaaL96		VPusGfsggaUfaAfAfaggaCfaCfugguasasa		UUUACCAAGUUGCCUUUUUAUCCCCA	
AD-1217004	usasucccAfaAfGfUfuguuuagaaL96		VPusGfsuuaCfaAfCfaacuUfuGfgguasasa		UUUAUCCCCAAAGUUGUUGUUAACC	
AD-1217007	cscscaaaGfuUfGfUfuguaaccugaL96		VPusCfsaggUfuAfCfaacaAfcUfuugggsasu		AUCCCCAAAGUUGUUGUUAACCUGC	
AD-70947	usascuugUfgGfAfUfugauucgaaL96		VPusUfsucgAfaUfCfaucCfaAfcagnasgsc		GCUACUGUUGGAUUUGAUUCGAAA	

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Table 7. Single Dose Screens in Hep3b Cells

Duplex	Avg 10 nM	SD	Avg 1 nM	SD	Avg 0.1 nM	SD
AD-70947	11.5	1.8	22.32	1.27	44.29	2.41
AD-1216613	16.7	3.2	22.86	1.17	45.84	1.90
AD-1214765	16.0	2.7	26.93	7.66	46.32	4.90
AD-70947	9.5	2.0	22.89	2.32	46.86	5.57
AD-1216904	19.6	1.7	33.00	4.75	55.35	1.63
AD-1211676	16.3	1.8	28.31	2.58	55.61	3.66
AD-1214770	14.7	3.3	27.87	2.51	55.97	3.06
AD-1216784	11.8	0.9	28.35	2.48	57.01	2.10
AD-1211065	25.9	12.7	37.93	15.20	57.44	2.20
AD-1216910	20.9	2.5	33.63	2.51	57.90	6.75
AD-1211064	16.1	2.9	32.53	3.90	58.39	1.17
AD-1213113	16.3	2.9	30.68	1.07	60.99	6.32
AD-1216790	17.9	4.1	35.54	2.50	61.19	1.44
AD-1216911	23.9	2.2	41.70	5.24	64.07	3.50
AD-1216792	21.3	1.9	39.55	4.48	65.87	3.48
AD-1216787	20.8	1.9	45.87	1.90	67.18	8.76
AD-1212720	20.0	5.4	42.28	5.20	67.21	2.28
AD-1216790	23.2	2.2	36.54	4.30	67.25	5.88
AD-1216913	20.9	1.6	39.18	6.07	68.42	5.84
AD-1212720	19.2	2.1	46.19	3.61	68.98	8.77
AD-1216792	20.8	3.0	36.61	6.33	69.00	7.37
AD-1213116	25.2	2.1	42.65	5.17	69.14	3.63
AD-1214768	22.3	1.5	34.43	3.82	69.90	4.53
AD-1213112	25.8	6.7	37.46	5.52	70.27	6.74
AD-1216597	41.0	2.9	57.74	5.54	70.35	3.58
AD-1216945	24.4	1.4	40.98	3.24	71.68	7.48
AD-1216556	39.6	3.8	51.67	4.94	72.05	3.10
AD-1216593	46.8	1.6	53.51	1.69	73.22	4.02
AD-1211675	21.8	2.6	46.93	5.04	73.29	5.65
AD-1216546	48.5	3.8	50.31	6.67	73.33	3.69
AD-1210479	21.7	2.1	41.58	3.60	73.56	2.79
AD-1216528	45.2	0.8	53.03	7.55	73.66	5.25
AD-1213749	24.9	3.0	41.47	2.95	73.82	2.96
AD-1216593	37.8	1.5	57.64	1.50	74.01	4.82
AD-1213118	23.9	2.0	40.28	1.63	74.06	9.20
AD-1216526	40.6	4.2	58.15	4.28	74.31	5.60
AD-1216907	20.3	1.5	44.23	1.79	75.29	6.79
AD-1216525	45.5	2.8	54.02	6.07	75.48	2.48
AD-1216990	35.0	3.2	53.14	1.71	75.92	6.54
AD-1213116	28.5	4.2	48.54	3.25	76.12	5.40
AD-1216594	45.7	8.6	53.08	4.53	76.37	8.88

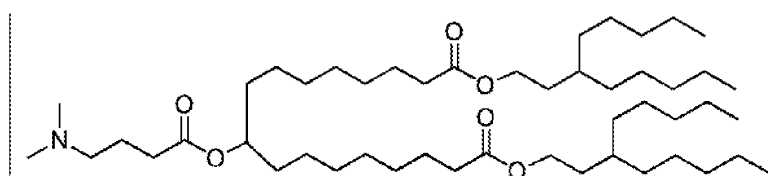
Duplex	Avg 10 nM	SD	Avg 1 nM	SD	Avg 0.1 nM	SD
AD-1211677	18.5	2.2	37.22	4.04	77.49	6.22
AD-1216940	29.4	5.5	49.40	3.49	77.55	4.88
AD-1216595	45.6	2.2	59.98	4.33	78.30	6.20
AD-1216594	43.7	2.7	52.17	5.56	78.38	9.40
AD-1216131	29.4	4.5	40.22	5.08	78.57	3.24
AD-1216942	27.5	5.1	50.47	2.40	78.95	7.99
AD-1216559	47.5	3.5	58.31	5.29	79.57	4.34
AD-1210480	27.4	1.4	55.25	6.03	80.03	7.65
AD-1213115	21.8	1.8	50.16	8.38	80.29	7.30
AD-1216786	29.8	3.2	49.96	7.35	81.03	9.54
AD-1216895	26.5	2.6	52.63	5.98	81.50	11.93
AD-1216132	34.0	5.1	49.52	2.35	81.50	3.94
AD-1216596	43.8	1.7	60.51	7.52	81.59	2.06
AD-1216906	31.6	2.9	51.31	7.11	81.65	4.04
AD-1210732	27.5	0.7	55.20	5.63	82.56	11.46
AD-1216989	29.2	2.9	50.63	3.85	82.99	5.31
AD-1217004	29.5	3.0	53.78	3.96	83.37	7.09
AD-1216592	56.6	2.7	65.11	5.53	83.40	5.10
AD-1216941	39.4	4.4	62.94	4.12	83.43	0.29
AD-1212721	54.0	6.9	65.65	8.12	83.48	3.38
AD-1216561	48.4	4.4	63.17	7.36	83.52	9.05
AD-1216506	46.0	1.9	56.58	2.41	83.91	3.34
AD-1216596	47.9	2.8	61.91	2.53	84.41	7.38
AD-1213119	34.2	2.7	60.89	5.55	85.22	6.55
AD-1216560	52.4	6.1	60.41	1.71	85.93	1.24
AD-1215018	28.4	3.1	51.14	6.12	86.11	2.76
AD-1216595	54.3	5.3	63.21	5.04	86.30	9.38
AD-1216349	41.1	2.3	64.12	7.16	86.49	5.12
AD-1215017	42.1	3.4	61.25	4.57	86.93	4.89
AD-1212721	52.4	2.6	70.04	5.96	86.98	5.97
AD-1217007	41.0	3.4	61.63	5.54	87.47	8.88
AD-1217007	39.5	3.3	66.47	2.70	87.52	4.62
AD-1216350	42.5	0.8	65.23	5.55	87.58	8.36
AD-1216938	43.4	5.9	58.25	4.31	87.74	1.60
AD-1216944	47.0	5.4	64.14	3.95	87.75	2.03
AD-1216560	57.5	2.0	78.55	10.47	88.32	5.75
AD-1216345	28.2	3.2	54.21	5.91	88.89	11.42
AD-1216558	52.0	2.9	66.25	12.01	90.56	3.45
AD-1211582	40.8	4.1	68.58	7.37	90.76	4.63
AD-1210734	66.0	8.0	75.91	5.09	92.38	5.61
AD-1210734	56.8	1.9	83.84	6.72	93.89	2.46
AD-1211578	81.4	6.2	91.70	1.40	96.01	0.86
AD-1213117	41.0	7.4	65.32	9.19	97.54	7.35

Duplex	Avg 10 nM	SD	Avg 1 nM	SD	Avg 0.1 nM	SD
AD-1216352	44.5	3.0	71.22	7.34	98.29	10.71

Example 4. *in Vivo* Assessment of CTNNB1 siRNAs in Non-Human Primates

Duplexes identified from the above *in vitro* analyses were assessed for their ability to inhibit CTNNB1 expression *in vivo*. Briefly, duplexes were formulated in lipid particles comprising a biodegradable lipid, *e.g.*, cationic lipid and intravenously administered to non-human primates.

In particular, duplexes AD-167990 (Negative Control), AD-1548393, AD-1548488, and AD-1548459 were combined with a cationic lipid having the structure below and DSPC/Chol/PEG-DMG in a ratio of 50:12:36:2, respectively.



At Day -10 pre-dose, percutaneous needle liver biopsies were obtained and the level of CTNNB1 mRNA was determined as described above.

At Day 0, cynomolgus monkeys were intravenously administered a single 0.1 mg/kg or 0.3 mg/kg dose of the lipid formulated duplex and at Days 5, 15, and 29, percutaneous needle liver biopsies were obtained and the level of CTNNB1 mRNA was determined as described above. The study design is provided in Table 8 below.

As shown in FIGs. 1A-1C, all three duplexes potently inhibit CTNNB1 expression at 0.1 mg/kg and 0.3 mg/kg.

Table 8. Study Design

Group	siRNA	LNP	N	Dose Route	Dose Day	Dose Level (mg/kg)	Biopsies
1	AD-167990 (Negative Control)	50/12/36/2 (AF-105)	3	IV	Day 1	0.3	<ul style="list-style-type: none"> • Pre-dose • Day 5 • Day 15 • Day 29
2	AD-1548393.1	AF-105	3	IV		0.1	
3	AD-1548393.1	AF-105	3	IV		0.3	
4	AD-1548488.1	AF-105	3	IV		0.1	
5	AD-1548488.1	AF-105	3	IV		0.3	
6	AD-1548459.1	AF-105	3	IV		0.1	
7	AD-1548459.1	AF-105	3	IV		0.3	

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

Informal Sequence Listing

SEQ ID NO: 1

>NM_001904.4 Homo sapiens catenin beta 1 (CTNNB1), transcript variant 1, mRNA

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SEQ ID NO: 3

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SEQ ID NO: 5

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SEQ ID NO: 6 REVERSE COMPLEMENT OF SEQ ID NO: 5

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40

SEQ ID NO: 7

>NM_001319394.1 Macaca fascicularis catenin beta 1 (CTNNB1), mRNA
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25 **SEQ ID NO: 8 REVERSE COMPLEMENT OF SEQ ID NO: 7**
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SEQ ID NO: 9

>NM_001257918.1 Macaca mulatta catenin beta 1 (CTNNB1), mRNA
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SEQ ID NO: 10 REVERSE COMPLEMENT OF SEQ ID NO: 9

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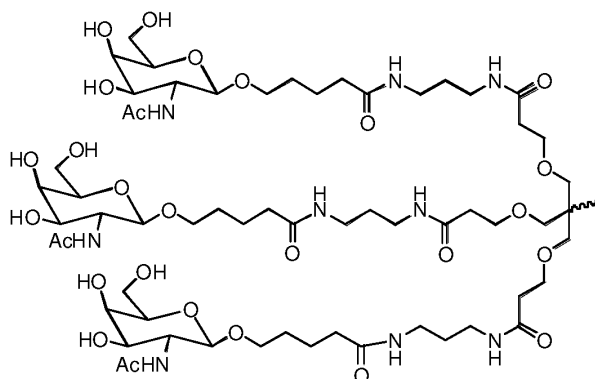
We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of beta-catenin (CTNNB1) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of
5 complementarity to an mRNA encoding CTNNB1, 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, 5, or 6.
2. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of beta-catenin
10 (CTNNB1) 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 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 603-625, 1489-1511, or 1739-1761 of SEQ ID NO: 1, and the antisense strand comprises at
15 least 15 contiguous nucleotides differing by no more than three nucleotides, from the corresponding nucleotide sequence of SEQ ID NO:2.
3. The dsRNA agent of claim 1 or 2, wherein the 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 a duplex selected from the group consisting of
20 AD-1548393, AD-1548488, and AD-1548459.
4. The dsRNA agent of any one of claims 1-3, wherein the dsRNA agent comprises at least one modified nucleotide.
- 25 5. The dsRNA agent of any one of claims 1-4, wherein substantially all of the nucleotides of the sense strand are modified nucleotides; 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.
- 30 6. The dsRNA agent of any one of claims 1-5, wherein 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.
- 35 7. The dsRNA agent of any one of claims 4-6, wherein at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxythymidine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified

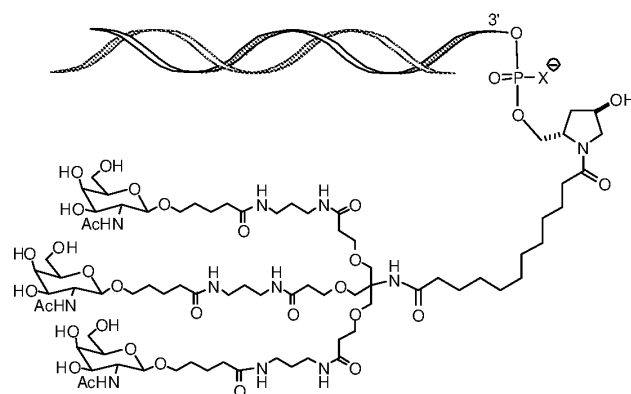
- nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl 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.
8. The dsRNA agent of any one of claims 4-6, wherein at least one of the modified nucleotides is selected from the group consisting of LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.
9. The dsRNA agent of any one of claims 4-6, 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 nucleotide comprising a phosphorothioate group, and a vinyl-phosphonate nucleotide; and combinations thereof.
10. The dsRNA agent of any one of claims 4-6, wherein at least one of the modified nucleotides is a nucleotide modified with a thermally destabilizing nucleotide modification.
11. The dsRNA agent of claim 10, 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; a destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acid (UNA), and a glycerol nucleic acid (GNA).
12. The dsRNA agent of any one of claims 1-11, wherein the double stranded region is 19-30 nucleotide pairs in length.
13. The dsRNA agent of claim 12, wherein the double stranded region is 19-25 nucleotide pairs in length.
14. The dsRNA agent of claim 12, wherein the double stranded region is 19-23 nucleotide pairs in length.

15. The dsRNA agent of claim 12, wherein the double stranded region is 23-27 nucleotide pairs in length.
16. The dsRNA agent of claim 12, wherein the double stranded region is 21-23 nucleotide pairs in length.
17. The dsRNA agent of any one of claims 1-16, wherein each strand is independently no more than 30 nucleotides in length.
18. The dsRNA agent of any one of claims 1-17, wherein the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.
19. The dsRNA agent of any one of claims 1-18, wherein the region of complementarity is at least 17 nucleotides in length.
20. The dsRNA agent of any one of claims 1-19, wherein the region of complementarity is between 19 and 23 nucleotides in length.
21. The dsRNA agent of any one of claims 1-20, wherein the region of complementarity is 19 nucleotides in length.
22. The dsRNA agent of any one of claims 1-21, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.
23. The dsRNA agent of any one of claims 1-21, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.
24. The dsRNA agent of any one of claims 1-23, further comprising a ligand.
25. The dsRNA agent of claim 24, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.
26. The dsRNA agent of claim 24 or 25, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.
27. The dsRNA agent of any one of claims 24-26, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

28. The dsRNA agent of claim 26 or 27, wherein the ligand is



29. The dsRNA agent of claim 28, wherein the dsRNA agent is conjugated to the ligand as
5 shown in the following schematic



and, wherein X is O or S.

30. The dsRNA agent of claim 29, wherein the X is O.
10

31. The dsRNA agent of any one of claims 1-30, wherein the dsRNA agent further comprises at
least one phosphorothioate or methylphosphonate internucleotide linkage.

32. The dsRNA agent of claim 31, wherein the phosphorothioate or methylphosphonate
15 internucleotide linkage is at the 3'-terminus of one strand.

33. The dsRNA agent of claim 32, wherein the strand is the antisense strand.

34. The dsRNA agent of claim 32, wherein the strand is the sense strand.
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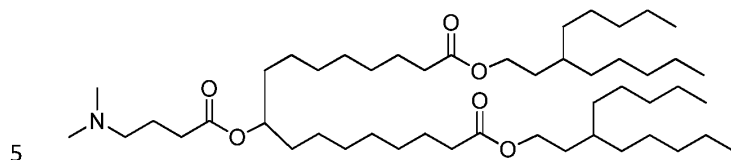
35. The dsRNA agent of claim 31, wherein the phosphorothioate or methylphosphonate
internucleotide linkage is at the 5'-terminus of one strand.

36. The dsRNA agent of claim 35, wherein the strand is the antisense strand.
37. The dsRNA agent of claim 35, wherein the strand is the sense strand.
- 5 38. The dsRNA agent of claim 31, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at both the 5'- and 3'-terminus of one strand.
39. The dsRNA agent of claim 38, wherein the strand is the antisense strand.
- 10 40. The dsRNA agent of any one of claims 1-39, wherein the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.
41. The dsRNA agent of any one of claims 1-40, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5' -
 15 UTUCGAAUCAATCCAACAGUAGC -3'.
42. The dsRNA agent of any one of claims 1-40, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5'-
 UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprising at least 15 contiguous
 20 nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5' -
 UTUCGAAUCAATCCAACAGUAGC -3'.
43. The dsRNA agent of claim 41 or 42, wherein the antisense strand comprises the nucleotide
 25 sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.
44. The dsRNA agent of claims 41 or 42, wherein the sense strand comprises the nucleotide
 sequence 5' - UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprises the
 nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.
- 30 45. The dsRNA agent of claims 44, wherein the sense strand differs by no more than 4 bases
 from the nucleotide sequence 5'-usascuguugGfAfUfugauucgasasa-3' and the antisense strand differs
 by no more than 4 bases from the nucleotide sequence 5' -VPudTucdGadAucaadTcCfaacaguasgsc -
 3',
 wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and
 35 Uf are 2'-fluoro A, G, C and U respectively; s is a phosphorothioate linkage; VP is a vinyl
 phosphonate; dT is 2'-deoxythymidine -3'-phosphate; dG is 2'-deoxyguanosine-3'-phosphate; and
 dA is 2'-deoxyadenosine-3'-phosphate.

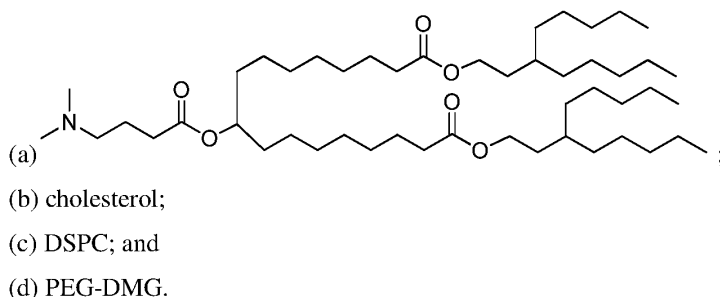
46. A double stranded RNA (dsRNA) agent for inhibiting expression of beta-catenin (CTNNB1) in a cell, comprising a sense strand comprising the nucleotide sequence 5'-usascuguugGfAfUfugauucgasasa-3' and the antisense strand comprises the nucleotide sequence 5'-VPudTucdGadAucaadTcCfaacaguasgsc-3',
- 5 wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; s is a phosphorothioate linkage; VP is a vinyl phosphonate; dT is 2'-deoxythymidine-3'-phosphate; dG is 2'-deoxyguanosine-3'-phosphate; and dA is 2'-deoxyadenosine-3'-phosphate.
- 10 47. The dsRNA agent of claim 46, further comprising a ligand.
48. A cell containing the dsRNA agent of any one of claims 1-47.
49. A pharmaceutical composition for inhibiting expression of a gene encoding beta-catenin
- 15 (CTNNB1) comprising the dsRNA agent of any one of claims 1-47 and a pharmaceutically acceptable carrier.
50. The pharmaceutical composition of claim 49, wherein dsRNA agent is in an unbuffered solution.
- 20 51. The pharmaceutical composition of claim 50, wherein the unbuffered solution is saline or water.
52. The pharmaceutical composition of claim 49, wherein said dsRNA agent is in a buffer
- 25 solution.
53. The pharmaceutical composition of claim 52, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.
- 30 54. The pharmaceutical composition of claim 53, wherein the buffer solution is phosphate buffered saline (PBS).
55. A pharmaceutical composition for inhibiting expression of a gene encoding beta-catenin (CTNNB1) comprising the dsRNA agent of any one of claims 1-47 and a lipid.
- 35 56. The pharmaceutical composition of claim 55, wherein the lipid is a cationic lipid.

57. The pharmaceutical composition of claim 56, wherein the cationic lipid comprises one or more biodegradable groups.

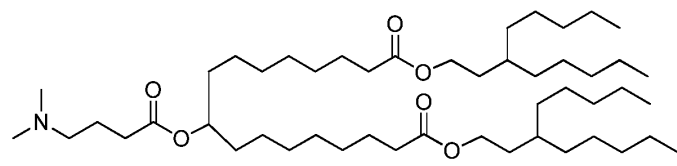
58. The pharmaceutical composition of claim 57, wherein the lipid comprises the structure



59. The pharmaceutical composition of claim 58, comprising



60. The pharmaceutical composition of claim 59, wherein the



15 DSPC, cholesterol, and PEG-DMG are present in a molar ratio of 50:12:36:2, respectively.

61. A pharmaceutical composition comprising a dsRNA agent for inhibiting expression of a gene encoding beta-catenin (CTNNB1), comprising a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5' -
 20 UTUCGAAUCAATCCAACAGUAGC -3', and a lipid.

62. The pharmaceutical composition of claim 61, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5' -
 25 UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5' -
 UTUCGAAUCAATCCAACAGUAGC -3'.

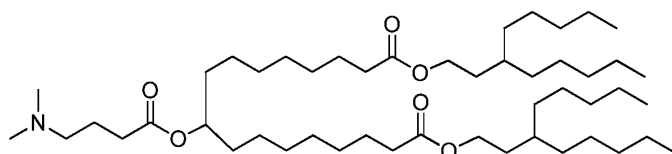
63. The pharmaceutical composition of claim 61 or 62, wherein the antisense strand comprises the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

64. The pharmaceutical composition of claim 61 or 62, wherein the sense strand comprises the nucleotide sequence 5' - UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprises the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

65. The pharmaceutical composition of claim 64, wherein the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-usascuguugGfAfUfugauucgasasa-3' and the antisense strand differs by no more than 4 bases from the nucleotide sequence 5' - VPudTucdGadAucaadTcCfaacaguasgsc -3',

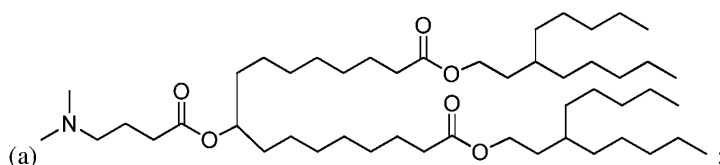
wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; s is a phosphorothioate linkage; VP is a vinyl phosphonate; dT is 2'-deoxythymidine -3'-phosphate; dG is 2'-deoxyguanosine-3'-phosphate; and dA is 2'-deoxyadenosine-3'-phosphate.

66. The pharmaceutical composition of any one of claims 61-65, wherein the lipid comprises the structure



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67. The pharmaceutical composition of claim 66, comprising

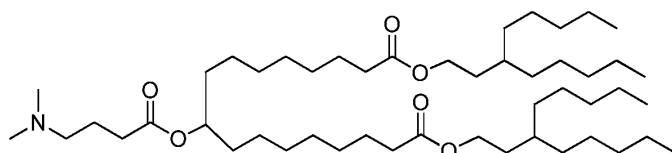


(b) cholesterol;

(c) DSPC; and

25 (d) PEG-DMG.

68. The pharmaceutical composition of claim 66, wherein the



, DSPC, cholesterol, and PEG-DMG are present in a molar ratio of 50:12:36:2 respectively.

69. A method of inhibiting expression of a beta-catenin (CTNNB1) gene in a cell, the method comprising contacting the cell with the dsRNA agent of any one of claims 1-47, or the pharmaceutical composition of any one of claims 49-68, thereby inhibiting expression of the CTNNB1 gene in the cell.
- 5
70. The method of claim 69, wherein the cell is within a subject.
71. The method of claim 70, wherein the subject is a human.
- 10 72. The method of claim 70, wherein the subject has a CTNNB1-associated disorder.
73. The method of claim 72, wherein the CTNNB1-associated disorder is a cancer.
- 15 74. The method of claim 73, wherein the cancer is hepatocellular carcinoma.
75. The method of any one of claims 69-74, wherein contacting the cell with the dsRNA agent inhibits the expression of CTNNB1 by at least 50%, 60%, 70%, 80%, 90%, or 95%.
- 20 76. The method of any one of claims 69-75, wherein inhibiting expression of CTNNB1 decreases CTNNB1 protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.
- 25 77. A method of treating a subject having a disorder that would benefit from reduction in beta-catenin (CTNNB1) expression, comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-47, or the pharmaceutical composition of any one of claims 49-68, thereby treating the subject having the disorder that would benefit from reduction in CTNNB1 expression.
- 30 78. A method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in beta-catenin (CTNNB1) expression, comprising administering to the subject a prophylactically effective amount of the dsRNA agent of any one of claims 1-47, or the pharmaceutical composition of any one of claims 49-68, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in CTNNB1 expression.
- 35 79. The method of claim 77 or 78, wherein the disorder is a CTNNB1-associated disorder.
80. The method of claim 79, wherein the CTNNB1-associated disorder is a cancer.

81. The method of claim 80, wherein the cancer is hepatocellular carcinoma.
82. The method of claim 79 or 80, wherein the subject is a human.
- 5 83. The method of any one of claims 79-82, wherein administration of the dsRNA agent to the subject causes a decrease in CTNNB1 protein accumulation in the subject.
84. The method of any one of claims 79-83, wherein the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.
- 10 85. The method of any one of claims 79-84, wherein the dsRNA agent is administered to the subject subcutaneously.
86. The method of any one of claims 79-84, wherein the dsRNA agent is administered to the subject intravenously.
- 15 87. The method of any one of claims 79-86, further comprising determining the level of CTNNB1 in a sample(s) from the subject.
- 20 88. The method of claim 87, wherein the level of CTNNB1 in the subject sample(s) is a CTNNB1 protein level in a blood or serum or liver tissue sample(s).
89. The method of any one of claims 79-88, further comprising administering to the subject an additional therapeutic agent for treatment of a CTNNB1-associated disorder.
- 25 90. The method of claim 89, wherein the additional therapeutic agent is selected from the group consisting of a chemotherapeutic agent, a growth inhibitory agent, an anti-angiogenesis agent, an anti-neoplastic composition and a combination of any of the foregoing.
- 30 91. A kit comprising the dsRNA agent of any one of claims 1-47 or the pharmaceutical composition of any one of claims 49-68.
92. A vial comprising the dsRNA agent of any one of claims 1-47 or the pharmaceutical composition of any one of claims 49-68.
- 35 93. A syringe comprising the dsRNA agent of any one of claims 1-47 or the pharmaceutical composition of any one of claims 49-68.

94. An RNA-induced silencing complex (RISC) comprising an antisense strand of any of the dsRNA agents of claims 1-47.