

hypertriglyceridemia can also lead to pancreatitis. Hyperlipidemia is another example of a lipid metabolism disorder that is characterized by elevated levels of any one or all lipids and/or lipoproteins in the blood. Current treatments for disorders of lipid metabolism, including dieting, exercise and treatment with statins and other drugs, are not always effective. Accordingly, there is a need in the art for alternative treatments for subjects having disorders of lipid metabolism.

Summary of the Invention

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an ANGPL3 gene. The ANGPL3 gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The present invention also provides methods of using the iRNA compositions of the invention for inhibiting the expression of an ANGPL3 gene and/or for treating a subject who would benefit from inhibiting or reducing the expression of an ANGPL3 gene, *e.g.*, a subject suffering or prone to suffering from a disorder of lipid metabolism, such as a subject suffering or prone to suffering from hyperlipidemia or hypertriglyceridemia.

Accordingly, in one aspect, the present invention provides double-stranded ribonucleic acids (dsRNAs) for inhibiting expression of ANGPTL3. The dsRNAs comprise a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:5.

In another aspect, the present invention provides double-stranded ribonucleic acids (dsRNAs) for inhibiting expression of ANGPTL3. The dsRNAs comprise a sense strand and an antisense strand, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no

more than 3 nucleotides from any one of the antisense sequences listed in Tables 2, 3, 7, 8, 9 and 10.

In one embodiment, the sense and antisense strands comprise sequences selected from the group consisting of AD-53063.1, AD-53001.1, AD-53015.1, AD-52986.1, AD-52981.1, AD-52953.1, AD-53024.1, AD-53033.1, AD-53030.1, AD-53080.1, AD-53073.1, AD-53132.1, AD-52983.1, AD-52954.1, AD-52961.1, AD-52994.1, AD-52970.1, AD-53075.1, AD-53147.1, AD-53077.1 of Tables 7 and 8.

In certain embodiments of the invention, the dsRNAs comprise at least one modified nucleotide. In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group. In another embodiment, the modified nucleotide is selected from the group consisting of a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

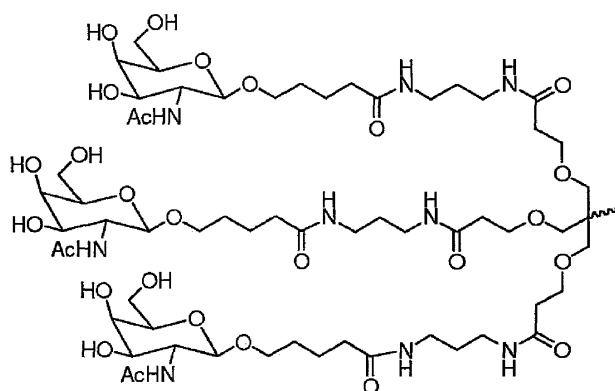
The region of complementarity of the dsRNAs may be at least 17 nucleotides in length, between 19 and 21 nucleotides in length, or 19 nucleotides in length.

In one embodiment, each strand of a dsRNA is no more than 30 nucleotides in length.

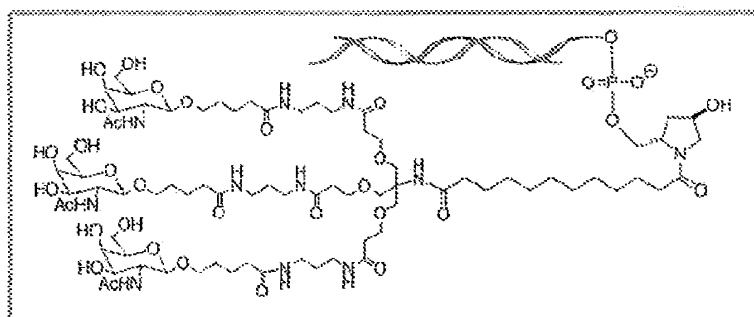
At least one strand of a dsRNA may comprise a 3' overhang of at least 1 nucleotide or at least 2 nucleotides.

In certain embodiments, a dsRNA further comprises a ligand. In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the dsRNA.

In some embodiments, the ligand is one or more N-acetylgalactosamine (GalNAc) derivatives attached through a bivalent or trivalent branched linker. In particular embodiments, the ligand is



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



5 In some embodiments, the RNAi agent further includes at least one phosphorothioate or methylphosphonate internucleotide linkage. In some embodiments, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminal of one strand. In some embodiments, the strand is the antisense strand. In other embodiments, the strand is the sense strand.

10 In one embodiment, the region of complementarity of a dsRNA consists of one of the antisense sequences of Tables 2, 3, 7, 8, 9 and 10.

 In another embodiment, a dsRNA comprises a sense strand consisting of a sense strand sequence selected from the sequences of Tables 2, 3, 7, 8, 9 and 10, and an antisense strand consisting of an antisense sequence selected from the sequences of
15 Tables 2, 3, 7, 8, 9 and 10.

In another aspect, the present invention provides a cell, *e.g.*, a hepatocyte, containing a dsRNA of the invention.

In yet another aspect, the present invention provides a vector encoding at least one strand of a dsRNA, wherein the dsRNA comprises a region of complementarity to at least a part of an mRNA encoding ANGPTL3, wherein the dsRNA is 30 base pairs or less in length, and wherein the dsRNA targets the mRNA for cleavage. The region of complementarity may be least 15 nucleotides in length or 19 to 21 nucleotides in length.

In a further aspect, the present invention provides a cell comprising a vector encoding at least one strand of a dsRNA, wherein the dsRNA comprises a region of complementarity to at least a part of an mRNA encoding ANGPTL3, wherein the dsRNA is 30 base pairs or less in length, and wherein the dsRNA targets the mRNA for cleavage.

In one aspect, the present invention provides a pharmaceutical composition for inhibiting expression of an ANGPTL3 gene comprising a dsRNA or vector of the invention.

In one embodiment, the pharmaceutical composition comprises a lipid formulation, such as a MC3, SNALP or XTC formulation.

In another aspect, the present invention provides methods of inhibiting ANGPTL3 expression in a cell. The methods include contacting the cell with a dsRNA or a vector of the invention, and maintaining the cell produced for a time sufficient to obtain degradation of the mRNA transcript of an ANGPTL3 gene, thereby inhibiting expression of the ANGPTL3 gene in the cell.

The cell may be within a subject, such as a human subject, for example a human subject suffering from a disorder of lipid metabolism, *e.g.*, hyperlipidemia or hypertriglyceridemia.

In one embodiment of the methods of the invention, ANGPTL3 expression is inhibited by at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at

least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

5 In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in ANGPTL3 expression, *e.g.*, a disorder of lipid metabolism, such as hyperlipidemia or hypertriglyceridemia. The methods include administering to the subject a therapeutically effective amount of a dsRNA or a vector of the invention, thereby treating the subject.

10 The disorder may be disorder of lipid metabolism, such as hyperlipidemia or hypertriglyceridemia

 In one embodiment, the administration of the dsRNA to the subject causes a decrease in the level of a serum lipid, triglycerides, cholesterol and/or free fatty acids; and/or a decrease in ANGPTL3 protein accumulation. In one embodiment,
15 administration of the dsRNA to the subject causes a decrease in the level of LDL-C, HDL-C, VLDL-C, IDL-C and/or total cholesterol.

 In one embodiment, the dsRNA is administered at a dose of about 0.01 mg/kg to about 10 mg/kg, *e.g.*, about 0.05 mg/kg to about 5 mg/kg, about 0.05 mg/kg to about 10 mg/kg, about 0.1 mg/kg to about 5 mg/kg, about 0.1 mg/kg to about 10 mg/kg, about 0.2 mg/kg to about 5 mg/kg, about 0.2 mg/kg to about 10 mg/kg, about 0.3 mg/kg to about 5 mg/kg, about 0.3 mg/kg to about 10 mg/kg, about 0.4 mg/kg to about 5 mg/kg, about 0.4 mg/kg to about 10 mg/kg, about 0.5 mg/kg to about 5 mg/kg, about 0.5 mg/kg to about 10 mg/kg, about 1 mg/kg to about 5 mg/kg, about 1 mg/kg to about 10 mg/kg, about 1.5 mg/kg to about 5 mg/kg, about 1.5 mg/kg to about 10 mg/kg, about 2 mg/kg to about 25 about 2.5 mg/kg, about 2 mg/kg to about 10 mg/kg, about 3 mg/kg to about 5 mg/kg, about 3 mg/kg to about 10 mg/kg, about 3.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 5 mg/kg, about 4.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 10 mg/kg, about 4.5 mg/kg to about 10 mg/kg, about 5 mg/kg to about 10 mg/kg, about 5.5 mg/kg to about 10 mg/kg, about 6 mg/kg to about 10 mg/kg, about 6.5 mg/kg to about 10 mg/kg, about 7 mg/kg to about 10 mg/kg, about 7.5 mg/kg to about 10 mg/kg, about 8
30 mg/kg, about 7 mg/kg to about 10 mg/kg, about 7.5 mg/kg to about 10 mg/kg, about 8

mg/kg to about 10 mg/kg, about 8.5 mg/kg to about 10 mg/kg, about 9 mg/kg to about 10 mg/kg, or about 9.5 mg/kg to about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, the dsRNA may be administered at a dose of about 0.01, 0.02, 0.03,
5 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3,
1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4,
3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5,
5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6,
7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7,
10 9.8, 9.9, or about 10 mg/kg. Values and ranges intermediate to the recited values are
also intended to be part of this invention.

In another embodiment, the dsRNA is administered at a dose of about 0.5 to
about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/kg, about 1.5 to
about 50 mg/kg, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to
15 about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to
about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to
about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 20 to
about 50 mg/kg, about 25 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to
about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to
20 about 50 mg/kg, about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to
about 45 mg/kg, about 1.5 to about 45 mg/kg, about 2 to about 45 mg/kg, about 2.5 to
about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to about 45 mg/kg, about 4 to
about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to
about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to
25 about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 25 to
about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to
about 45 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to
about 40 mg/kg, about 1.5 to about 40 mg/kg, about 2 to about 40 mg/kg, about 2.5 to
about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to
30 about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to
about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to
about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 25 to

about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.5 to about 30 mg/kg, about 0.75 to about 30 mg/kg, about 1 to about 30 mg/kg, about 1.5 to about 30 mg/kg, about 2 to about 30 mg/kg, about 2.5 to about 30 mg/kg, about 3 to about 30 mg/kg, about 3.5 to about 30 mg/kg, about 4 to about 30 mg/kg, about 4.5 to about 30 mg/kg, about 5 to about 30 mg/kg, about 7.5 to about 30 mg/kg, about 10 to about 30 mg/kg, about 15 to about 30 mg/kg, about 20 to about 30 mg/kg, about 20 to about 30 mg/kg, about 25 to about 30 mg/kg, about 0.5 to about 20 mg/kg, about 0.75 to about 20 mg/kg, about 1 to about 20 mg/kg, about 1.5 to about 20 mg/kg, about 2 to about 20 mg/kg, about 2.5 to about 20 mg/kg, about 3 to about 20 mg/kg, about 3.5 to about 20 mg/kg, about 4 to about 20 mg/kg, about 4.5 to about 20 mg/kg, about 5 to about 20 mg/kg, about 7.5 to about 20 mg/kg, about 10 to about 20 mg/kg, or about 15 to about 20 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, subjects can be administered a therapeutic amount of iRNA, such as about 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In another aspect, the present invention provides methods of inhibiting the expression of ANGPTL3 in a subject. The methods include administering to the subject a therapeutically effective amount of a dsRNA or a vector of the invention, thereby inhibiting the expression of ANGPTL3 in the subject.

In yet another aspect, the invention provides kits for performing the methods of the invention. In one aspect, the invention provides a kit for performing a method of inhibiting expression of ANGPTL3 gene in a cell by contacting a cell with a double stranded RNAi agent in an amount effective to inhibit expression of the ANGPTL3 in

the cell. The kit comprises an RNAi agent and instructions for use and, optionally, means for administering the RNAi agent to a subject.

Brief Description of the Drawings

5 Figure 1 is a schematic of the experimental procedure used for in vivo tests described in Example 2.

 Figure 2, Panel A is a graph showing measured levels of ANGPTL3 protein in WT mice after treatment with the indicated iRNA or a control. Figure 2, Panel B, is a graph showing measured levels of ANGPTL3 protein in ob/ob mice after treatment with
10 the indicated iRNA or a control.

 Figure 3, Panel A, is a graph showing measured levels of LDL-c in WT mice after treatment with the indicated iRNA or a control. Figure 3, Panel B, is a graph showing measured levels of LDL-c in ob/ob mice after treatment with the indicated iRNA or a control.

15 Figure 4, Panel A, is a graph showing measured levels of triglycerides in WT mice after treatment with the indicated iRNA or a control. Figure 4, Panel B, is a graph showing measured levels of triglycerides in ob/ob mice after treatment with the indicated iRNA or a control.

 Figure 5, Panel A, is a graph showing measured levels of total cholesterol (TC)
20 in WT mice after treatment with the indicated iRNA or a control. Figure 5, Panel B, is a graph showing measured levels of total cholesterol (TC) in ob/ob mice after treatment with the indicated iRNA or a control.

 Figure 6, Panel A, is a graph showing measured levels of HDL-c in WT mice after treatment with the indicated iRNA or a control. Figure 6, Panel B, is a graph
25 showing measured levels of HDL-c in ob/ob mice after treatment with the indicated iRNA or a control.

Figure 7 is a graph showing measured levels of ANGPTL3 protein in human PCS transgenic mice after treatment with a single dose of the indicated iRNA or a control.

Detailed Description of the Invention

5 The present invention provides iRNA compositions, which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an ANGPTL3 gene. The ANGPTL3 gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The present invention also provides methods of using the iRNA compositions of the invention for inhibiting the expression of an ANGPTL3 gene and/or
10 for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of an ANGPTL3 gene, *e.g.*, a disorder of lipid metabolism, such as hyperlipidemia or hypertriglyceridemia.

 The iRNAs of the invention include an RNA strand (the antisense strand) having a region which is about 30 nucleotides or less in length, *e.g.*, 15-30, 15-29, 15-28, 15-27,
15 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, which region is substantially complementary to at least
20 part of an mRNA transcript of an ANGPTL3 gene. The use of these iRNAs enables the targeted degradation of mRNAs of an ANGPTL3 gene in mammals. Very low dosages of ANGPTL3 iRNAs, in particular, can specifically and efficiently mediate RNA interference (RNAi), resulting in significant inhibition of expression of an ANGPTL3 gene. Using cell-based assays, the present inventors have demonstrated that iRNAs
25 targeting ANGPTL3 can mediate RNAi, resulting in significant inhibition of expression of an ANGPTL3 gene. Thus, methods and compositions including these iRNAs are useful for treating a subject who would benefit by a reduction in the levels and/or activity of an ANGPTL3 protein, such as a subject having a disorder of lipid metabolism, such as hyperlipidemia or hypertriglyceridemia.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of an ANGPTL3 gene, as well as compositions and methods for treating subjects having diseases and disorders that would benefit from inhibition and/or reduction of the expression of this gene.

5 **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.

10 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.

15 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.

20 The term "ANGPTL3" refers to an angiopoietin like protein 3 having an amino acid sequence from any vertebrate or mammalian source, including, but not limited to, human, bovine, chicken, rodent, mouse, rat, porcine, ovine, primate, monkey, and guinea pig, unless specified otherwise. The term also refers to fragments and variants of native ANGPTL3 that maintain at least one *in vivo* or *in vitro* activity of a native ANGPTL3. The term encompasses full-length unprocessed precursor forms of ANGPTL3 as well as mature forms resulting from post-translational cleavage of the signal peptide and forms resulting from proteolytic processing of the fibrinogen-like domain. The sequence of a human ANGPTL3 mRNA transcript can be found at, for example, GenBank Accession
25 No. GI: 41327750 (NM_014495.2; SEQ ID NO:1). The predicted sequence of rhesus ANGPTL3 mRNA can be found at, for example, GenBank Accession No. GI: 297278846 (XM_001086114.2; SEQ ID NO:2). The sequence of mouse ANGPTL3 mRNA can be found at, for example, GenBank Accession No. GI: 142388354 (NM_

013913.3; SEQ ID NO:3). The sequence of rat ANGPTL3 mRNA can be found at, for example, GenBank Accession No. GI: 68163568 (NM_001025065.1; SEQ ID NO:4).

The term“ANGPTL3” as used herein also refers to a particular polypeptide expressed in a cell by naturally occurring DNA sequence variations of the ANGPTL3 gene, such as a single nucleotide polymorphism in the ANGPTL3 gene. Numerous SNPs within the ANGPTL3 gene have been identified and may be found at, for example, NCBI dbSNP (see, *e.g.*, www.ncbi.nlm.nih.gov/snp). Non-limiting examples of SNPs within the ANGPTL3 gene may be found at, NCBI dbSNP Accession Nos. rs193064039; rs192778191; rs192764027; rs192528948; rs191931953; rs191293319; rs191171206; rs191145608; rs191086880; rs191012841; or rs190255403.

As used herein, “target sequence” refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an ANGPTL3gene, including mRNA that is a 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 an ANGPTL3gene.

The target sequence may be from about 9-36 nucleotides in length, *e.g.*, about 15-30 nucleotides in length. For example, the target sequence can be from about 15-30 nucleotides, 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. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

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

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

The terms “iRNA”, “RNAi agent,” “iRNA agent,” “RNA interference agent” as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The iRNA modulates, *e.g.*, inhibits, the expression of ANGPTL3 in a cell, *e.g.*, a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNA that interacts with a target RNA sequence, *e.g.*, an ANGPTL3 target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp et al., Genes Dev. 2001, 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, et al., (2001) Nature 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the

complementary antisense strand to guide target recognition (Nykanen, et al., (2001) Cell 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, et al., (2001) Genes Dev. 15:188). Thus, in one aspect the invention relates to a single stranded RNA (siRNA) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.*, an ANGPTL3 gene. Accordingly, the term “siRNA” is also used herein to refer to an RNAi as described above.

In another aspect, the RNAi agent is a single-stranded antisense RNA molecule. An antisense RNA molecule is complementary to a sequence within the target mRNA. Antisense RNA can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. *et al.*, (2002) *Mol. Cancer Ther.* 1:347-355. The single-stranded antisense RNA molecule may be about 13 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense RNA molecule may comprise a sequence that is at least about 13, 14, 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the antisense sequences in Tables 2, 3, 7, 8, 9 and 10.

In another embodiment, an “iRNA” for use in the compositions and methods of the invention is a double-stranded RNA and is referred to herein as a “double stranded RNAi agent,” “double-stranded RNA (dsRNA) molecule,” “dsRNA agent,” or “dsRNA”. The term “dsRNA”, refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having “sense” and “antisense” orientations with respect to a target RNA, *i.e.*, an ANGPTL3 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.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 9 to 36 base pairs in length, *e.g.*, about 15-30 base pairs in length, for example, about 9, 10, 11, 12,

13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 5 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

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 10 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 15 embodiments, the hairpin loop can comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23 or more unpaired nucleotides.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. 20 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 25 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.

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of an iRNA, *e.g.*, a dsRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other 30 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 and/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 and/or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

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

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.*, an ANGPTL3 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.*, an ANGPTL3 nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, 3, or 2 nucleotides of the 5'- and/or 3'-terminus of the iRNA.

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, and unless otherwise indicated, the term “complementary,” when
5 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
10 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
15 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
20 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
25 pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.*, inhibition of gene expression via a RISC pathway. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of
30 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.

5 “Complementary” sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs and/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.

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

As used herein, a polynucleotide that is “substantially complementary to at least part of” a messenger RNA (mRNA) refers to a polynucleotide that is substantially
15 complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding ANGPTL3). For example, a polynucleotide is complementary to at least a part of an ANGPTL3mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding ANGPTL3.

In general, the majority of nucleotides of each strand are ribonucleotides, but as
20 described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. In addition, an “iRNA” may include 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 an iRNA molecule, are encompassed by “iRNA”
25 for the purposes of this specification and claims.

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 an ANGPTL3,” as used herein, includes inhibition of expression of any ANGPTL3 gene (such as, *e.g.*, a mouse ANGPTL3 gene, a rat ANGPTL3 gene, a monkey ANGPTL3 gene, or a human ANGPTL3 gene) as well as variants or mutants of an ANGPTL3 gene that encode an ANGPTL3 protein.

5 “Inhibiting expression of an ANGPTL3 gene” includes any level of inhibition of an ANGPTL3 gene, *e.g.*, at least partial suppression of the expression of an ANGPTL3 gene, such as an inhibition by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

15 The expression of an ANGPTL3 gene may be assessed based on the level of any variable associated with ANGPTL3 gene expression, *e.g.*, ANGPTL3 mRNA level or ANGPTL3 protein level. The expression of an ANGPTL3 may also be assessed indirectly based on the levels of a serum lipid, a triglyceride, cholesterol (including LDL-C, HDL-C, VLDL-C, IDL-C and total cholesterol), or free fatty acids. Inhibition may be assessed by a decrease in an absolute or relative level of one or more of these variables compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

25 In one embodiment, at least partial suppression of the expression of an ANGPTL3 gene, is assessed by a reduction of the amount of ANGPTL3 mRNA which can be isolated from or detected in a first cell or group of cells in which an ANGPTL3 gene is transcribed and which has or have been treated such that the expression of an ANGPTL3 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 or have not been so treated (control cells). The degree of inhibition may be expressed in terms of:

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

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

Contacting a cell *in vitro* may be done, for example, by incubating the cell with the RNAi agent. Contacting a cell *in vivo* may be done, for example, by injecting the RNAi agent into or near the tissue where the cell is located, or by injecting the RNAi agent into another area, *e.g.*, the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the RNAi agent may contain and/or be coupled to a ligand, *e.g.*, GalNAc3, that directs the RNAi agent 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 RNAi agent and subsequently transplanted into a subject.

In one embodiment, 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 diffusive or active cellular processes, or by auxiliary agents or devices. Introducing an iRNA into a cell may be *in vitro* and/or *in vivo*. For example, for *in vivo* introduction, iRNA can be injected into a tissue site or administered systemically. *In vivo* delivery can also be done by a beta-glucan delivery system, such as those described in U.S. Patent Nos. 5,032,401 and 5,607,677, and U.S. Publication No. 2005/0281781, the entire contents of which are hereby incorporated herein by reference. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below and/or are known in the art.

The term "SNALP" refers to a stable nucleic acid-lipid particle. A SNALP is a vesicle of lipids coating a reduced aqueous interior comprising a nucleic acid such as an iRNA or a plasmid from which an iRNA is transcribed. SNALPs are described, *e.g.*, in U.S. Patent Application Publication Nos. 20060240093, 20070135372, and in
5 International Application No. WO 2009082817, the entire contents of which are hereby incorporated herein by reference. Examples of "SNALP" formulations are described below.

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-
10 primate (such as a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, a horse, and a whale), or a bird (*e.g.*, a duck or a goose). In an embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder or condition that would benefit from reduction in ANGPTL3 expression; a human at risk for a disease, disorder or condition that would
15 benefit from reduction in ANGPTL3 expression; a human having a disease, disorder or condition that would benefit from reduction in ANGPTL3 expression; and/or human being treated for a disease, disorder or condition that would benefit from reduction in ANGPTL3 expression as described herein. As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result including, such as lowering levels of
20 triglycerides in a subject. The terms "treating" or "treatment" also include, but are not limited to, alleviation or amelioration of one or more symptoms of a disorder of lipid metabolism, such as, *e.g.*, a decrease in the size of eruptive xanthomas. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

25 By "lower" in the context of a disease marker or symptom is meant a statistically significant decrease in such level. The decrease can be, for example, at least 10%, at least 20%, at least 30%, at least 40% or more, and is preferably down to a level accepted as within the range of normal for an individual without such disorder. As used herein, "prevention" or "preventing," when used in reference to a disease, disorder or condition
30 thereof, that would benefit from a reduction in expression of an ANGPTL3 gene, refers to a reduction in the likelihood that a subject will develop a symptom associated with

such disease, disorder, or condition, *e.g.*, high triglyceride levels or eruptive xanthoma. The likelihood of developing a high triglyceride levels or eruptive xanthoma is reduced, for example, when an individual having one or more risk factors for a high triglyceride levels or eruptive xanthoma either fails to develop high triglyceride levels or eruptive xanthoma or develops high triglyceride levels or eruptive xanthoma with less severity relative to a population having the same risk factors and not receiving treatment as described herein. The failure to develop a disease, disorder or condition, or the reduction in the development of a symptom associated with such a disease, disorder or condition i (*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 "serum lipid" refers to any major lipid present in the blood. Serum lipids may be present in the blood either in free form or as a part of a protein complex, *e.g.*, a lipoprotein complex. Non-limiting examples of serum lipids may include triglycerides and cholesterol, such as total cholesterol (TG), low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C) and intermediate-density lipoprotein cholesterol (IDL-C).

As used herein, a "disorder of lipid metabolism" refers to any disorder associated with or caused by a disturbance in lipid metabolism. For example, this term includes any disorder, disease or condition that can lead to hyperlipidemia, or condition characterized by abnormal elevation of levels of any or all lipids and/or lipoproteins in the blood. This term refers to an inherited disorder, such as familial hypertriglyceridemia, or an acquired disorder, such as a disorder acquired as a result of a diet or intake of certain drugs. Exemplary disorders of lipid metabolism include, but are not limited to, atherosclerosis, dyslipidemia, hypertriglyceridemia (including drug-induced hypertriglyceridemia, diuretic-induced hypertriglyceridemia, alcohol-induced hypertriglyceridemia, β -adrenergic blocking agent-induced hypertriglyceridemia, estrogen-induced hypertriglyceridemia, glucocorticoid-induced hypertriglyceridemia, retinoid-induced hypertriglyceridemia, cimetidine-induced hypertriglyceridemia, and familial hypertriglyceridemia), acute pancreatitis associated with hypertriglyceridemia,

chylomicron syndrom, familial chylomicronemia, Apo-E deficiency or resistance, LPL deficiency or hypoactivity, hyperlipidemia (including familial combined hyperlipidemia), hypercholesterolemia, gout associated with hypercholesterolemia, xanthomatosis (subcutaneous cholesterol deposits).

5 Cardiovascular diseases associated with disorders of lipid metabolism are also considered “disorders of lipid metabolism”, as defined herein. These diseases may include coronary artery disease (also called ischemic heart disease), inflammation associated with coronary artery disease, restenosis, peripheral vascular diseases, and stroke.

10 Disorders related to body weight are also considered “disorders of lipid metabolism”, as defined herein. Such disorders may include obesity, metabolic syndrome including independent components of metabolic syndrome (*e.g.*, central obesity, FBG/pre-diabetes/diabetes, hypercholesterolemia, hypertriglyceridemia, and hypertension), hypothyroidism, uremia, and other conditions associated with weight gain
15 (including rapid weight gain), weight loss, maintenance of weight loss, or risk of weight regain following weight loss.

Blood sugar disorders are further considered “disorders of lipid metabolism”, as defined herein. Such disorders may include diabetes, hypertension, and polycystic ovarian syndrome related to insulin resistance. Other exemplary disorders of lipid
20 metabolism may also include renal transplantation, nephrotic syndrome, Cushing's syndrome, acromegaly, systemic lupus erythematosus, dysglobulinemia, lipodystrophy, glycogenosis type I, and Addison's disease.

“Therapeutically effective amount,” as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a disorder of lipid
25 metabolism, 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
30 other individual characteristics of the subject to be treated.

“Prophylactically effective amount,” as used herein, is intended to include the amount of an iRNA that, when administered to a subject having a disorder of lipid metabolism, 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
5 reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the iRNA, 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.

10 A "therapeutically-effective amount" or “prophylactically effective amount” also includes an amount of an RNAi agent that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. 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.

15 The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/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.

20 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
25 organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as
30 sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered

tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

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 blood or plasma drawn from the subject.

II. iRNAs of the Invention

Described herein are iRNAs which inhibit the expression of an ANGPTL3 gene. In one embodiment, the iRNA agent includes double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of an ANGPTL3 gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human having a disorder of lipid metabolism, *e.g.*, familial hyperlipidemia. The dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of an ANGPTL3 gene. The region of complementarity is about 30 nucleotides

or less in length (*e.g.*, about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 nucleotides or less in length). Upon contact with a cell expressing the ANGPTL3 gene, the iRNA inhibits the expression of the ANGPTL3 gene (*e.g.*, a human, a primate, a non-primate, or a bird ANGPTL3 gene) by at least about 10% as assayed by, for
5 example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, Western Blotting or flowcytometric techniques.

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
10 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 an ANGPTL3 gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and
15 form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is between 15 and 30 base pairs in length, *e.g.*,
20 between, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths
25 intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

Similarly, the region of complementarity to the target sequence is between 15 and 30 nucleotides in length, *e.g.*, between 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,
30 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. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

5 In some embodiments, the dsRNA is between about 15 and about 20 nucleotides in length, or between about 25 and 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 can serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of
10 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
15 functional portion of a dsRNA, *e.g.*, a duplex region of about 9 to 36 base pairs, *e.g.*, about 10-36, 11-36, 12-36, 13-36, 14-36, 15-36, 9-35, 10-35, 11-35, 12-35, 13-35, 14-35, 15-35, 9-34, 10-34, 11-34, 12-34, 13-34, 14-34, 15-34, 9-33, 10-33, 11-33, 12-33, 13-33, 14-33, 15-33, 9-32, 10-32, 11-32, 12-32, 13-32, 14-32, 15-32, 9-31, 10-31, 11-31, 12-31, 13-32, 14-31, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-
20 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. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*,
25 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 ANGPTL3 expression is not
30 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, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide overhang can have unexpectedly 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 either an antisense or sense strand of a dsRNA.

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

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

In one 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 Tables 2, 3, 7, 8, 9 and 10, and the corresponding antisense strand of the sense strand is selected from the group of sequences of Tables 2, 3, 7, 8, 9 and 10. 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 an ANGPTL3 gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in Tables 2, 3, 7, 8, 9 and 10, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in Tables 2, 3, 7, 8, 9 and 10. In one embodiment, the substantially

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

The skilled person is well aware that dsRNAs having a duplex structure of between about 20 and 23 base pairs, *e.g.*, 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, (2001) *EMBO J.*, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Tables 2, 3, 7, 8, 9 and 10, 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 one of the sequences of Tables 2, 3, 7, 8, 9 and 10 minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences of Tables 2, 3, 7, 8, 9 and 10, and differing in their ability to inhibit the expression of an ANGPTL3 gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs provided in Tables 2, 3, 7, 8, 9 and 10 identify a site(s) in an ANGPTL3 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 15 contiguous nucleotides from one of the sequences provided in Tables 2, 3, 7, 8, 9 and 10 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in an ANGPTL3 gene.

While a target sequence is generally about 15-30 nucleotides in length, there is wide variation in the suitability of particular sequences in this range for directing

cleavage of any given target RNA. Various software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given gene target, but an empirical approach can also be taken in which a “window” or “mask” of a given size (as a non-limiting example, 21 nucleotides) is literally or figuratively (including, *e.g.*, in silico) placed on the target RNA sequence to identify sequences in the size range that can serve as target sequences. By moving the sequence “window” progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified sequences (using assays as described herein or as known in the art) to identify those sequences that perform optimally can identify those RNA sequences that, when targeted with an iRNA agent, mediate the best inhibition of target gene expression. Thus, while the sequences identified, for example, in Tables 2, 3, 7, 8, 9 and 10 represent effective target sequences, it is contemplated that further optimization of inhibition efficiency can be achieved by progressively “walking the window” one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

Further, it is contemplated that for any sequence identified, *e.g.*, in Tables 2, 3, 7, 8, 9 and 10, further optimization could be achieved by systematically either adding or removing nucleotides to generate longer or shorter sequences and testing those sequences generated by walking a window of the longer or shorter size up or down the target RNA from that point. Again, coupling this approach to generating new candidate targets with testing for effectiveness of iRNAs based on those target sequences in an inhibition assay as known in the art and/or as described herein can lead to further improvements in the efficiency of inhibition. Further still, such optimized sequences can be adjusted by, *e.g.*, the introduction of modified nucleotides as described herein or as known in the art, addition or changes in overhang, or other modifications as known in the art and/or discussed herein to further optimize the molecule (*e.g.*, increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with

silencing pathway enzymes, increasing release from endosomes) as an expression inhibitor.

An iRNA as described herein can contain one or more mismatches to the target sequence. In one embodiment, an iRNA as described herein contains no more than 3 mismatches. If the antisense strand of the iRNA contains mismatches to a target sequence, it is preferable that the area of mismatch is not located in the center of the region of complementarity. If the antisense strand of the iRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, for a 23 nucleotide iRNA agent the strand which is complementary to a region of an ANGPTL3 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 iRNA containing a mismatch to a target sequence is effective in inhibiting the expression of an ANGPTL3 gene. Consideration of the efficacy of iRNAs with mismatches in inhibiting expression of an ANGPTL3 gene is important, especially if the particular region of complementarity in an ANGPTL3 gene is known to have polymorphic sequence variation within the population.

III. Modified iRNAs of the Invention

In one embodiment, the RNA of an iRNA of the invention, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. The nucleic acids featured in the invention can be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Modifications include, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (*e.g.*, at the 2'-position or

4'-position) or replacement of the sugar; and/or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

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

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

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

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240;
15 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.

In other embodiments, suitable RNA mimetics are contemplated for use in iRNAs, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of
20 the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an
25 aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. 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
30 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--[wherein the native phosphodiester backbone is represented as --O--P--O--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.

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(CH₂)₂.

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 U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application. The entire contents of each of the foregoing are hereby incorporated herein by reference.

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 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.*, (1991) *Angewandte Chemie, International Edition*, 30:613, and those disclosed by Sanghvi, Y S., Chapter 15, *dsRNA Research and Applications*, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC

Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2 °C (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., *dsRNA Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

10 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; 15 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

The RNA of an iRNA can also be modified to include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 20 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193).

Representative U.S. Patents that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Patent Nos. 6,268,490; 6,670,461; 6,794,499; 6,998,484; 7,053,207; 7,084,125; and 7,399,845, the entire contents of each of which are hereby incorporated herein by reference.

Potentially stabilizing modifications to the ends of RNA molecules can include N- (acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-0-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-
 5 docosanoyl-uridine-3"- phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

IV. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves
 10 chemically linking to the RNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the iRNA. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, (1989) *Proc. Natl. Acad. Sci. USA*, 86: 6553-6556), cholic acid (Manoharan *et al.*, (1994) *Biorg. Med. Chem. Lett.*, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*,
 15 (1992) *Ann. N.Y. Acad. Sci.*, 660:306-309; Manoharan *et al.*, (1993) *Biorg. Med. Chem. Lett.*, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, (1992) *Nucl. Acids Res.*, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, (1991) *EMBO J*, 10:1111-1118; Kabanov *et al.*, (1990) *FEBS Lett.*, 259:327-330; Svinarchuk *et al.*, (1993) *Biochimie*, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate
 20 (Manoharan *et al.*, (1995) *Tetrahedron Lett.*, 36:3651-3654; Shea *et al.*, (1990) *Nucl. Acids Res.*, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, (1995) *Nucleosides & Nucleotides*, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, (1995) *Tetrahedron Lett.*, 36:3651-3654), a palmityl moiety (Mishra
 25 *et al.*, (1995) *Biochim. Biophys. Acta*, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, (1996) *J. Pharmacol. Exp. Ther.*, 277:923-937).

In one embodiment, a ligand alters the distribution, targeting or lifetime of an iRNA agent into which it is incorporated. In preferred embodiments a ligand provides
 30 an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment,

e.g., a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. Preferred ligands will not take part in duplex pairing in a duplexed nucleic acid.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*,
5 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
10 (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:
15 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,
20 *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-galucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose,
25 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.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin),
30 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-gulucosamine 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.

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

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins *etc.* Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin *etc.* Oligonucleotides that

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

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

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

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

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the

present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

5

A. Lipid Conjugates

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

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

In another preferred embodiment, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be preferably distributed to the kidney. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid based ligand.

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

B. Cell Permeation Agents

In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-
10 permeation agent. Preferably, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopodia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent is preferably an alpha-helical agent, which preferably has a lipophilic and a lipophobic phase.

15 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
20 peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide
25 or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 9). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 10) containing a hydrophobic MTS can also
30 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: 11) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 12) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

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

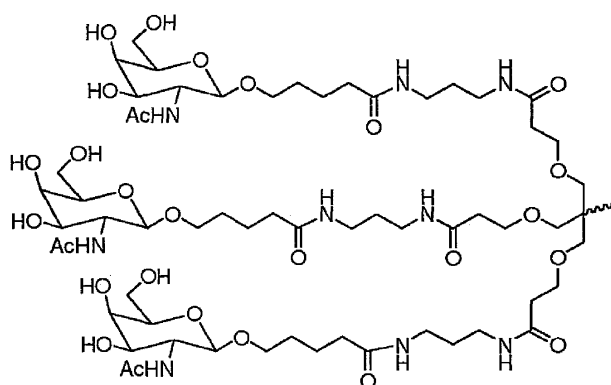
A "cell permeation peptide" is capable of permeating a cell, *e.g.*, a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, a α -helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing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

30

C. Carbohydrate Conjugates

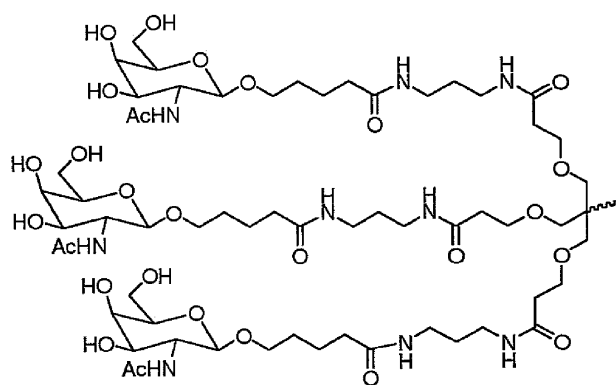
In some embodiments of the compositions and methods of the invention, an iRNA oligonucleotide further comprises a carbohydrate. The carbohydrate conjugated iRNA are advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein.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

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

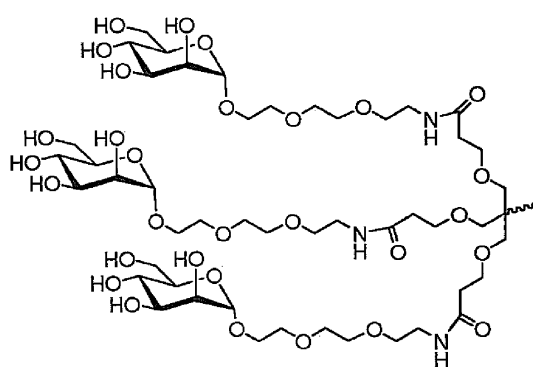


Formula II.

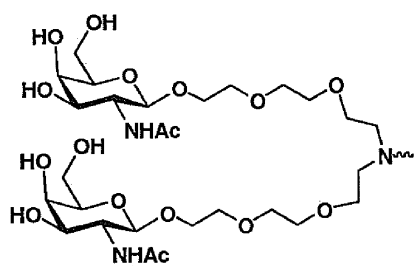
In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



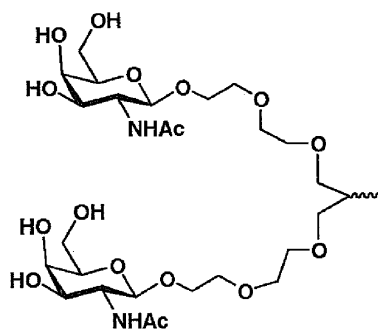
Formula II,



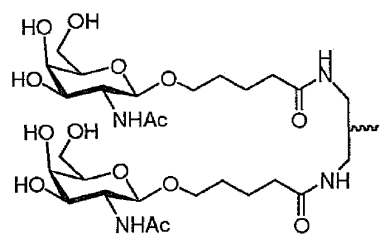
Formula III,



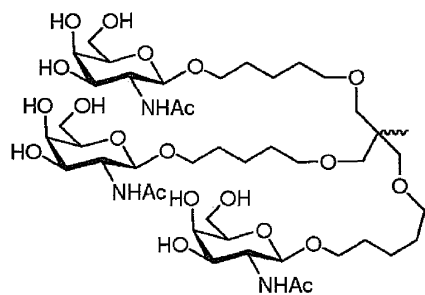
Formula IV,



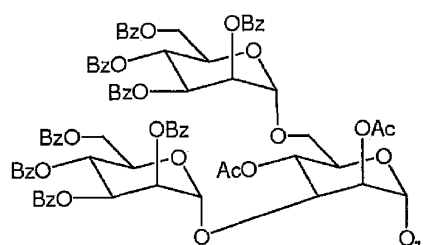
Formula V,



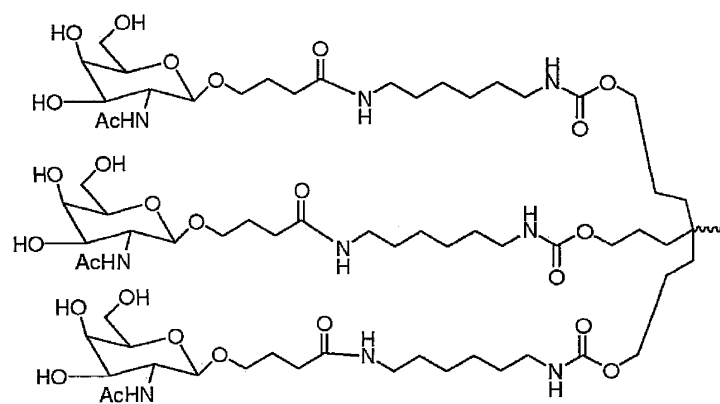
Formula VI,



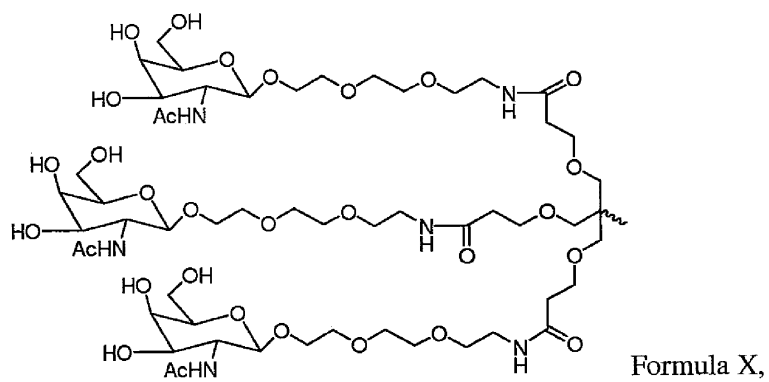
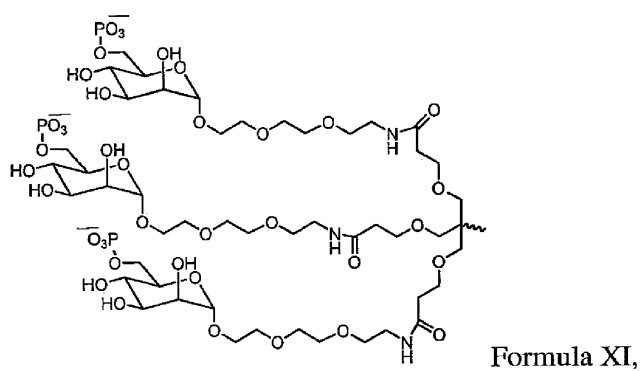
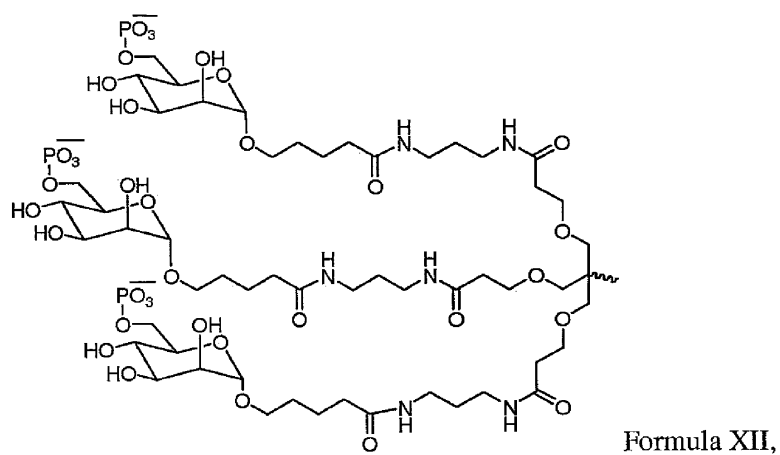
Formula VII,

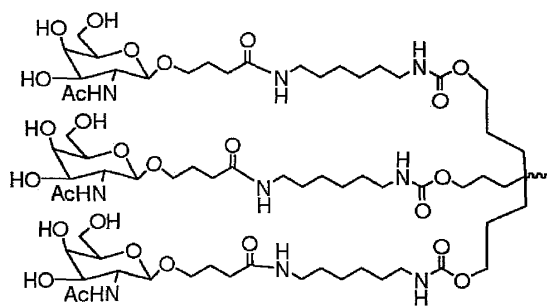


Formula VIII,

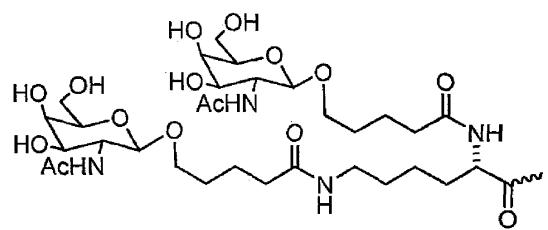


Formula IX,

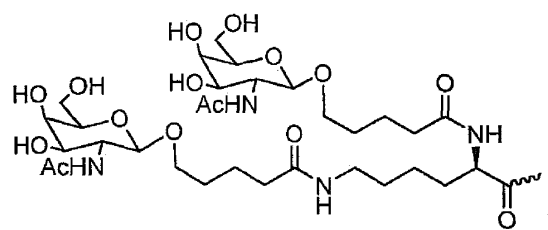




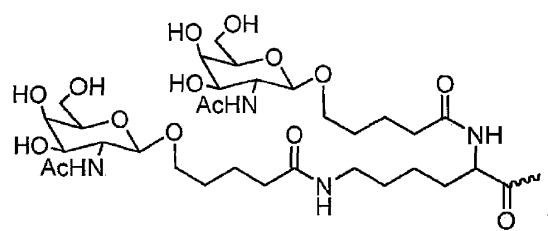
Formula XIII,



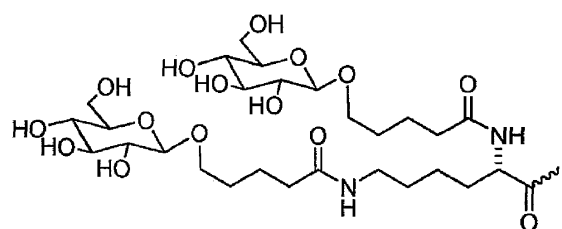
Formula XIV,



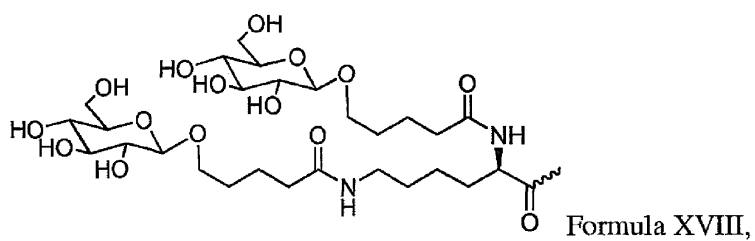
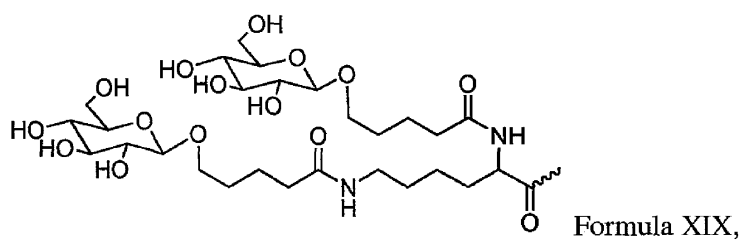
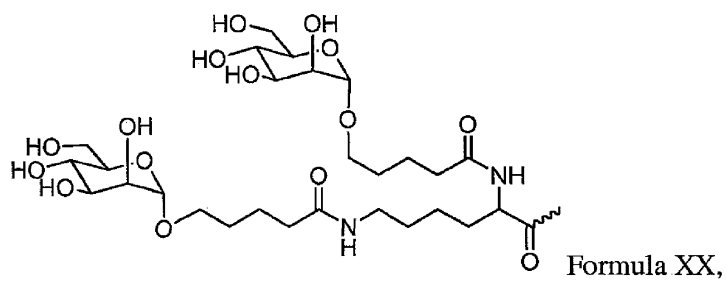
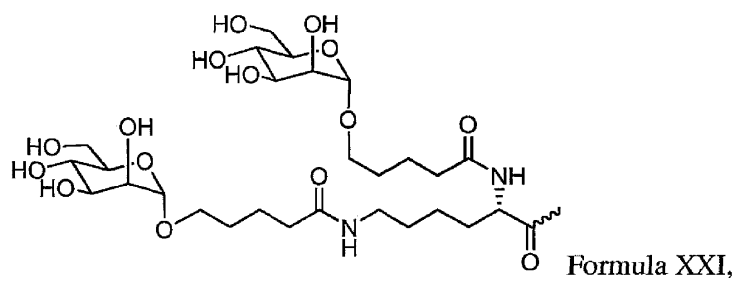
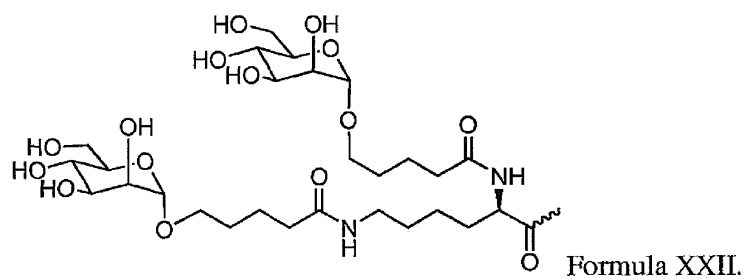
Formula XV,



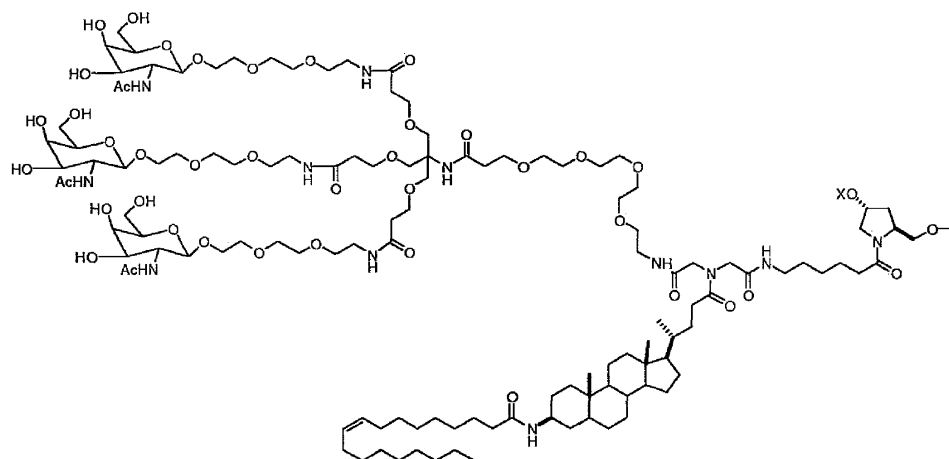
Formula XVI,



Formula XVII,



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



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

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

D. Linkers

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

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.*, covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR₂, 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,
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 5 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,
 10 N(R₈), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl,
 substituted or unsubstituted heterocyclic; where R₈ is hydrogen, acyl, aliphatic or
 substituted aliphatic. In one embodiment, the linker is between about 1-24 atoms, 2-24,
 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-17, or 8-16 atoms.

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

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

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

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

Linkers that contain peptide bonds can be used when targeting cell types rich in
15 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.
20 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
25 make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic
30 extracellular conditions).

i. Redox cleavable linking groups

In one embodiment, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable
5 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
10 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
15 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.

20 *ii. Phosphate-based cleavable linking groups* In another embodiment, 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-
25 P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S-. Preferred embodiments are -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -S-P(S)(OH)-O-, -O-
30 P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O-, -S-P(S)(H)-O-, -S-P(O)(H)-S-, -O-P(S)(H)-

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

iii. Acid cleavable linking groups

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

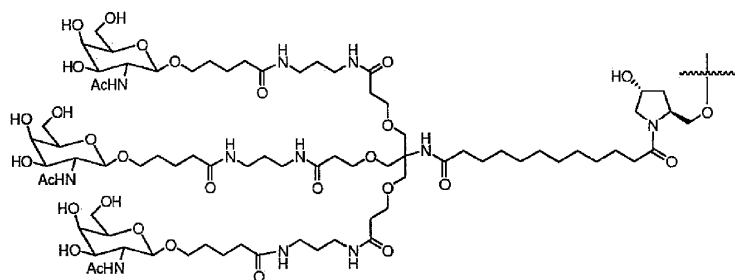
iv. Ester-based linking groups In another embodiment, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited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

v. Peptide-based cleaving groups

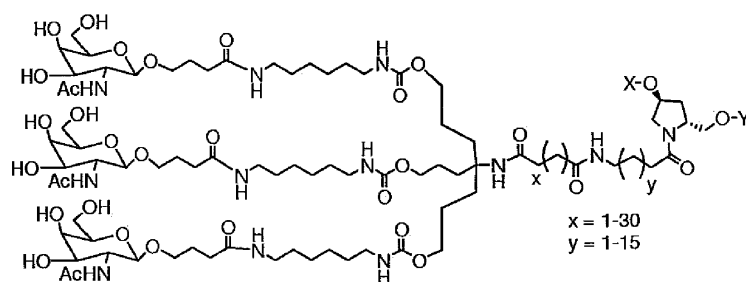
In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides *etc.*) and polypeptides. Peptide-based cleavable groups do not include the

amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynylene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula – NHCHRAC(O)NHCHRBC(O)- (SEQ ID NO: 13), where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

10 In one embodiment, 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 XXIV),

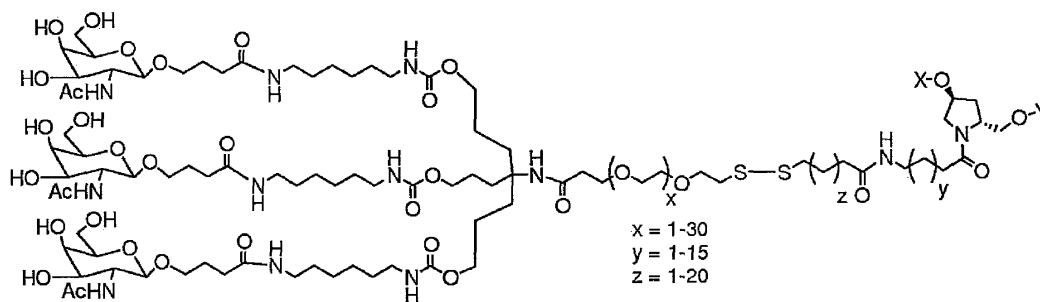


(Formula XXV),

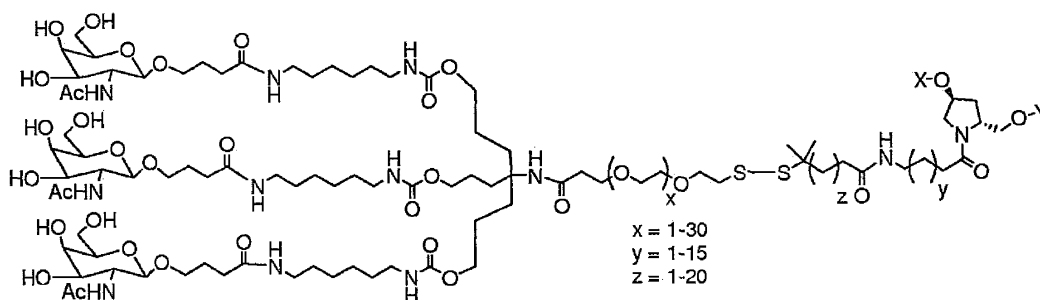
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(Formula XXIX), and

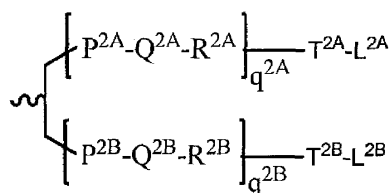


(Formula XXX), when one of X or Y is an oligonucleotide, the other is a hydrogen.

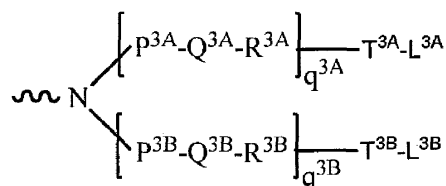
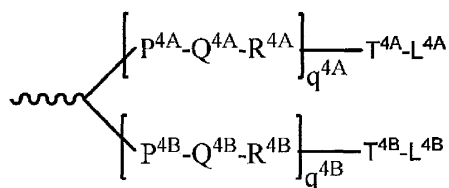
5 In certain embodiments of the compositions and methods of the invention, a ligand is one or more GalNAc (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

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 (XXXI) – (XXXIV):

Formula XXXI

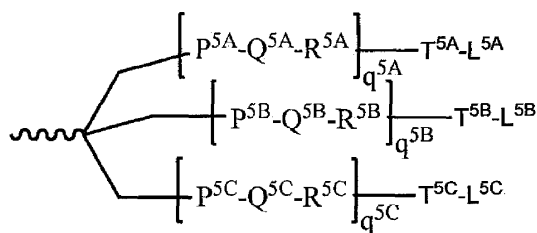


Formula XXXII



Formula (VI)

, or



Formula (VII)

;

5

Formula XXXIII

Formula XXXIV

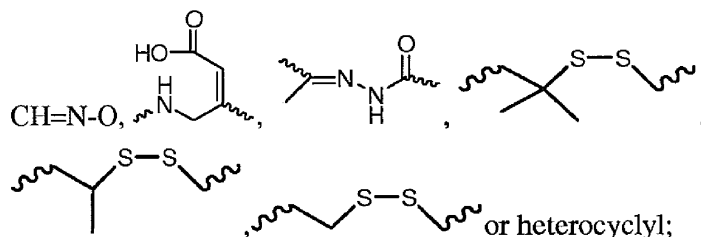
wherein:

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;

- 10 P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH₂, CH₂NH or CH₂O;

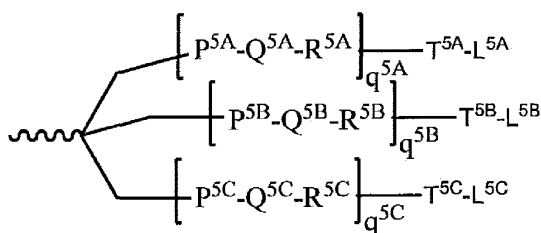
$Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C}$ are independently for each occurrence absent, alkylene, substituted alkylene wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO₂, N(R^N), C(R')=C(R''), C≡C or C(O);

$R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C}$ 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,



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

Formula XXXV



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

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

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

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

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

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-

5 ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. *et al.*, Biochem. Biophys. Res. Comm., 2007, 365(1):54-61; Letsinger *et al.*, Proc. Natl. Acad. Sci. USA, 1989, 86:6553), cholic acid (Manoharan *et al.*, Bioorg. Med. Chem. Lett., 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al.*, Ann. N.Y. Acad. Sci., 1992, 660:306; Manoharan *et al.*, Bioorg. Med. Chem. Let., 1993, 3:2765), a

10 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,

15 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.,

20 1996, 277:923). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of an RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with

25 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,

30 such as a human subject (*e.g.*, a subject in need thereof, such as a subject having a

disorder of lipid metabolism) 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.

- 5 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.,
10 (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. The non-specific effects of an iRNA can be minimized by
15 local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the iRNA molecule to be administered. Several studies
20 have shown successful knockdown of gene products when an iRNA is administered locally. For example, intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, MJ. *et al.*, (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, SJ. *et al.* (2003) *Mol. Vis.* 9:210-216) were both shown to prevent neovascularization in an experimental model of age-related macular
25 degeneration. In addition, direct intratumoral injection of a dsRNA in mice reduces tumor volume (Pille, J. *et al.* (2005) *Mol. Ther.* 11:267-274) and can prolong survival of tumor-bearing mice (Kim, WJ. *et al.*, (2006) *Mol. Ther.* 14:343-350; Li, S. *et al.*, (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G. *et al.*, (2004) *Nucleic Acids* 32:e49; Tan, PH. *et al.* (2005) *Gene Ther.* 12:59-66; Makimura, H. *et al.* (2002) *BMC Neurosci.* 3:18;
30 Shishkina, GT., *et al.* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al.* (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya, Y., *et al.* (2005) *J.*

Neurophysiol. 93:594-602) and to the lungs by intranasal administration (Howard, KA. *et al.*, (2006) *Mol. Ther.* 14:476-484; Zhang, X. *et al.*, (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V. *et al.*, (2005) *Nat. Med.* 11:50-55). For administering an iRNA systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-nucleases in vivo. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA composition to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J. *et al.*, (2004) *Nature* 432:173-178). Conjugation of an iRNA to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, JO. *et al.*, (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the iRNA can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an iRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (see *e.g.*, Kim SH. *et al.*, (2008) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al.* (2003) *J. Mol. Biol* 327:761-766; Verma, UN. *et al.*, (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS *et al.*, (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of iRNAs include DOTAP (Sorensen, DR., *et al.* (2003), *supra*; Verma, UN. *et al.*, (2003), *supra*), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS. *et al.*, (2006) *Nature* 441:111-114), cardiolipin (Chien, PY. *et al.*, (2005) *Cancer Gene Ther.* 12:321-

328; Pal, A. *et al.*, (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME. *et al.*, (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA. *et al.*, (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H. *et al.*, (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be found in U.S. Patent No. 7, 427, 605, which is herein incorporated by reference in its entirety.

10 A. Vector encoded iRNAs of the Invention

iRNA targeting the ANGPTL3 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. Pat. No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, (1995) *Proc. Natl. Acad. Sci. USA* 92:1292).

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

iRNA expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, preferably those compatible with

vertebrate cells, can be used to produce recombinant constructs for the expression of an iRNA as described herein. Eukaryotic cell expression vectors are well known in the art and are available from a number of commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired nucleic acid segment. Delivery of iRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

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

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

promoters, enhancers, *etc.*, to ensure the expression of the iRNA in target cells. Other aspects to consider for vectors and constructs are further described below.

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

Expression of the iRNA can be precisely regulated, for example, by using an inducible regulatory sequence that is sensitive to certain physiological regulators, *e.g.*, circulating glucose levels, or hormones (Docherty *et al.*, 1994, FASEB J. 8:20-24).
10 Such inducible expression systems, suitable for the control of dsRNA expression in cells or in mammals include, for example, regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 - thiogalactopyranoside (IPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the iRNA
15 transgene.

Viral vectors that contain nucleic acid sequences encoding an iRNA can be used. For example, a retroviral vector can be used (see Miller *et al.*, (1993) *Meth. Enzymol.* 217:581-599). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid
20 sequences encoding an iRNA are cloned into one or more vectors, which facilitate delivery of the nucleic acid into a patient. More detail about retroviral vectors can be found, for example, in Boesen *et al.*, *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the
25 use of retroviral vectors in gene therapy are: Clowes *et al.*, (1994) *J. Clin. Invest.* 93:644-651; Kiem *et al.*, (1994) *Blood* 83:1467-1473; Salmons and Gunzberg, (1993) *Human Gene Therapy* 4:129-141; and Grossman and Wilson, (1993) *Curr. Opin. in Genetics and Devel.* 3:110-114. Lentiviral vectors contemplated for use include, for example, the HIV based vectors described in U.S. Patent Nos. 6,143,520; 5,665,557; and
30 5,981,276, which are herein incorporated by reference.

Adenoviruses are also contemplated for use in delivery of iRNAs of the invention. Adenoviruses are especially attractive vehicles, *e.g.*, for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, (1993) *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout *et al.*, (1994) *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, (1991) *Science* 252:431-434; Rosenfeld *et al.*, (1992) *Cell* 68:143-155; Mastrangeli *et al.*, (1993) *J. Clin. Invest.* 91:225-234; PCT Publication WO94/12649; and Wang *et al.*, (1995) *Gene Therapy* 2:775-783. A suitable AV vector for expressing an iRNA featured in the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H *et al.* (2002), *Nat. Biotech.* 20: 1006-1010.

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

Another viral vector suitable for delivery of an iRNA of the invention is a pox virus such as a vaccinia virus, for example an attenuated vaccinia such as Modified Virus Ankara (MVA) or NYVAC, an avipox such as fowl pox or canary pox.

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

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

V. Pharmaceutical Compositions of the Invention

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for treating a disease or disorder associated with the expression or activity of an ANGPTL3 gene, *e.g.*, a disorder of lipid metabolism, such as hypertriglyceridemia.

Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery, *e.g.*, by intravenous (IV) or for subcutaneous delivery. Another example is compositions that are formulated for direct delivery into the liver, *e.g.*, by infusion into the liver, such as by continuous pump infusion.

The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a ANGPTL3 gene. In general, a suitable dose of an iRNA of the invention will be in the range of about 0.001 to about 200.0

milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. For example, the dsRNA can be administered at about 0.01 mg/kg, about 0.05 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 3 mg/kg, about 10 mg/kg, about 20 mg/kg, about
5 30 mg/kg, about 40 mg/kg, or about 50 mg/kg per single dose.

For example, the dsRNA may be administered at a dose of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6,
10 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, or about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In another embodiment, the dsRNA is administered at a dose of about 0.1 to about 50 mg/kg, about 0.25 to about 50 mg/kg, about 0.5 to about 50 mg/kg, about 0.75
15 to about 50 mg/kg, about 1 to about 50 mg/kg, about 1.5 to about 50 mg/kg, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 20 to about 50 mg/kg, about 25 to
20 about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 0.1 to about 45 mg/kg, about 0.25 to about 45 mg/kg, about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to about 45 mg/kg, about 1.5 to about 45 mg/kg, about 2 to about 45 mg/kg, about 2.5 to about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to
25 about 45 mg/kg, about 4 to about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 25 to about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to about 45 mg/kg, about 0.1 to about 40 mg/kg, about 0.25 to
30 about 40 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to about 40 mg/kg, about 1.5 to about 40 mg/kg, about 2 to about 40 mg/kg, about 2.5 to

about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 25 to about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.1 to about 30 mg/kg, about 0.25 to about 30 mg/kg, about 0.5 to about 30 mg/kg, about 0.75 to about 30 mg/kg, about 1 to about 30 mg/kg, about 1.5 to about 30 mg/kg, about 2 to about 30 mg/kg, about 2.5 to about 30 mg/kg, about 3 to about 30 mg/kg, about 3.5 to about 30 mg/kg, about 4 to about 30 mg/kg, about 4.5 to about 30 mg/kg, about 5 to about 30 mg/kg, about 7.5 to about 30 mg/kg, about 10 to about 30 mg/kg, about 15 to about 30 mg/kg, about 20 to about 30 mg/kg, about 20 to about 30 mg/kg, about 25 to about 30 mg/kg, about 0.1 to about 20 mg/kg, about 0.25 to about 20 mg/kg, about 0.5 to about 20 mg/kg, about 0.75 to about 20 mg/kg, about 1 to about 20 mg/kg, about 1.5 to about 20 mg/kg, about 2 to about 20 mg/kg, about 2.5 to about 20 mg/kg, about 3 to about 20 mg/kg, about 3.5 to about 20 mg/kg, about 4 to about 20 mg/kg, about 4.5 to about 20 mg/kg, about 5 to about 20 mg/kg, about 7.5 to about 20 mg/kg, about 10 to about 20 mg/kg, or about 15 to about 20 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, the dsRNA may be administered at a dose of about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, or about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In another embodiment, the dsRNA is administered at a dose of about 0.5 to about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/kg, about 1.5 to about 50 mg/kg, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 20 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 50 to about 50 mg/kg, or about 55 to about 50 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

about 50 mg/kg, about 25 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to about 45 mg/kg, about 1.5 to about 45 mg/kg, about 2 to about 45 mg/kg, about 2.5 to about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to about 45 mg/kg, about 4 to about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to about 45 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to about 40 mg/kg, about 1.5 to about 40 mg/kg, about 2 to about 40 mg/kg, about 2.5 to about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.5 to about 30 mg/kg, about 0.75 to about 30 mg/kg, about 1 to about 30 mg/kg, about 1.5 to about 30 mg/kg, about 2 to about 30 mg/kg, about 2.5 to about 30 mg/kg, about 3 to about 30 mg/kg, about 3.5 to about 30 mg/kg, about 4 to about 30 mg/kg, about 4.5 to about 30 mg/kg, about 5 to about 30 mg/kg, about 7.5 to about 30 mg/kg, about 10 to about 30 mg/kg, about 15 to about 30 mg/kg, about 20 to about 30 mg/kg, about 25 to about 30 mg/kg, about 0.5 to about 20 mg/kg, about 0.75 to about 20 mg/kg, about 1 to about 20 mg/kg, about 1.5 to about 20 mg/kg, about 2 to about 20 mg/kg, about 2.5 to about 20 mg/kg, about 3 to about 20 mg/kg, about 3.5 to about 20 mg/kg, about 4 to about 20 mg/kg, about 4.5 to about 20 mg/kg, about 5 to about 20 mg/kg, about 7.5 to about 20 mg/kg, about 10 to about 20 mg/kg, or about 15 to about 20 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, subjects can be administered a therapeutic amount of iRNA, such as about 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5,

6.6, 6.7, 6.8. 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8. 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8. 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8. 9.9, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or
5 about 50 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

The pharmaceutical composition can be administered once daily, or the iRNA can be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release
10 formulation. In that case, the iRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, *e.g.*, using a conventional sustained release formulation which provides sustained release of the iRNA over a several day period. Sustained release formulations are well known in the art and are particularly useful for
15 delivery of agents at a particular site, such as could be used with the agents of the present invention. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

The effect of a single dose on ANGPTL3 levels can be long lasting, such that subsequent doses are administered at not more than 3, 4, or 5 day intervals, or at not
20 more than 1, 2, 3, or 4 week intervals.

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a
25 therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and *in vivo* half-lives for the individual iRNAs encompassed by the invention can be made using conventional methodologies or on the basis of *in vivo* testing using an appropriate animal model, as described elsewhere herein.

Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as disorders of lipid metabolism that would benefit from reduction in the expression of ANGPTL3. Such models can be used for *in vivo* testing of iRNA, as well as for determining a therapeutically effective dose.

- 5 Suitable mouse models are known in the art and include, for example, an obese (ob/ob) mouse containing a mutation in the obese (ob) gene (Wiegman *et al.*, (2003) *Diabetes*, 52:1081-1089); a mouse containing homozygous knock-out of an LDL receptor (LDLR -/- mouse; Ishibashi *et al.*, (1993) *J Clin Invest* 92(2):883-893); diet-induced atherosclerosis mouse model (Ishida *et al.*, (1991) *J. Lipid. Res.*, 32:559-568); and
10 heterozygous lipoprotein lipase knockout mouse model (Weistock *et al.*, (1995) *J. Clin. Invest.* 96(6):2555-2568).

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

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

- Pharmaceutical compositions and formulations for topical administration can include transdermal patches, ointments, lotions, creams, gels, drops, suppositories,
25 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 iRNAs featured in the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and
30 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). iRNAs featured in the invention can be encapsulated within liposomes or
5 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, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-
10 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

15 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
20 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
25 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
30 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.*, (1987) *Proc. Natl. Acad. Sci. USA* 8:7413-7417; U.S. Pat. No. 4,897,355; U.S. Pat. No. 5,171,678; Bangham *et al.*, (1965) *M. Mol. Biol.* 23:238; Olson *et al.*, (1979) *Biochim. Biophys. Acta* 557:9; Szoka *et al.*, (1978) *Proc. Natl. Acad. Sci.* 75: 4194; Mayhew *et al.*, (1984) *Biochim. Biophys. Acta* 775:169; Kim *et al.*, (1983) *Biochim. Biophys. Acta* 728:339; and Fukunaga *et al.*, (1984) *Endocrinol.* 115:757. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, e.g., Mayer *et al.*, (1986) *Biochim. Biophys. Acta* 858:161. Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew *et al.*, (1984) *Biochim. Biophys. Acta* 775:169. These methods are readily adapted to packaging RNAi agent preparations into liposomes.

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
5 within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang *et al.* (1987) *Biochem. Biophys. Res. Commun.*, 147:980-985).

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

One major type of liposomal composition includes phospholipids other than
15 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
20 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.

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;
25 WO 91/16024; Felgner, (1994) *J. Biol. Chem.* 269:2550; Nabel, (1993) *Proc. Natl. Acad. Sci.* 90:11307; Nabel, (1992) *Human Gene Ther.* 3:649; Gershon, (1993) *Biochem.* 32:7143; and Strauss, (1992) *EMBO J.* 11:417.

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
30 and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl

dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporine A into
5 different layers of the skin (Hu *et al.*, (1994) *S.T.P. Pharma. Sci.*, 4(6):466).

Liposomes also include “sterically stabilized” liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes
10 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,
15 or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen *et al.*, (1987) *FEBS Letters*, 223:42; Wu *et al.*, (1993) *Cancer Research*, 53:3765).

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

In one embodiment, cationic liposomes are used. Cationic liposomes possess the
30 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
5 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.

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

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
20 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
25 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 dioctaoyleamide ("DOGS") (Transfectam™, Promega, Madison, Wisconsin) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-
5 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., (1991) *Biochim. Biophys. Res. Commun.* 179:280).
10 Lipopolylysine, made by conjugating polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. *et al.*, (1991) *Biochim. Biophys. Acta* 1065:8). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions. Other commercially available cationic lipid
15 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,
20 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
25 agent into dermal tissues, e.g., into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, e.g., Weiner *et al.*, (1992) *Journal of Drug Targeting*, vol. 2,405-410 and du Plessis *et al.*, (1992) *Antiviral Research*, 18:259-265; Mannino, R. J. and Fould-Fogerite, S., (1998) *Biotechniques* 6:682-690; Itani, T. *et al.*, (1987) *Gene* 56:267-276;
30 Nicolau, C. *et al.* (1987) *Meth. Enzymol.* 149:157-176; Straubinger, R. M. and

Papahadjopoulos, D. (1983) *Meth. Enzymol.* 101:512-527; Wang, C. Y. and Huang, L., (1987) *Proc. Natl. Acad. Sci. USA* 84:7851-7855).

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
5 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
10 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
15 Dekker, Inc., New York, N.Y., 1988, p. 285).

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

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

The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

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

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

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

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 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.

5 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.

10 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.

15 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
20 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.

25 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. Nucleic acid lipid particles

iRNAs, *e.g.*, dsRNAs of in the invention may be fully encapsulated in the lipid formulation, *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
5 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.*)
10 injection and accumulate at distal sites (*e.g.*, sites physically separated from the administration site). SPLPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. The particles of the present invention typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more
15 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;
20 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 intermediate to the above recited ranges
25 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 (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane
30

(DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxyp propane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-
5 3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyoxy-3-dimethylaminopropane (DLin-2-DMA), 1,2-Dilinoleyoxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyoxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-
10 propanedio (DOAP), 1,2-Dilinoleyoxy-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-
15 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)didodecan-2-ol (Tech G1), or a mixture 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.

20 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.

25 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.

30 The ionizable/non-cationic lipid can be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC),

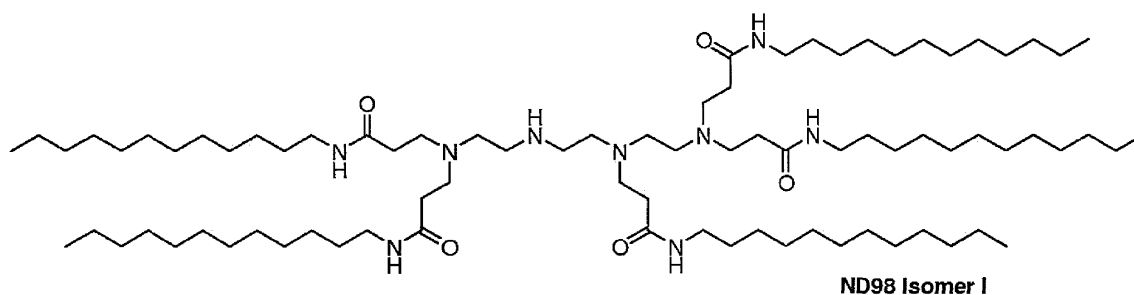
dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC),
dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG),
dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine
(POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-
5 phosphatidylethanolamine 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
10 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-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or
15 a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-
dilauryloxypropyl (Ci₂), a PEG-dimyristyloxypropyl (Ci₄), a PEG-dipalmitoyloxypropyl
(Ci₆), or a PEG- distearyloxypropyl (Ci₈). 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.

20 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),
25 Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used
to prepare lipid-dsRNA nanoparticles (*i.e.*, LNP01 particles). Stock solutions of each in
ethanol can be prepared as follows: ND98, 133 mg/ml; Cholesterol, 25 mg/ml, PEG-
Ceramide C16, 100 mg/ml. The ND98, Cholesterol, and PEG-Ceramide C16 stock
solutions can then be combined in a, *e.g.*, 42:48:10 molar ratio. The combined lipid
30 solution can be mixed with aqueous dsRNA (*e.g.*, in sodium acetate pH 5) such that the

final ethanol concentration is about 35-45% and the final sodium acetate concentration is about 100-300 mM. Lipid-dsRNA nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (*e.g.*, 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion step can be omitted. Ethanol removal and simultaneous buffer exchange can be accomplished by, for example, dialysis or tangential flow filtration. Buffer can be exchanged with, for example, phosphate buffered saline (PBS) at about pH 7, *e.g.*, about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, or about pH 7.4.



Formula 1

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

Additional exemplary lipid-dsRNA formulations are described in the table below.

	Ionizable/Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio
SNALP-1	1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1

2-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA ~ 7:1
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediy)didodecan-2-ol (Tech G1)	Tech G1/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5

		Lipid:siRNA: 11:1
LNP15	MC3	MC3/DSPC/Chol/PEG-DSG/GalNAc-PEG-DSG 50/10/35/4.5/0.5 Lipid:siRNA: 11:1
LNP16	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP17	MC3	MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP18	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 12:1
LNP19	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:siRNA: 8:1
LNP20	MC3	MC3/DSPC/Chol/PEG-DPG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP21	C12-200	C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP22	XTC	XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

PEG-DMG: PEG-didimyristoyl glycerol (C14-PEG, or PEG-C14) (PEG with avg
5 mol wt of 2000)

PEG-DSG: PEG-distyryl glycerol (C18-PEG, or PEG-C18) (PEG with avg mol wt of 2000)

PEG-cDMA: PEG-carbamoyl-1,2-dimyristyloxypropylamine (PEG with avg mol wt of 2000)

- 5 SNALP (1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed April 15, 2009, which is hereby incorporated by reference.

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

MC3 comprising formulations are described, *e.g.*, in U.S. Publication No. 2010/0324120, filed June 10, 2010, the entire contents of which are hereby incorporated by reference.

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

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

Synthesis of ionizable/cationic lipids

Any of the compounds, *e.g.*, cationic lipids and the like, used in the nucleic acid-lipid particles of the invention can be prepared by known organic synthesis techniques, including the methods described in more detail in the Examples. All substituents are as defined below unless indicated otherwise.

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

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

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

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

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

tetrahydrothiophenyl, tetrahydrothiopyranyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, and the like.

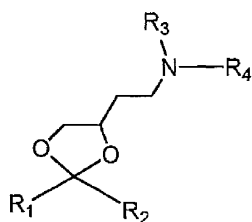
The terms “optionally substituted alkyl”, “optionally substituted alkenyl”, “optionally substituted alkynyl”, “optionally substituted acyl”, and “optionally substituted heterocycle” means that, when substituted, at least one hydrogen atom is replaced with a substituent. In the case of an oxo substituent (=O) two hydrogen atoms are replaced. In this regard, substituents include oxo, halogen, heterocycle, -CN, -OR_x, -NR_xR_y, -NR_xC(=O)R_y, -NR_xSO₂R_y, -C(=O)R_x, -C(=O)OR_x, -C(=O)NR_xR_y, -SOnR_x and -SOnNR_xR_y, wherein n is 0, 1 or 2, R_x and R_y are the same or different and independently hydrogen, alkyl or heterocycle, and each of said alkyl and heterocycle substituents can be further substituted with one or more of oxo, halogen, -OH, -CN, alkyl, -OR_x, heterocycle, -NR_xR_y, -NR_xC(=O)R_y, -NR_xSO₂R_y, -C(=O)R_x, -C(=O)OR_x, -C(=O)NR_xR_y, -SOnR_x and -SOnNR_xR_y.

“Halogen” means fluoro, chloro, bromo and iodo.

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

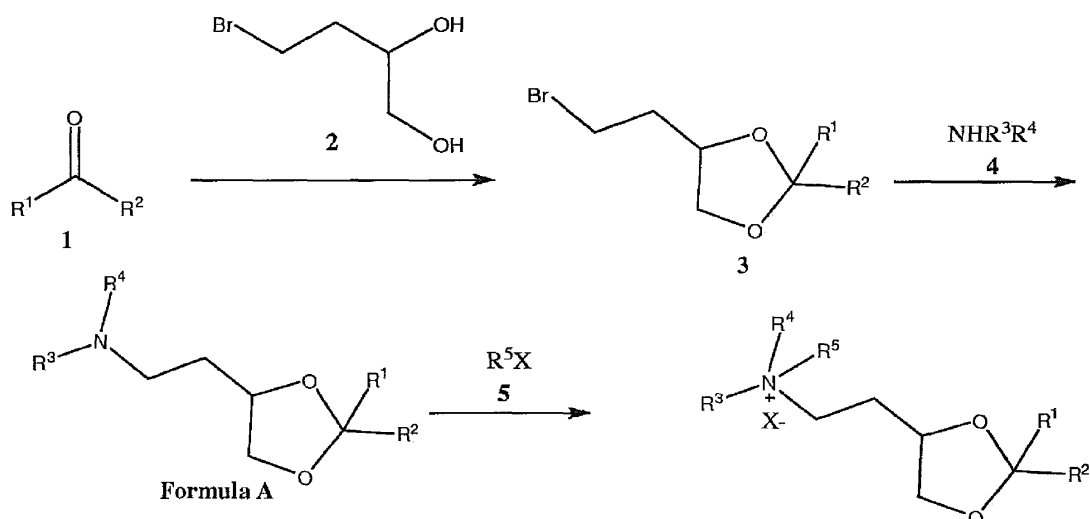
Synthesis of Formula A

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



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

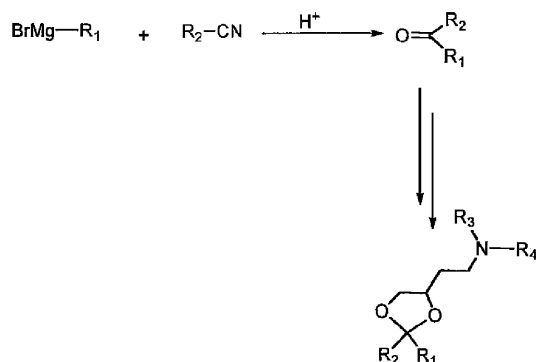
Scheme 1



- 10 Lipid A, where R1 and R2 are independently alkyl, alkenyl or alkynyl, each can be optionally substituted, and R3 and R4 are independently lower alkyl or R3 and R4 can be taken together to form an optionally substituted heterocyclic ring, can be prepared according to Scheme 1. Ketone 1 and bromide 2 can be purchased or prepared according to methods known to those of ordinary skill in the art. Reaction of 1 and 2
- 15 yields ketal 3. Treatment of ketal 3 with amine 4 yields lipids of formula A. The lipids of formula A can be converted to the corresponding ammonium salt with an organic salt

of formula 5, where X is anion counter ion selected from halogen, hydroxide, phosphate, sulfate, or the like.

Scheme 2



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

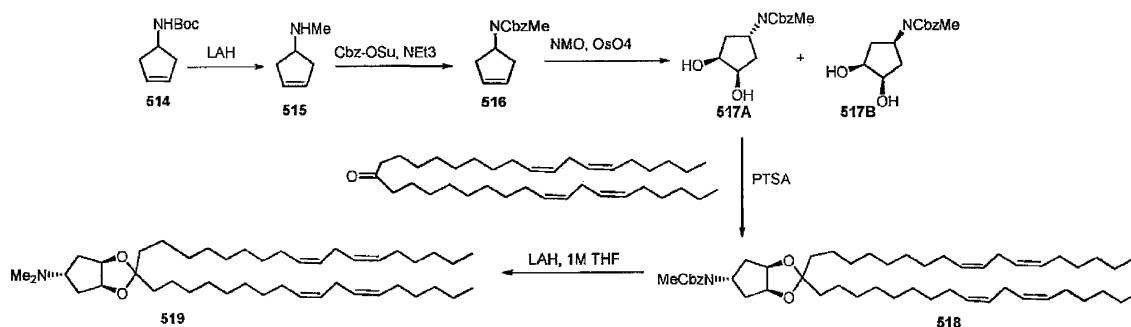
10 *Synthesis of MC3*

Preparation of DLin-M-C3-DMA (*i.e.*, (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate) was as follows. A solution of (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol (0.53 g), 4-N,N-dimethylaminobutyric acid hydrochloride (0.51 g), 4-N,N-dimethylaminopyridine (0.61g) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.53 g) in
 15 dichloromethane (5 mL) was stirred at room temperature overnight. The solution was washed with dilute hydrochloric acid followed by dilute aqueous sodium bicarbonate. The organic fractions were dried over anhydrous magnesium sulphate, filtered and the solvent removed on a rotovap. The residue was passed down a silica gel column (20 g)
 20 using a 1-5% methanol/dichloromethane elution gradient. Fractions containing the purified product were combined and the solvent removed, yielding a colorless oil (0.54 g).

Synthesis of ALNY-100

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

3:



5 Synthesis of 515

To a stirred suspension of LiAlH_4 (3.74 g, 0.09852 mol) in 200 ml anhydrous THF in a two neck RBF (1L), was added a solution of 514 (10g, 0.04926mol) in 70 mL of THF slowly at 0°C under nitrogen atmosphere. After complete addition, reaction mixture was warmed to room temperature and then heated to reflux for 4 h. Progress of the reaction was monitored by TLC. After completion of reaction (by TLC) the mixture was cooled to 0°C and quenched with careful addition of saturated Na_2SO_4 solution. Reaction mixture was stirred for 4 h at room temperature and filtered off. Residue was washed well with THF. The filtrate and washings were mixed and diluted with 400 mL dioxane and 26 mL conc. HCl and stirred for 20 minutes at room temperature. The volatilities were stripped off under vacuum to furnish the hydrochloride salt of 515 as a white solid. Yield: 7.12 g $^1\text{H-NMR}$ (DMSO, 400MHz): δ = 9.34 (broad, 2H), 5.68 (s, 2H), 3.74 (m, 1H), 2.66-2.60 (m, 2H), 2.50-2.45 (m, 5H).

Synthesis of 516

To a stirred solution of compound 515 in 100 mL dry DCM in a 250 mL two neck RBF, was added NEt_3 (37.2 mL, 0.2669 mol) and cooled to 0°C under nitrogen atmosphere. After a slow addition of N-(benzyloxy-carbonyloxy)-succinimide (20 g, 0.08007 mol) in 50 mL dry DCM, reaction mixture was allowed to warm to room temperature. After completion of the reaction (2-3 h by TLC) mixture was washed

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

Synthesis of 517A and 517B

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

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

Synthesis of 518

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

General Procedure for the Synthesis of Compound 519

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

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

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

acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Patent 6,887,906, US Publn. No. 20030027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

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

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. Particularly preferred are formulations that target the liver when treating hepatic disorders such as hepatic carcinoma.

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 or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention can also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions can further contain substances which increase the viscosity of the

suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension can also contain stabilizers.

C. Additional Formulations

i. Emulsions

5 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 μ m in diameter (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, 10 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 15 *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 20 composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution in either aqueous phase, oily phase or itself as a separate phase. 25 Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do 30 not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose

small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

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

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

York, NY Rieger, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

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

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

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

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that can readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium

salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used can be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or
5 reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

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

ii. Microemulsions

In one embodiment of the present invention, the compositions of iRNAs and
25 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
30 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

5 thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant,

10 cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

15 The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, L.V., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms,

20 Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets

25 that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate

30 (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in

combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions can, however,
5 be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase can typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase can include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM,
10 fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

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

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

iii. Microparticles

10 an RNAi agent 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

15 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
20 membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

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

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

Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprinate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C₁₋₂₀ alkyl esters thereof (*e.g.*, methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (*i.e.*, oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, *etc.*) (see *e.g.*, Touitou, E., *et al.* *Enhancement in Drug Delivery*, CRC Press, Danvers, MA, 2006; Lee *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p.92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33; El Hariri *et al.*, *J. Pharm. Pharmacol.*, 1992, 44, 651-654).

The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (see *e.g.*, Malmsten, M. *Surfactants and polymers in drug delivery*, Informa Health Care, New York, NY, 2002; Brunton, Chapter 38 in: Goodman & Gilman's *The Pharmacological Basis of Therapeutics*, 9th Ed., Hardman *et al.* Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucolic acid (sodium

glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto *et al.*, J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita *et al.*, J. Pharm. Sci., 1990, 79, 579-583).

Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of iRNAs through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315-339). Suitable chelating agents include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (*e.g.*, sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines)(see *e.g.*, Katdare, A. *et al.*, Excipient development for pharmaceutical, biotechnology, and drug delivery, CRC Press, Danvers, MA, 2006; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur *et al.*, J. Control Rel., 1990, 14, 43-51).

As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of iRNAs through the alimentary mucosa (see *e.g.*, Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers includes, for example,

unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita *et al.*, J. Pharm. Pharmacol., 1987, 39, 621-626).

- 5 Agents that enhance uptake of iRNAs at the cellular level can also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi *et al.*, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo *et al.*, PCT Application WO 97/30731), are also known to enhance the cellular uptake of dsRNAs.
- 10 Examples of commercially available transfection reagents include, for example Lipofectamine™ (Invitrogen; Carlsbad, CA), Lipofectamine 2000™ (Invitrogen; Carlsbad, CA), 293fectin™ (Invitrogen; Carlsbad, CA), Cellfectin™ (Invitrogen; Carlsbad, CA), DMRIE-C™ (Invitrogen; Carlsbad, CA), FreeStyle™ MAX (Invitrogen; Carlsbad, CA), Lipofectamine™ 2000 CD (Invitrogen; Carlsbad, CA), Lipofectamine™
- 15 (Invitrogen; Carlsbad, CA), RNAiMAX (Invitrogen; Carlsbad, CA), Oligofectamine™ (Invitrogen; Carlsbad, CA), Optifect™ (Invitrogen; Carlsbad, CA), X-tremeGENE Q2 Transfection Reagent (Roche; Grenzacherstrasse, Switzerland), DOTAP Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), DOSPER Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), or Fugene (Grenzacherstrasse,
- 20 Switzerland), Transfectam® Reagent (Promega; Madison, WI), TransFast™ Transfection Reagent (Promega; Madison, WI), Tfx™-20 Reagent (Promega; Madison, WI), Tfx™-50 Reagent (Promega; Madison, WI), DreamFect™ (OZ Biosciences; Marseille, France), EcoTransfect (OZ Biosciences; Marseille, France), TransPass® D1 Transfection Reagent (New England Biolabs; Ipswich, MA, USA),
- 25 LyoVec™/LipoGen™ (Invitrogen; San Diego, CA, USA), PerFectin Transfection Reagent (Genlantis; San Diego, CA, USA), NeuroPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), GenePORTER Transfection reagent (Genlantis; San Diego, CA, USA), GenePORTER 2 Transfection reagent (Genlantis; San Diego, CA, USA), Cytofectin Transfection Reagent (Genlantis; San Diego, CA, USA),
- 30 BaculoPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), TroganPORTER™ transfection Reagent (Genlantis; San Diego, CA, USA), RiboFect (Bioline; Taunton, MA, USA), PlasFect (Bioline; Taunton, MA, USA), UniFECTOR

(B-Bridge International; Mountain View, CA, USA), SureFECTOR (B-Bridge International; Mountain View, CA, USA), or HiFect™ (B-Bridge International, Mountain View, CA, USA), among others.

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

v. Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, “carrier compound” or “carrier” can refer to a
10 nucleic acid, or analog thereof, which is inert (*i.e.*, does not possess biological activity per se) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The
15 coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate dsRNA in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic
20 acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao *et al.*, DsRNA Res. Dev., 1995, 5, 115-121; Takakura *et al.*, DsRNA & Nucl. Acid Drug Dev., 1996, 6, 177-183.

vi. Excipients

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

carriers include, but are not limited to, binding agents (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, *etc.*); fillers (*e.g.*, lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, *etc.*); lubricants (*e.g.*, magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, *etc.*); disintegrants (*e.g.*, starch, sodium starch glycolate, *etc.*); and wetting agents (*e.g.*, sodium lauryl sulphate, *etc.*).

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

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

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

vii. 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,

5 astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/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 and/or dextran. The suspension can also contain stabilizers.

15 In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA compounds and (b) one or more agents which function by a non-RNAi mechanism and which are useful in treating a disorder of lipid metabolism. Examples of such agents include, but are not limited to an anti-inflammatory agent, anti-steatosis agent, anti-viral, and/or anti-fibrosis agent. In addition, other substances commonly used to protect the liver, such as silymarin, can also be used in conjunction with the *iRNAs described herein*. Other agents useful for treating liver diseases include telbivudine, entecavir, and protease inhibitors such as telaprevir and other disclosed, for example, in Tung *et al.*, U.S. Application Publication Nos. 2005/0148548, 2004/0167116, and 2003/0144217; and in Hale *et al.*, U.S. Application Publication No. 2004/0127488.

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

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

15 In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by ANGPTL3 expression. 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
20 described herein.

VI. Methods of the Invention

The present invention also provides methods of using an iRNA of the invention and/or a composition containing an iRNA of the invention to reduce and/or inhibit
25 ANGPTL3 expression in a cell. The methods include contacting the cell with a dsRNA of the invention and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of an ANGPTL3 gene, thereby inhibiting expression of the ANGPTL3 gene in the cell. Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of ANGPTL3
30 may be determined by determining the mRNA expression level of ANGPTL3 using

methods routine to one of ordinary skill in the art, *e.g.*, Northern blotting, qRT-PCR; by determining the protein level of ANGPTL3 using methods routine to one of ordinary skill in the art, such as Western blotting, immunological techniques. A reduction in the expression of ANGPTL3 may also be assessed indirectly by measuring a decrease in
5 biological activity of ANGPTL3, *e.g.*, a decrease in the level of serum lipid, triglycerides, cholesterol and/or free fatty acids.

In the methods of the invention the cell may be contacted *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
10 that expresses an ANGPTL3 gene. 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 or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), a non-primate cell (such as a cow cell, a pig cell, a camel cell, a llama cell, a horse cell, a goat cell, a rabbit cell, a sheep cell, a hamster, a guinea pig cell, a cat cell, a dog cell, a rat cell, a mouse cell, a lion cell,
15 a tiger cell, a bear cell, or a buffalo cell), a bird cell (*e.g.*, a duck cell or a goose cell), or a whale cell. In one embodiment, the cell is a human cell, *e.g.*, a human liver cell.

ANGPTL3 expression is inhibited in the cell by at least about 5, 6, 7, 8, 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, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57,
20 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80,
81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or about 100%.

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 ANGPTL3 gene of the
25 mammal to be treated. When the organism to be treated is a mammal such as a human, the composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal
30 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 some embodiments, the administration is via a depot injection. A depot injection may release the iRNA in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, *e.g.*, a desired inhibition of ANGPTL3, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

In some embodiments, the administration is via a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the iRNA to the liver.

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 an ANGPTL3 gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets an ANGPTL3 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the ANGPTL3 gene, thereby inhibiting expression of the ANGPTL3 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. Reduction in protein production can be assessed by any methods known in the art and by methods, *e.g.* ELISA, described herein. In one embodiment, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in ANGPTL3 gene and/or protein expression.

The present invention further provides methods of treatment of 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 and/or inhibition of ANGPTL3 expression, in a therapeutically effective amount of an iRNA
5 targeting an ANGPTL3 gene or a pharmaceutical composition comprising an iRNA targeting an ANGPTL3 gene.

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,
10 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
15 pharmaceutical composition, such as a dsRNA liposomal formulation.

Subjects that would benefit from a reduction and/or inhibition of ANGPTL3 gene expression are those having a disorder of lipid metabolism, *e.g.*, an inherited disorder of lipid metabolism or an acquired disorder of lipid metabolism. In one embodiment, a subject having disorder of lipid metabolism has hyperlipidemia. In
20 another embodiment, a subject having a disorder of lipid metabolism has hypertriglyceridemia. Treatment of a subject that would benefit from a reduction and/or inhibition of ANGPTL3 gene expression includes therapeutic treatment (*e.g.*, a subject is having eruptive xanthomas) and prophylactic treatment (*e.g.*, the subject is not having eruptive xanthomas or a subject may be at risk of developing eruptive xanthomas).

25 The invention further provides methods for the use of an iRNA or a pharmaceutical composition thereof, *e.g.*, for treating a subject that would benefit from reduction and/or inhibition of ANGPTL3 expression, *e.g.*, a subject having a disorder of lipid metabolism, in combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as,
30 for example, those which are currently employed for treating these disorders. For

example, in certain embodiments, an iRNA targeting ANGPTL3 is administered in combination with, *e.g.*, an agent useful in treating a disorder of lipid metabolism as described elsewhere herein. For example, additional agents suitable for treating a subject that would benefit from reduction in ANGPTL3 expression, *e.g.*, a subject having
5 a disorder of lipid metabolism, may include agents that lower one or more serum lipids. Non-limiting examples of such agents may include cholesterol synthesis inhibitors, such as HMG-CoA reductase inhibitors, *e.g.*, statins. Statins may include atorvastatin (Lipitor), fluvastatin (Lescol), lovastatin (Mevacor), lovastatin extended-release (Altoprev), pitavastatin (Livalo), pravastatin (Pravachol), rosuvastatin (Crestor), and
10 simvastatin (Zocor). Other agents useful in treating a disorder of lipid metabolism may include bile sequestering agents, such as cholestyramine and other resins; VLDL secretion inhibitors, such as niacin; lipophilic antioxidants, such as Probucol; acyl-CoA cholesterol acyl transferase inhibitors; farnesoid X receptor antagonists; sterol regulatory binding protein cleavage activating protein (SCAP) activators; microsomal triglyceride
15 transfer protein (MTP) inhibitors; ApoE-related peptide; and therapeutic antibodies against ANGPTL3. The additional therapeutic agents may also include agents that raise high density lipoprotein (HDL), such as cholesteryl ester transfer protein (CETP) inhibitors. Furthermore, the additional therapeutic agents may also include dietary supplements, *e.g.*, fish oil. The iRNA and additional therapeutic agents may be
20 administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

In one embodiment, the method includes administering a composition featured herein such that expression of the target ANGPTL3 gene is decreased, such as for about
25 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 18, 24 hours, 28, 32, or about 36 hours. In one embodiment, expression of the target ANGPTL3 gene is decreased for an extended duration, *e.g.*, at least about two, three, four days or more, *e.g.*, about one week, two weeks, three weeks, or four weeks or longer.

Preferably, the iRNAs useful for the methods and compositions featured herein
30 specifically target RNAs (primary or processed) of the target ANGPTL3 gene.

Compositions and methods for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

Administration of the dsRNA according to the methods of the invention may result in a reduction of the severity, signs, symptoms, and/or markers of such diseases or disorders in a patient with a disorder of lipid metabolism. By “reduction” in this context is meant a statistically significant decrease in such level. The reduction can be, for example, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%.

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of a disorder of lipid metabolism may be assessed, for example, by periodic monitoring of one or more serum lipid levels. Comparisons of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of an iRNA targeting ANGPTL3 or pharmaceutical composition thereof, “effective against” a disorder of lipid metabolism indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as a improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating disorder of lipid metabolisms and the related causes.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a

favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment. Efficacy for a given iRNA drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

Alternatively, the efficacy can be measured by a reduction in the severity of disease as determined by one skilled in the art of diagnosis based on a clinically accepted disease severity grading scale, as but one example the Child-Pugh score (sometimes the Child-Turcotte-Pugh score). Any positive change resulting in *e.g.*, lessening of severity of disease measured using the appropriate scale, represents adequate treatment using an iRNA or iRNA formulation as described herein.

Subjects can be administered a therapeutic amount of dsRNA, such as about 0.01 mg/kg to about 5 mg/kg, about 0.01 mg/kg to about 10 mg/kg, about 0.05 mg/kg to about 5 mg/kg, about 0.05 mg/kg to about 10 mg/kg, about 0.1 mg/kg to about 5 mg/kg, about 0.1 mg/kg to about 10 mg/kg, about 0.2 mg/kg to about 5 mg/kg, about 0.2 mg/kg to about 10 mg/kg, about 0.3 mg/kg to about 5 mg/kg, about 0.3 mg/kg to about 10 mg/kg, about 0.4 mg/kg to about 5 mg/kg, about 0.4 mg/kg to about 10 mg/kg, about 0.5 mg/kg to about 5 mg/kg, about 0.5 mg/kg to about 10 mg/kg, about 1 mg/kg to about 5 mg/kg, about 1 mg/kg to about 10 mg/kg, about 1.5 mg/kg to about 5 mg/kg, about 1.5 mg/kg to about 10 mg/kg, about 2 mg/kg to about 2.5 mg/kg, about 2 mg/kg to about 10 mg/kg, about 3 mg/kg to about 5 mg/kg, about 3 mg/kg to about 10 mg/kg, about 3.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 5 mg/kg, about 4.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 10 mg/kg, about 4.5 mg/kg to about 10 mg/kg, about 5 mg/kg to about 10 mg/kg, about 5.5 mg/kg to about 10 mg/kg, about 6 mg/kg to about 10 mg/kg, about 6.5 mg/kg to about 10 mg/kg, about 7 mg/kg to about 10 mg/kg, about 7.5 mg/kg to about 10 mg/kg, about 8 mg/kg to about 10 mg/kg, about 8.5 mg/kg to about 10 mg/kg, about 9 mg/kg to about 10 mg/kg, or about 9.5 mg/kg to about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, the dsRNA may be administered at a dose of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, or about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In other embodiments, for example, when a composition of the invention comprises a dsRNA as described herein and an N-acetylgalactosamine, subjects can be administered a therapeutic amount of dsRNA, such as a dose of about 0.1 to about 50 mg/kg, about 0.25 to about 50 mg/kg, about 0.5 to about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/kg, about 1.5 to about 50 mg/kg, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 0.1 to about 45 mg/kg, about 0.25 to about 45 mg/kg, about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to about 45 mg/kg, about 1.5 to about 45 mg/kg, about 2 to about 45 mg/kg, about 2.5 to about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to about 45 mg/kg, about 4 to about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to about 45 mg/kg, about 0.1 to about 40 mg/kg, about 0.25 to about 40 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to about 40 mg/kg, about 1.5 to about 40 mg/kg, about 2 to about 40 mg/kg, about 2.5 to about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 40 to about 40 mg/kg, about 45 to about 40 mg/kg.

mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.1 to about 30
mg/kg, about 0.25 to about 30 mg/kg, about 0.5 to about 30 mg/kg, about 0.75 to about
30 mg/kg, about 1 to about 30 mg/mg, about 1.5 to about 30 mg/kg, about 2 to about 30
mg/kg, about 2.5 to about 30 mg/kg, about 3 to about 30 mg/kg, about 3.5 to about 30
5 mg/kg, about 4 to about 30 mg/kg, about 4.5 to about 30 mg/kg, about 5 to about 30
mg/kg, about 7.5 to about 30 mg/kg, about 10 to about 30 mg/kg, about 15 to about 30
mg/kg, about 20 to about 30 mg/kg, about 20 to about 30 mg/kg, about 25 to about 30
mg/kg, about 0.1 to about 20 mg/kg, about 0.25 to about 20 mg/kg, about 0.5 to about 20
mg/kg, about 0.75 to about 20 mg/kg, about 1 to about 20 mg/mg, about 1.5 to about 20
10 mg/kg, about 2 to about 20 mg/kg, about 2.5 to about 20 mg/kg, about 3 to about 20
mg/kg, about 3.5 to about 20 mg/kg, about 4 to about 20 mg/kg, about 4.5 to about 20
mg/kg, about 5 to about 20 mg/kg, about 7.5 to about 20 mg/kg, about 10 to about 20
mg/kg, or about 15 to about 20 mg/kg. Values and ranges intermediate to the recited
values are also intended to be part of this invention.

15 For example, subjects can be administered a therapeutic amount of dsRNA, such
as about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8,
1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9,
4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6,
6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1,
20 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.5, 11,
11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 21,
22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43,
44, 45, 46, 47, 48, 49, or about 50 mg/kg. Values and ranges intermediate to the recited
values are also intended to be part of this invention.

25 The iRNA can be administered by intravenous infusion over a period of time,
such as over a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or
about a 25 minute period. The administration may be repeated, for example, on a
regular basis, such as biweekly (*i.e.*, every two weeks) for one month, two months, three
months, four months or longer. After an initial treatment regimen, the treatments can be
30 administered on a less frequent basis. For example, after administration biweekly for
three months, administration can be repeated once per month, for six months or a year or

longer. Administration of the iRNA can reduce ANGPTL3 levels, *e.g.*, in a cell, tissue, blood, urine or other compartment of the patient by at least about 5%, 6, 7, 8, 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, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 39, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or at least about 99% or more.

Before administration of a full dose of the iRNA, patients can be administered a smaller dose, such as a 5% infusion reaction, and monitored for adverse effects, such as an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (*e.g.*, TNF-alpha or INF-alpha) levels.

Alternatively, the iRNA can be administered subcutaneously, *i.e.*, by subcutaneous injection. One or more injections may be used to deliver the desired daily dose of iRNA to a subject. The injections may be repeated over a period of time, such as over 2, 3, 4, 5, 6, 7, 8, 9, 10 or 15 days. The administration may be repeated, for example, on a regular basis, such as biweekly (*i.e.*, every two weeks) for one month, two months, three months, four months or longer. After an initial treatment regimen, the treatments can be administered on a less frequent basis. In some embodiments, a single dose of iRNA is followed by monthly dosing. In some embodiments, the dosing may comprise a loading phase of multiple doses on consecutive days.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the iRNAs and methods featured in the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

Example 1. siRNA Synthesis

Source of reagents

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

Transcripts

- siRNA design was carried out to identify siRNAs targeting the human
ANGPTL3 transcript annotated in the NCBI Gene database
10 (<http://www.ncbi.nlm.nih.gov/gene/>) and a cynomolgus monkey (*Macaca fascicularis*;
henceforth “cyno”) ANGPTL3 transcript produced via sequencing of cDNA prepared
from liver RNA. Sequencing of cyno ANGPTL3 mRNA was done in-house, and the
mRNA sequence is shown in SEQ ID NO:9.

- Design used the following transcripts from the NCBI collection: Human -
15 NM_014495.2 (SEQ ID NO:1) ; Mouse - NM_013913.3 (SEQ ID NO:2). All siRNA
duplexes were designed that shared 100% identity with the listed human and cyno
transcripts. A subset of siRNA duplexes, described below, also shared 100% identity
with the mouse (*Mus musculus*) ANGPTL3 transcript found in NCBI Gene database.

- 20 *siRNA Design, Specificity, and Efficacy Prediction*

- The predicted specificity of all possible 19mers was predicted from each
sequence. Candidate 19mers were then selected that lacked repeats longer than 7
nucleotides. These 977 candidate human/cyno siRNAs, and a subset of 38 that also
matched mouse (“human/cyno/mouse candidate siRNAs”) were then used in a
25 comprehensive search against the human transcriptome (defined as the set of NM_ and
XM_ records within the human NCBI Refseq set) using an exhaustive “brute-force”
algorithm implemented in the python script ‘BruteForce.py’. The script next parsed the

transcript-oligo alignments to generate a score based on the position and number of mismatches between the siRNA and any potential 'off-target' transcript. The off-target score is weighted to emphasize differences in the 'seed' region of siRNAs, in positions 2-9 from the 5' end of the molecule. Each oligo-transcript pair from the brute-force search

5 was given a mismatch score by summing the individual mismatch scores; mismatches in the position 2-9 were counted as 2.8, mismatches in the cleavage site positions 10-11 were counted as 1.2, and mismatches in region 12-19 counted as 1.0. An additional off-target prediction was carried out by comparing the frequency of heptamers and octomers derived from 3 distinct, seed-derived hexamers of each oligo. The hexamers from

10 positions 2-7 relative to the 5' start were used to create 2 heptamers and one octomer. 'Heptamer1' was created by adding a 3' A to the hexamer; 'heptamer2' was created by adding a 5' A to the hexamer; octomer was created by adding an A to both 5' and 3' ends of the hexamer. The frequency of octomers and heptamers in the human 3'UTRome (defined as the subsequence of the transcriptome from NCBI's Refseq

15 database where the end of the coding region, the 'CDS', is clearly defined) was pre-calculated. The octomer frequency was normalized to the heptamer frequency using the median value from the range of octomer frequencies. A 'mirSeedScore' was then calculated by calculating the sum of ((3 X normalized octomer count) + (2 X heptamer2 count) + (1 X heptamer1 count)).

20 Both siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. Sorting was carried out by the specificity of the antisense strand. Duplexes were then selected from the human/cyno set with antisense oligos lacking miRNA seed matches, scores of 3 or better, less than 65%

25 overall GC content, no GC at the first position, 4 or more Us or As in the seed region, and GC at the nineteenth position. Duplexes from the human/cyno/mouse set with antisense oligos having scores of 2 or better, less than 65% overall GC content, and no GC at the first position were also selected.

siRNA sequence selection

A total of 47 sense and 47 antisense derived siRNA oligos from the human/cyno set were synthesized and formed into duplexes. A total of 15 sense and 15 antisense derived siRNAs from the human/cyno/mouse set were synthesized and formed into
5 duplexes.

Synthesis of ANGPTL3 sequences

ANGPTL3 sequences were synthesized on a MerMade 192 synthesizer at either a 1 or 0.2 μ mol scale. Single strands were synthesized with 2'-O-methyl modifications for transfection based in vitro screening. For use in free uptake screening assays, 3'
10 GalNAc conjugates were made with 2'-F and 2'-O-methyl chemical modifications. In these designs, GalNAc moiety was placed at the 3' end of the sense strand. The antisense sequence was 23 nucleotides in length and also contained 2'-F and 2'-OMethyl chemical modifications with two phosphorothioate linkages at the 3' end.

On one set of 21mer single strands and duplexes, 'endolight' chemistry was
15 applied as detailed below.

- All pyrimidines (cytosine and uridine) in the sense strand were modified with 2'-O-Methyl nucleotides (2'-O-Methyl C and 2'-O-Methyl U)
- In the antisense strand, pyrimidines adjacent (towards 5' position) to ribo
20 A nucleoside were replaced with their corresponding 2'-O-Methyl nucleosides
- A two base dTsdT extension at the 3' end of both sense and anti sense sequences was introduced

For GalNAc conjugated 21mer sense and complementary 23mer antisense sequences, 2'-F and 2'-OMethyl modified single strands were synthesized. The synthesis
25 was performed on a GalNAc modified CPG support for the sense strand and CPG modified with universal support for the antisense sequence at a 1 μ mol scale. The sequence motif named 'TOFFEE' was applied, in which the sense strand contained a

three-nucleotide 2'F-modified motif at positions 9, 10 and 11 and in the antisense, a 2'OMethyl-modified motif was included at positions 11, 12 and 13.

Synthesis, Cleavage and Deprotection

The synthesis of ANGPTL3 sequences used solid supported oligonucleotide
5 synthesis using phosphoramidite chemistry. For 21 mer endolight sequences, a deoxy thymidine CPG was used as the solid support while for the GalNAc conjugates, GalNAc solid support for the sense strand and a universal CPG for the antisense strand were used.

The synthesis of the above sequences was performed at either a 1 or 0.2 μ m scale
10 in 96 well plates. The amidite solutions were prepared at 0.1M concentration and ethyl thio tetrazole (0.6M in Acetonitrile) was used as the activator.

The synthesized sequences were cleaved and deprotected in 96 well plates, using methylamine in the first step and fluoride reagent in the second step. For GalNAc and 2'F nucleoside containing sequences, deprotection conditions were modified. Sequences
15 after cleavage and deprotection were precipitated using an acetone: ethanol (80:20) mix and the pellets were re-suspended in 0.2M sodium acetate buffer. Samples from each sequence were analyzed by LC-MS to confirm the identity, UV for quantification and a selected set of samples by IEX chromatography to determine purity.

Purification, Desalting and Annealing

20 ANGPTL3 sequences were precipitated and purified on an AKTA Purifier system using a Sephadex column. The ANGPTL3 was run at ambient temperature. Sample injection and collection was performed in 96 well plates with 1.8 mL deep wells. A single peak corresponding to the full length sequence was collected in the eluent. The desalted ANGPTL3 sequences were analyzed for concentration (by UV measurement at
25 A_{260}) and purity (by ion exchange HPLC). The complementary single strands were then combined in a 1:1 stoichiometric ratio to form siRNA duplexes.

Example 2. *In vitro* screening

Cell culture and transfections

- Hep3B cells (ATCC, Manassas, VA) were grown to near confluence at 37 °C in an atmosphere of 5% CO₂ in RPMI (ATCC) supplemented with 10% FBS, streptomycin, and glutamine (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 14.8 µl of Opti-MEM plus 0.2 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 µl of siRNA duplexes per well into a 96-well plate and incubated at room temperature for 15 minutes. 80 µl of complete growth media without antibiotic containing $\sim 2 \times 10^4$ Hep3B cells were then added to the siRNA mixture. Cells were incubated for either 24 or 120 hours prior to RNA purification. Single dose experiments were performed at 10 nM and 0.1 nM final duplex concentration and dose response experiments were done at 10, 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00005 and 0.00001 nM final duplex concentration unless otherwise stated.

15 *Free uptake transfection*

- 5 µl of each GalNac conjugated siRNA in PBS was combined with 4×10^4 freshly thawed cryopreserved Cynomolgus monkey hepatocytes resuspended in 95 µl of In Vitro Gro CP media (In Vitro Technologies- Celsis, Baltimore, MD) in each well of a 96 well plate. The mixture was incubated for about 24 hrs at 37 °C in an atmosphere of 5% CO₂. siRNAs were tested at final concentrations of 500nM, 100nM and 10nM for efficacy free uptake assays. For dose response screens, final siRNA concentrations were 500nM, 100nM, 20nM, 4nM, 0.8nM, 0.16nM, 0.032nM and 0.0064nM.

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

- 25 Cells were harvested and lysed in 150 µl of Lysis/Binding Buffer then mixed for 5 minute at 850 rpm using an Eppendorf Thermomixer (the mixing speed was the same throughout the process). Ten microliters of magnetic beads and 80 µl of Lysis/Binding Buffer mixture were added to a round bottom plate and mixed for 1 minute. Magnetic beads were captured using magnetic stand and the supernatant was removed without

disturbing the beads. After removing supernatant, the lysed cells were added to the remaining beads and mixed for 5 minutes. After removing supernatant, magnetic beads were washed 2 times with 150 μ l Wash Buffer A and mixed for 1 minute. Beads were captured again and supernatant removed. Beads were then washed with 150 μ l of Wash
5 Buffer B, captured, and the supernatant was removed. Beads were next washed with 150 μ l Elution Buffer, captured, and the supernatant was removed. Beads were allowed to dry for 2 minutes. After drying, 50 μ l of Elution Buffer was added and mixed for 5 minutes at 70 °C. Beads were captured on magnet for 5 minutes. 40 μ l of supernatant was removed and added to another 96 well plate.

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

A master mix of 2 μ l 10X Buffer, 0.8 μ l 25X dNTPs, 2 μ l Random primers, 1 μ l Reverse Transcriptase, 1 μ l RNase inhibitor and 3.2 μ l of H₂O per reaction were added into 10 μ l total RNA. cDNA was generated using a Bio-Rad C-1000 or S-1000 thermal
15 cycler (Hercules, CA) through the following steps: 25 °C 10 min, 37 °C 120 min, 85 °C 5 sec, 4 °C hold.

Real time PCR

2 μ l of cDNA was added to a master mix containing 0.5 μ l GAPDH TaqMan Probe (Applied Biosystems Cat #4326317E), 0.5 μ l ANGPTL TaqMan probe (Applied
20 Biosystems cat # Hs00205581_m1) and 5 μ l Lightcycler 480 probe master mix (Roche Cat #04887301001) per well in a 384 well 50 plates (Roche cat # 04887301001). Real time PCR was done in an ABI 7900HT Real Time PCR system (Applied Biosystems) using the $\Delta\Delta$ Ct(RQ) assay. Each duplex was tested in two independent transfections, and each transfection was assayed in duplicate, unless otherwise noted in the summary
25 tables.

To calculate relative fold change, real time data was analyzed using the $\Delta\Delta$ Ct method and normalized to assays performed with cells transfected with 10 nM 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 naïve cells over the same

dose range, or to its own lowest dose. AD-1955 sequence, used as a negative control, targets luciferase and has the following sequence: sense: cuuAcGcuGAGuAcuucGAdTsdT; antisense: UCGAAGuACUcAGCGuAAGdTsdT.

Viability screens

- 5 Cell viability was measured on days 3 and 6 in HeLa and Hep3B cells following transfection with 10, 1, 0.5, 0.1, 0.05 nM siRNA. Cells were plated at a density of 10,000 cells per well in 96 well plates. Each siRNA was assayed in triplicate and the data averaged. siRNAs targeting PLK1 and AD-19200 were included as positive controls for loss of viability, and AD-1955 and mock transfected cells as negative controls. PLK1 and AD-19200 result in a dose dependent loss of viability. To measure viability, 20 μ l of CellTiter Blue (Promega) was added to each well of the 96 well plates after 3 or 6 days and incubated at 37 °C for 2 hours. Plates were then read in a Spectrophotometer (Molecular Devices) at 560Ex/590Em. Viability was expressed as the average value of light units from three replicate transfections +/- standard deviation.
- 10
- 15 Relative viability was assessed by first averaging the three replicate transfections and then normalizing Mock transfected cells. Data is expressed as % viable cells.

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.
- 20

Abbreviation	Nucleotide(s)
A	adenosine
C	cytidine
G	guanosine
T	thymidine
U	uridine
N	any nucleotide (G, A, C, T or U)
a	2'-O-methyladenosine
c	2'-O-methylcytidine

Abbreviation	Nucleotide(s)
g	2'-O-methylguanosine
u	2'-O-methyluridine
dT	2'-deoxythymidine
s	phosphorothioate linkage

Table 2. Unmodified sense and antisense strand sequences of ANGPTL3 dsRNAs

Duplex ID	Sense Name	Sense Sequence	Position in NM_014495.2	Antisense Name	Antisense Sequence	Position in NM_014495.2
AD-45939.1	A-96225.1	UAUUUGAUCAGUCUUUUUA	281-299	A-96226.1	UAAAAAGACUGAACAAUA	281-299
AD-45858.1	A-96149.1	GAGCAACUAAACUUA	478-496	A-96150.1	UUAAGUUAGUUAGUUGCUC	478-496
AD-45869.1	A-96137.1	GGCCAAAUUAAUGACAU	247-265	A-96138.1	AUAUGUCAUUUUUUGGCC	247-265
AD-45884.1	A-96189.1	CGAAUUGAGUUGGAAGACU	1045-1063	A-96190.1	AGUCUUCCAACUCAUUUCG	1045-1063
AD-45892.1	A-96129.1	CCUCCUUCAGUUGGGACAU	198-216	A-96130.1	AUGUCCCAACUGAAGGAGG	198-216
AD-45899.1	A-96147.1	CACUUGAACUCAACUCA	401-419	A-96148.1	UUUGAGUUGAGUUCAGUG	401-419
AD-45915.1	A-96231.1	GUCCAUGGACAUUAAUUA	890-908	A-96232.1	UGAAUUAAUUGUCAUGGAC	890-908
AD-45924.1	A-96219.1	AAUCAAGAUUUGCUAUGUU	152-170	A-96220.1	AACAUAGCAAUCUUGAUU	152-170
AD-45860.1	A-96181.1	CUAGAGAAGAUUACUCCA	1000-1018	A-96182.1	UGGAGUAUAUCUUCUCUAG	1000-1018
AD-45870.1	A-96153.1	CUAACUAACUUAUUUCAA	484-502	A-96154.1	UUUGAAUUAAAGUUAGUUAG	484-502
AD-45870.2	A-96153.2	CUAACUAACUUAUUUCAA	484-502	A-96154.2	UUUGAAUUAAAGUUAGUUAG	484-502
AD-45877.1	A-96171.1	CAUUAUUUCAAACUUGAAU	899-917	A-96172.1	AUUCGAUUGUUGAAUUAAUG	899-917
AD-45885.1	A-96205.1	CAAAUUGUUGAUCCA	1392-1410	A-96206.1	UGGAUGGAUCAACAUUUUG	1392-1410
AD-45893.1	A-96145.1	CAUAUAAACUACAAGUCA	359-377	A-96146.1	UUUGACUUUGUAGUUUAUAG	359-377
AD-45900.1	A-96163.1	GACCCAGCAACUCUCAAGU	839-857	A-96164.1	ACUUGAGAGUUUGCUGGGUC	839-857
AD-45925.1	A-96235.1	GGUUGGGCCUAGAGAAGAU	992-1010	A-96236.1	AUCUUCUCUAGGCCCAACC	992-1010
AD-45861.1	A-96197.1	GUGUGGAGAAAACAACCUA	1272-1290	A-96198.1	UAGGUUGUUUUCUCCACAC	1272-1290

AD-45903.1	A-96211.1	CAGAGCCAAAAUCAAGAUU	143-161	A-96212.1	AAUCUUGAUUUUGGCUCUG	143-161
AD-45919.1	A-96217.1	AAAUCAAAGAUUUGCUAUGU	151-169	A-96218.1	ACAUAGCAAAUUCUUGAUUU	151-169
AD-45865.1	A-96167.1	CAUGGACAUUAAUUAACA	893-911	A-96168.1	UGUUGAAUUAUUGUCCAUG	893-911
AD-45874.1	A-96123.1	GAUUUGCUAUGUUAGACGA	158-176	A-96124.1	UCGUCUAAACAUAGCAAUC	158-176
AD-45881.1	A-96141.1	GAACUACAUUAAACUACA	353-371	A-96142.1	UGUAGUUUAUUAUGUAGUUC	353-371
AD-45889.1	A-96175.1	CGAAUAGAUGGAUCACAAA	913-931	A-96176.1	UUUGUGAUCCAUCUAUUCG	913-931
AD-45897.1	A-96209.1	CUUGUUAAAACUCUAAAACU	1817-1835	A-96210.1	AGUUUAGAGUUUUAACAAG	1817-1835
AD-45904.1	A-96227.1	AUUUGAUCAGUCUUUUUAU	282-300	A-96228.1	AUAAAAAGACUGAUCAAAU	282-300
AD-45920.1	A-96233.1	UCCAUGGACAUUAAUUAACA	891-909	A-96234.1	UUGAAUUAAUGUCCAUGGA	891-909
AD-45856.1	A-96117.1	CACAAUUAAAGCUCCUUCUU	57-75	A-96118.1	AAGAAGGAGCUUAAUUUGUG	57-75
AD-45929.1	A-96221.1	CAACAUUUUUGAUCAGUCU	276-294	A-96222.1	AGACUGAUCAAAUAUGUUUG	276-294
AD-45866.1	A-96183.1	CUCCAUAUGAAGCAAUUCU	1014-1032	A-96184.1	AGAUUGCUUCACUAUUGGAG	1014-1032
AD-45875.1	A-96139.1	GCCAAAUUAAUUGACAUUUU	248-266	A-96140.1	AAUAUGUCAUUAAUUUUGGC	248-266
AD-45882.1	A-96157.1	CAACAGCAUAGUCAAAUAA	622-640	A-96158.1	UUAAUUUGACUAUGCUGUUG	622-640
AD-45890.1	A-96191.1	GGAAUACGAAACCAACU	1105-1123	A-96192.1	AGUUGGUUUCUGAUUUUCC	1105-1123
AD-45898.1	A-96131.1	CAGUUGGACAUUGGUCUUA	205-223	A-96132.1	UAAGACCAUGUCCCAACUG	205-223
AD-45857.1	A-96133.1	GACAUUGGUCUUAAAGACUU	212-230	A-96134.1	AAGUCUUUAAGACCAUGUC	212-230
AD-45930.1	A-96237.1	UGUGGAGAAAACAACCUAA	1273-1291	A-96238.1	UUAGGUUUGUUUUCUCCACA	1273-1291
AD-45867.1	A-96199.1	GUGGAGAAAACAACCUAAA	1274-1292	A-96200.1	UUUAGGUUGUUUUCUCCAC	1274-1292
AD-45876.1	A-96155.1	CCAACAGCAUAGUCAAAUA	621-639	A-96156.1	UAUUUGACUAUGCUGUUGG	621-639
AD-45883.1	A-96173.1	CAACAUCGAAUAGAUGGAU	907-925	A-96174.1	AUCCAUCUAUUCGAUGUUG	907-925

AD-45891.1	A-96207.1	GCAAAUUUUAAAAAGGCAAU	1441-1459	A-96208.1	UAUUGCCUUUUUAAAAUUUGC	1441-1459
AD-45914.1	A-96215.1	CAAAAUCAAGAUUUUGCUAU	149-167	A-96216.1	AUAGCAAAUUCUUGAUUUUG	149-167
AD-15838.1	A-26242.1	AGAGCCAAAAUCAAGAUUU	144-162	A-26243.2	AAAUUUUGAUUUUGGCUCU	144-162

Table 3. Modified sense and antisense strand sequences of ANGPTL3 dsRNAs

Duplex ID	Sense OligoName	Sense Sequence	Antisense OligoName	Antisense Sequence
AD-45939.1	A-96225.1	uAuuuGAucAGucuuuuuAdTsdT	A-96226.1	uAAAAAGACUGAucAAAAuAdTsdT
AD-45858.1	A-96149.1	GAGcAAcuAAcuAAcuAAAdTsdT	A-96150.1	UuAAGUuAGUuAGUUGCUCdTsdt
AD-45869.1	A-96137.1	GGccAAAAuuAAuGAcAuAudTsdT	A-96138.1	AuAUGUcAUuAAUuuUUGGCCdTsdt
AD-45884.1	A-96189.1	cGAAuuGAGuuGGAAGAcudTsdT	A-96190.1	AGUCUUCcAACUCcAAUUCGdTsdt
AD-45892.1	A-96129.1	ccuccuucAGuuGGGAcAudTsdT	A-96130.1	AUGUCCcAACUGAAGGAGGdTsdt
AD-45899.1	A-96147.1	cAuuGAAAcucAAcucAAAdTsdT	A-96148.1	UUUGAGUUUGAGUuAAAGUGdTsdt
AD-45915.1	A-96231.1	GuccAuGGAcAuAAuucAdTsdT	A-96232.1	UGAAUuAAUGUCcAUGGACdTsdt
AD-45924.1	A-96219.1	AAucAAGAAuuGcuAuGuudTsdT	A-96220.1	AAcAuAGcAAAAUCUUAUdTsdt
AD-45860.1	A-96181.1	cuAGAGAAAGAuAcuccAdTsdT	A-96182.1	UGGAGuAuAUCUUCUCuAGdTsdt
AD-45870.1	A-96153.1	cuAAcuAAcuuAAuucAAAdTsdT	A-96154.1	UUUGAAUuAAAGUuAGUuAGdTsdt
AD-45870.2	A-96153.2	cuAAcuAAcuuAAuucAAAdTsdT	A-96154.2	UUUGAAUuAAAGUuAGUuAGdTsdt
AD-45877.1	A-96171.1	cAuAAuucAAcAucGAAudTsdT	A-96172.1	AUUCGAUGUUUGAAUuAAUGdTsdt
AD-45885.1	A-96205.1	cAAAAuGuuGAuccAuccAdTsdT	A-96206.1	UGGAUUGGAUcAAcAUUUUGdTsdt
AD-45893.1	A-96145.1	cAuAuAAAAcuAcAAGucAAAdTsdT	A-96146.1	UUUGACUUGuAGUuAuAUGdTsdt
AD-45900.1	A-96163.1	GAcccAGcAAcucucAAGudTsdT	A-96164.1	ACUUGAGAGUUUGCUGGUCdTsdt
AD-45925.1	A-96235.1	GGuuGGGccuAGAGAGAuAdTsdT	A-96236.1	AUCUUCUCuAGGCCcAACcAdTsdt
AD-45861.1	A-96197.1	GuGuGGGAGAAAAcAAccuAdTsdT	A-96198.1	uAGGUUGUUUUCUCcAcAdTsdt
AD-45871.1	A-96169.1	GAcAuAAuucAAcAucGAdTsdT	A-96170.1	UCGAUGUUUGAAUuAAUGUCdTsdt

AD-45878.1	A-96187.1	cAuAGuGAAGcAAucAAudTsdT	A-96188.1	AUuAGAUUGCUUcACuAUGdTsdT
AD-45886.1	A-96127.1	cuAuGuuAGAcGAGuGAAAAdTsdT	A-96128.1	UUuAcAUCGUCuAAcAuAGdTsdT
AD-45894.1	A-96161.1	cAcAGAAuuucucuAucdTsdT	A-96162.1	AGAuAGAGAAAUUUUCUGGdTsdT
AD-45901.1	A-96179.1	GuuGGGccuAGAGAGAuAdTsdT	A-96180.1	uAUCUUUCUCuAGGCCcCAAcdTsdT
AD-45909.1	A-96213.1	GccAAAAuAcAGAAuuuGcudTsdT	A-96214.1	AGcAAAUUCUUGAUUUUUGGCdTsdT
AD-45934.1	A-96223.1	AcAuAuuuGAucAGucuuudTsdT	A-96224.1	AAAGACUGAUcAAAUuAGUdTsdT
AD-45934.2	A-96223.2	AcAuAuuuGAucAGucuuudTsdT	A-96224.2	AAAGACUGAUcAAAUuAGUdTsdT
AD-45863.1	A-96135.1	cuuAAAGAcuuuGuccAuAdTsdT	A-96136.1	uAUGGAcAAAGUCUuAAAGdTsdT
AD-45872.1	A-96185.1	ccAuAGuGAAGcAAucAAdTsdT	A-96186.1	UUuAGAUUGCUUcACuAUGGdTsdT
AD-45879.1	A-96203.1	cAAcAAAAuGuuGAuccAdTsdT	A-96204.1	UGGAUcAAcAUUUUUGGUUGdTsdT
AD-45887.1	A-96143.1	cuAcAuAuAAAcuAcAAGudTsdT	A-96144.1	ACUUGuAGUuAuAuAGuAGdTsdT
AD-45895.1	A-96177.1	GGGAGGcuuGAUGGAGAAudTsdT	A-96178.1	AUUCUCcAUcAAGCCUCCcdTsdT
AD-45902.1	A-96195.1	GGuGuuuuuAcuuGGGAudTsdT	A-96196.1	AUCCcAAGuAGAAAAcACcdTsdT
AD-45910.1	A-96229.1	AAGAGcAccAAGAAcuAcudTsdT	A-96230.1	AGuAGUUCUUGGUGCUCUudTsdT
AD-45935.1	A-96239.1	uGGAGAAAAcAAccuAAAudTsdT	A-96240.1	AUUuAGGUUGUUUUUCUCCAdTsdT
AD-45864.1	A-96151.1	GcAAcuAAcuAAcuAAudTsdT	A-96152.1	AAUuAAGUuAGUuAGUUGCdTsdT
AD-45873.1	A-96201.1	cAAccuAAAUgGuAAAUAdTsdT	A-96202.1	AuAUUuACcAUuAGGUUGdTsdT
AD-45880.1	A-96125.1	GcuAuGuuAGAcGAGuAAdTsdT	A-96126.1	UUuAcAUCGUCuAAcAuAGCdTsdT
AD-45888.1	A-96159.1	cccAcAGAAuuuucucuAudTsdT	A-96160.1	AuAGAGAAAUUCUGUGGGdTsdT
AD-45896.1	A-96193.1	GAuuuGGGuuuuucuAcuudTsdT	A-96194.1	AAGuAGAAAAcACcAAAUcCdTsdT
AD-45903.1	A-96211.1	cAGAGcAAAAuAcAAGAuudTsdT	A-96212.1	AAUCUUGAUUUUUGGCUCUGdTsdT

AD-45919.1	A-96217.1	AAAucAAGAuuuGcuAuGudTsdT	A-96218.1	AcAuAGcAAAAUCUUGAUUUUdTsdT
AD-45865.1	A-96167.1	cAuGGAcAuAAuucAAcAdTsdT	A-96168.1	UGUUGAAUuAAUUGUCcAuGdTsdT
AD-45874.1	A-96123.1	GAuuuGcuAuGuuAGAcGAdTsdT	A-96124.1	UCGUcUAACcAuAGcAAAUcDdTsdT
AD-45881.1	A-96141.1	GAACuAcAuAuAAAACuAcAdTsdT	A-96142.1	UGuAGUuAuAuAGuAGUUCdTsdT
AD-45889.1	A-96175.1	cGAAuAGAuGGAucAAAAAdTsdT	A-96176.1	UUUGUGAUcCAUCuAUUCGdTsdT
AD-45897.1	A-96209.1	cuuGuuAAAAcucuAAAAdTsdT	A-96210.1	AGUuAuAGAGUUuAAcAAgdTsdT
AD-45904.1	A-96227.1	AuuuGAucAGucuuuuuAudTsdT	A-96228.1	AuAAAAAGACUGAUcAAAAUdTsdT
AD-45920.1	A-96233.1	uccAuGGAcAuAAuucAAAdTsdT	A-96234.1	UUGAAUuAAUUGUCcAuGGAdTsdT
AD-45856.1	A-96117.1	cAcAAuuAAGcuccuucudTsdT	A-96118.1	AAGAAGGAGCUuAAUUGUGdTsdT
AD-45929.1	A-96221.1	cAAcAuAuuuGAucAGucudTsdT	A-96222.1	AGACUGAUcAAAUUGUUGdTsdT
AD-45866.1	A-96183.1	cuccAuAGUGAAGcAAucudTsdT	A-96184.1	AGAUUGCUUcACuAUUGGAGdTsdT
AD-45875.1	A-96139.1	GccAAAUuAAuGAcAuAuudTsdT	A-96140.1	AAuAUGUcAuAAUuUUGGcDdTsdT
AD-45882.1	A-96157.1	cAAcAGcAuAGucAAAAuAdTsdT	A-96158.1	UuAUUUGACuAUGCUGUUGdTsdT
AD-45890.1	A-96191.1	GGAAuAcGAAAcAAcudTsdT	A-96192.1	AGUUGGUUUCGUGAUUUCCdTsdT
AD-45898.1	A-96131.1	cAGuuGGGAcAuGGucuuAdTsdT	A-96132.1	uAAGAcCAUGUCCcAACUGdTsdT
AD-45857.1	A-96133.1	GAcAuGGucuuAAAGAcuudTsdT	A-96134.1	AAGUCUUuAAGAcCAUGUCdTsdt
AD-45930.1	A-96237.1	uGuGGAGAAAAcAAccuAdTsdT	A-96238.1	UuAGGUUGUUUUCUCcAcAdTsdT
AD-45867.1	A-96199.1	GuGGAGAAAAcAAccuAAAdTsdT	A-96200.1	UUuAGGUUGUUUUCUCcAcDdTsdT
AD-45876.1	A-96155.1	ccAAcAGcAuAGucAAAUAdTsdT	A-96156.1	uAUUUGACuAUGCUGUUGGdTsdT
AD-45883.1	A-96173.1	cAAcAucGAuAGuGGAudTsdT	A-96174.1	AUCcAuCUAUUCGAUGUUGdTsdT
AD-45891.1	A-96207.1	GcAAAUuuAAAAAGGcAAuAdTsdT	A-96208.1	uAUUGCCUUuAAAAUUGCdTsdt

AD-45914.1	A-96215.1	cAAAAucAAGAuuuGcuAudTsdT	A-96216.1	AuAGcAAAUCUUGAUUUUGdTsdT
AD-15838.1	A-26242.1	AGAGccAAAAAucAAGAuuuTsdT	A-26243.2	AAAUcUuGAUUUuGGCUCUdTsdT

Lowercase nucleotides (a, u, g, c) are 2'-O-methyl nucleotides; s is a phosphothiorate linkage.

Table 4. Results of single dose screen using ANGPTL3 dsRNA sequences

The experiments were conducted using modified oligonucleotide duplexes listed in Table 3. The sequence of AD-15838.2 is identical to the sequence of AD-15838.1. Delivery of siRNA duplexes was done using LNPs.

Human Hep3B				
Duplex	10nM	0.1nM	STDEV, 10 nM	STDEV,0.1 nM
AD-15838.2	0.09	0.66	0.008	0.030
AD-45856.1	0.32	0.91	0.026	0.032
AD-45857.1	2.46	1.07	0.140	0.044
AD-45858.1	0.10	0.74	0.010	0.070
AD-45860.1	0.02	0.47	0.002	0.097
AD-45861.1	0.03	0.68	0.004	0.062
AD-45863.1	1.42	0.95	0.145	0.126
AD-45864.1	0.02	0.17	0.002	0.045
AD-45865.1	0.32	0.93	0.022	0.062
AD-45866.1	0.10	0.92	0.010	0.041
AD-45867.1	0.04	0.61	0.000	0.048
AD-45869.1	0.45	1.08	0.028	0.081
AD-45870.1	0.01	0.10	0.003	0.010
AD-45871.1	0.05	0.57	0.006	0.071
AD-45872.1	0.07	0.71	0.007	0.034
AD-45873.1	0.02	0.23	0.001	0.011
AD-45874.1	0.08	0.75	0.013	0.049
AD-45875.1	0.13	0.82	0.017	0.040
AD-45876.1	0.03	0.54	0.000	0.013
AD-45877.1	0.06	0.47	0.002	0.025
AD-45878.1	0.02	0.44	0.002	0.031
AD-45879.1	0.03	0.35	0.003	0.023
AD-45880.1	0.49	1.00	0.039	0.088
AD-45881.1	0.20	0.90	0.019	0.095
AD-45882.1	0.20	0.95	0.012	0.086
AD-45883.1	0.16	0.98	0.011	0.058

AD-45884.1	0.09	0.94	0.003	0.044
AD-45885.1	0.22	0.91	0.020	0.145
AD-45886.1	0.04	0.40	0.008	0.080
AD-45887.1	0.03	0.35	0.002	0.057
AD-45888.1	0.05	0.80	0.006	0.042
AD-45889.1	0.31	0.91	0.013	0.052
AD-45890.1	0.06	0.90	0.001	0.047
AD-45891.1	0.06	0.82	0.007	0.034
AD-45892.1	1.01	1.09	0.033	0.211
AD-45893.1	0.04	0.58	0.002	0.046
AD-45894.1	0.04	0.59	0.003	0.024
AD-45895.1	0.84	1.00	0.047	0.047
AD-45896.1	0.84	0.98	0.032	0.095
AD-45897.1	0.36	0.61	0.032	0.053
AD-45898.1	0.98	1.09	0.021	0.117
AD-45899.1	0.04	0.59	0.005	0.095
AD-45900.1	0.06	0.80	0.005	0.091
AD-45901.1	0.33	0.94	0.025	0.096
AD-45902.1	0.24	1.03	0.010	0.079
AD-45903.1	0.74	1.02	0.003	0.092
AD-45904.1	0.39	0.87	0.010	0.010
AD-45909.1	0.04	0.73	0.008	0.013
AD-45910.1	1.08	1.01	0.037	0.089
AD-45914.1	0.52	0.99	0.018	0.071
AD-45915.1	0.06	0.48	0.004	0.046
AD-45919.1	0.67	0.98	0.048	0.064
AD-45920.1	0.61	1.00	0.031	0.038
AD-45924.1	0.09	0.67	0.005	0.012
AD-45925.1	0.13	0.90	0.008	0.100
AD-45929.1	0.02	0.42	0.001	0.083
AD-45930.1	0.05	0.63	0.005	0.052
AD-45934.1	0.04	0.41	0.001	0.062
AD-45935.1	0.08	0.76	0.006	0.058

AD-45939.1	0.23	0.82	0.030	0.028
AD-1955.1	0.93	0.93	0.068	0.073
AD-1955.1	0.94	1.01	0.028	0.113
AD-1955.1	1.00	1.02	0.032	0.065
AD-1955.1	1.15	1.06	0.053	0.019

Table 5. Dose response screen results for ANGPTL3 dsRNA sequences

The experiments were conducted using modified oligonucleotide duplexes listed in Table 3. The sequence of AD-15838.2 is identical to the sequence of AD-15838.1.

Hep3B IC ₅₀						
24 hrs				120 hrs		
Duplex	IC ₅₀ I (nM)	IC ₅₀ II (nM)	IC ₅₀ weighted (nM)	IC ₅₀ I (nM)	IC ₅₀ II (nM)	IC ₅₀ weighted (nM)
AD-15838.2	0.027	0.006	0.017	0.657	0.937	0.800
AD-45860.1	0.006	0.002	0.004	0.045	0.032	0.039
AD-45864.1	0.002	0.001	0.002	0.046	0.042	0.044
AD-45870.1	0.002	0.001	0.001	0.011	0.008	0.010
AD-45873.1	0.005	0.004	0.005	0.037	0.025	0.031
AD-45876.1	0.032	0.006	0.019	0.269	0.045	0.156
AD-45877.1	0.018	0.012	0.015	1.660	0.538	1.091
AD-45878.1	0.023	0.015	0.019	0.252	0.131	0.190
AD-45879.1	0.002	0.003	0.003	0.023	0.029	0.026
AD-45886.1	0.004	0.004	0.004	0.030	0.018	0.025
AD-45887.1	0.010	0.009	0.010	0.058	0.059	0.059
AD-45915.1	0.016	0.015	0.015	0.110	0.056	0.083
AD-45929.1	0.023	0.008	0.016	0.227	0.025	0.124
AD-45934.1	0.006	0.006	0.006	0.110	0.045	0.077

Table 6. Results of cell viability screens using modified ANGPTL3 dsRNA sequences

The experiments were conducted using modified oligonucleotide duplexes listed in Table 3. The sequence of AD-15838.2 is identical to the sequence of AD-15838.1. Viability data is expressed as % viable relative to mock treated cells.

Target	Duplex	HeLa day 3									
		Ave	Ave	Ave	Ave	SD	SD	SD	SD	SD	SD
ANGPTL3	AD-15838.2	37.34	58.67	70.92	89.86	94.98	9.45	12.28	15.06	22.37	18.23
ANGPTL3	AD-15838.2	29.13	48.99	63.18	79.21	94.47	1.62	5.56	4.34	11.15	11.31
ANGPTL3	AD-45860.1	67.10	75.49	77.93	86.57	90.51	6.99	12.93	6.39	6.97	3.57
ANGPTL3	AD-45864.1	99.13	96.95	86.77	89.20	84.36	7.90	7.22	12.60	4.85	6.87
ANGPTL3	AD-45870.1	82.36	97.02	95.33	95.67	92.27	8.07	5.12	7.97	7.05	10.29
ANGPTL3	AD-45873.1	67.96	90.01	90.60	94.20	103.63	11.26	22.61	15.92	22.92	16.97
ANGPTL3	AD-45876.1	64.00	76.71	80.21	81.71	91.23	6.60	13.94	10.15	10.81	13.89
ANGPTL3	AD-45877.1	79.55	77.33	79.98	91.96	93.46	1.66	9.80	8.73	16.63	11.41
ANGPTL3	AD-45878.1	81.95	78.22	78.74	87.93	85.03	15.37	22.72	22.59	30.84	40.04
ANGPTL3	AD-45878.1	66.83	70.71	82.14	82.80	83.14	17.48	6.49	6.86	19.92	21.15
ANGPTL3	AD-45879.1	37.56	45.55	59.28	76.35	78.38	3.50	7.96	19.73	34.33	33.99
ANGPTL3	AD-45886.1	72.75	57.90	64.51	81.92	82.89	14.73	12.64	11.78	25.60	23.14
ANGPTL3	AD-45887.1	38.01	53.91	59.31	76.44	85.73	0.58	10.81	6.27	11.12	10.92
ANGPTL3	AD-45915.1	48.06	52.17	67.90	95.45	100.77	8.13	15.15	29.11	32.49	38.79
ANGPTL3	AD-45929.1	29.27	44.58	52.87	76.45	88.03	4.17	9.67	14.49	31.74	28.82
ANGPTL3	AD-45934.1	68.20	64.11	76.92	79.57	92.11	15.79	11.25	19.99	26.08	26.30
(+) control	AD-19200	41.09	85.94	95.13	101.29	96.60	9.99	25.31	24.56	32.26	26.35
(+) control	AD-19200	23.99	72.76	86.51	108.10	111.13	5.35	34.52	29.24	35.99	31.88

(-) control	AD-1955	89.65	99.87	94.59	104.04	105.10	4.57	5.94	4.19	5.78	7.46
(-) control	AD-1955	104.74	99.78	105.79	109.19	108.08	10.94	7.74	11.12	7.91	10.30
(-) control	mock	100.00					6.92				
(-) control	mock	100.00					9.85				
(+) control	PLK	10.66	26.65	46.16	92.42	98.78	1.70	8.65	13.47	22.99	23.48
(+) control	PLK	10.74	11.41	17.33	61.02	86.59	3.39	2.61	1.49	27.42	37.31

HeLa day 6											
Target	Duplex	Ave 10nM	Ave 1nM	Ave 500pM	Ave 100pM	Ave 50pM	SD 10nM	SD 1nM	SD 500pM	SD 100pM	SD 50pM
ANGPTL3	AD-15838.2	47.94	80.97	90.44	94.37	96.10	29.05	25.12	13.62	8.88	4.72
ANGPTL3	AD-15838.2	40.32	83.80	89.88	95.94	98.27	22.47	16.51	10.03	3.83	4.19
ANGPTL3	AD-45860.1	57.38	84.84	88.90	96.74	94.03	24.55	17.35	9.67	3.17	6.58
ANGPTL3	AD-45864.1	98.65	100.87	101.13	96.86	98.24	4.35	1.91	2.22	3.41	1.80
ANGPTL3	AD-45870.1	92.69	98.71	98.49	100.07	99.28	3.94	2.67	2.36	1.19	2.65
ANGPTL3	AD-45873.1	91.78	97.38	98.81	97.57	96.22	12.47	6.26	4.08	6.22	8.64
ANGPTL3	AD-45876.1	63.54	85.68	92.13	96.48	95.97	14.74	16.50	10.03	5.81	7.51
ANGPTL3	AD-45877.1	94.17	93.21	96.39	96.70	96.98	7.12	8.00	4.58	3.05	6.15
ANGPTL3	AD-45878.1	66.46	85.75	89.73	94.60	96.59	8.20	7.41	5.27	3.21	3.91
ANGPTL3	AD-45878.1	70.80	89.30	92.54	96.60	95.09	5.18	2.13	1.61	0.50	4.15
ANGPTL3	AD-45879.1	8.29	48.25	73.54	87.47	92.19	4.66	20.05	16.04	9.06	7.90
ANGPTL3	AD-45886.1	23.69	60.65	78.49	93.41	94.15	8.19	13.90	7.15	3.35	4.06
ANGPTL3	AD-45887.1	7.24	26.03	57.68	95.99	98.80	3.07	13.10	14.94	1.40	2.54

ANGPTL3	AD-45915.1	10.38	58.38	85.69	97.24	99.76	6.83	15.66	8.39	1.33	4.15
ANGPTL3	AD-45929.1	11.73	36.67	51.90	76.71	85.08	4.80	14.19	15.34	12.37	10.60
ANGPTL3	AD-45934.1	73.57	88.48	92.94	91.50	95.97	5.36	2.96	5.50	5.44	4.39
(+) control	AD-19200	63.58	90.14	95.44	94.65	93.28	34.11	14.32	8.78	10.90	12.13
(+) control	AD-19200	16.05	78.65	85.78	93.09	96.22	9.77	15.57	19.50	13.34	10.96
(-) control	AD-1955	93.52	97.36	97.90	99.65	100.07	5.02	1.78	0.84	0.58	1.14
(-) control	AD-1955	75.39	93.61	97.79	99.60	100.96	8.37	2.50	2.27	2.68	3.16
(-) control	mock	100.00					1.32				
(-) control	mock	100.00					3.35				
(+) control	PLK	3.68	55.22	63.00	89.39	95.33	1.42	30.96	33.97	15.85	8.54
(+) control	PLK	2.69	3.74	9.74	67.07	82.96	0.15	0.96	3.60	22.70	19.34

Hep3B day 3											
Target	Duplex	Ave 10nM	Ave 1nM	Ave 500pM	Ave 100pM	Ave 50pM	SD 10nM	SD 1nM	SD 500pM	SD 100pM	SD 50pM
ANGPTL3	AD-15838.2	35.33	61.00	68.79	82.74	90.41	2.41	6.21	4.21	2.61	7.07
ANGPTL3	AD-15838.2	35.34	61.04	72.14	89.71	106.88	1.49	2.61	7.37	6.48	7.13
ANGPTL3	AD-45860.1	17.79	39.25	60.57	94.28	99.85	1.07	3.51	3.57	13.09	16.41
ANGPTL3	AD-45864.1	80.35	88.19	87.01	89.39	92.09	6.93	6.98	9.42	7.41	17.05
ANGPTL3	AD-45870.1	75.00	93.30	96.64	106.29	99.08	7.10	12.24	4.01	5.95	9.64
ANGPTL3	AD-45873.1	42.68	78.45	82.26	97.11	96.58	5.17	5.04	8.31	12.11	11.33

ANGPTL3	AD-45876.1	31.37	55.00	70.69	93.49	91.00	4.39	6.09	5.47	15.11	6.38
ANGPTL3	AD-45877.1	74.45	94.60	96.70	103.77	106.75	3.27	2.44	3.45	6.10	7.40
ANGPTL3	AD-45878.1	50.22	69.65	80.49	92.77	97.37	2.51	14.94	10.44	8.21	5.30
ANGPTL3	AD-45878.1	44.85	65.39	75.67	92.83	109.67	10.10	7.76	8.56	7.78	4.97
ANGPTL3	AD-45879.1	23.73	60.81	84.59	95.72	108.68	6.43	21.36	19.62	13.69	5.95
ANGPTL3	AD-45886.1	27.19	55.35	64.97	100.18	102.09	0.97	6.65	11.46	6.91	4.08
ANGPTL3	AD-45887.1	41.70	97.18	101.91	111.27	105.18	9.26	6.81	7.36	1.72	2.23
ANGPTL3	AD-45915.1	45.10	66.31	82.22	97.97	103.30	6.91	11.84	14.79	6.54	2.48
ANGPTL3	AD-45929.1	48.58	79.14	89.96	95.00	101.37	10.40	10.29	10.52	18.24	10.53
ANGPTL3	AD-45934.1	80.15	102.93	112.82	114.16	113.98	5.28	0.62	4.19	0.75	3.99
(+) control	AD-19200	14.79	55.23	72.90	89.64	94.30	2.17	5.42	7.19	10.28	16.39
(+) control	AD-19200	22.76	92.02	101.56	106.68	113.09	6.61	18.99	7.41	9.83	10.64
(-) control	AD-1955	77.77	81.25	82.23	88.21	95.02	2.83	5.40	5.08	5.42	6.63
(-) control	AD-1955	80.42	86.70	90.23	93.46	97.04	10.53	5.70	8.14	3.27	3.45
(-) control	mock	100.00					5.77				
(-) control	mock	100.00					9.79				
(+) control	PLK	10.91	12.89	14.31	23.87	50.93	0.17	0.87	1.64	1.13	7.80
(+) control	PLK	13.19	16.12	22.89	55.03	94.35	0.78	0.88	8.36	18.88	9.85

Hep3B day 6												
Target	Duplex	Ave 10nM	Ave 1nM	Ave 500pM	Ave 100pM	Ave 50pM	SD 10nM	SD 1nM	SD 500pM	SD 100pM	SD 50pM	
ANGPTL3	AD-15838.2	78.88	89.58	93.08	91.10	100.66	11.60	9.15	12.04	10.51	5.87	
ANGPTL3	AD-15838.2	81.17	85.91	87.27	103.95	103.59	7.75	3.29	8.07	7.93	9.82	
ANGPTL3	AD-45860.1	84.11	87.77	93.22	99.15	96.75	14.22	13.36	20.98	13.15	17.62	
ANGPTL3	AD-45864.1	99.27	111.82	106.28	99.15	97.55	7.77	16.31	14.24	15.40	9.18	
ANGPTL3	AD-45870.1	95.49	109.60	104.16	104.65	106.76	11.92	12.98	9.25	10.29	19.12	
ANGPTL3	AD-45873.1	71.45	90.62	93.44	102.07	107.72	4.71	4.40	15.02	11.96	10.16	
ANGPTL3	AD-45876.1	76.92	82.09	89.44	95.27	105.41	9.39	13.55	7.93	9.77	10.42	
ANGPTL3	AD-45877.1	82.98	98.05	95.07	103.55	104.14	11.22	13.45	1.27	8.88	6.49	
ANGPTL3	AD-45878.1	75.14	82.48	89.68	92.71	95.72	8.65	10.07	10.77	12.44	15.04	
ANGPTL3	AD-45878.1	65.90	77.37	78.33	84.54	99.49	10.21	13.22	9.95	11.65	11.17	
ANGPTL3	AD-45879.1	86.42	89.45	101.50	97.30	100.66	10.59	10.12	19.77	13.19	9.54	
ANGPTL3	AD-45886.1	91.15	79.31	80.76	86.52	94.04	12.89	11.88	5.38	4.92	6.80	
ANGPTL3	AD-45887.1	91.67	103.38	107.88	100.05	102.05	10.80	14.84	19.18	13.72	18.00	
ANGPTL3	AD-45915.1	81.97	85.91	91.81	94.95	102.13	18.49	19.30	7.19	12.72	16.64	
ANGPTL3	AD-45929.1	61.92	79.39	87.28	88.09	96.00	6.80	10.76	5.80	10.68	16.66	
ANGPTL3	AD-45934.1	85.84	89.66	97.67	99.91	102.54	12.39	14.25	4.74	9.51	4.28	
(+) control	AD-19200	50.48	65.62	79.67	98.61	96.87	4.60	4.64	7.20	5.08	7.37	
(+) control	AD-19200	52.01	75.89	92.59	101.47	99.66	4.35	20.87	13.57	6.50	11.76	
(-) control	AD-1955	91.77	95.87	93.06	95.10	97.52	8.87	3.46	1.46	2.00	3.84	
(-) control	AD-1955	93.65	94.41	89.42	100.59	103.91	9.91	14.90	6.80	11.99	10.31	

(-) control	mock	100.00							5.10								
(-) control	mock	100.00							7.35								
(+) control	PLK	36.43	37.75	40.19	55.25	64.59	3.44	2.75	3.65	5.33	5.02						
(+) control	PLK	38.70	43.68	50.32	75.17	89.62	3.40	3.85	8.10	10.54	10.69						

Table 7. Unmodified sense and antisense strand sequences of ANGPTL3 GalNac-conjugated dsRNAs

Duplex ID	Sense Name	Sense Sequence	Position in NM_014495.2	Antisense Name	Antisense Sequence	Position in NM_014495.2
AD-53063.1	A-108558.1	AAAGACAAACAACAUUAUUAUx	1066-1086	A-108559.1	AAUUAUUGUUUGUUCUUUCC	1064-1086
AD-52965.1	A-108310.1	ACAAUUUAGCUCCUUCUUUUUx	58-78	A-108311.1	AAAAAGAGGAGCUUAAUUGUGA	56-78
AD-53030.1	A-108410.1	UGUCACUUUGAACUCACUCUAx	398-418	A-108411.1	UUGAGUUUGAGUUCAAAGUGACAUA	396-418
AD-52953.1	A-108306.1	UCACAAUUUAAAGCUCCUUCUUUx	56-76	A-108307.1	AAAGAAGGAGCUUAAUUGUGAAC	54-76
AD-53001.1	A-108416.1	CUUGAACUCAACUCAAAAACUx	403-423	A-108417.1	AAGUUUUGAGUUGAGUUCAAGUG	401-423
AD-53080.1	A-108548.1	CUCCAUAGUGAAGCAACUCUAx	1014-1034	A-108549.1	UUAGAUUGCUUCACUAUGGGAGUA	1012-1034
AD-52971.1	A-108312.1	CAUUUAGCUCUUCUUUUUUx	59-79	A-108313.1	UAAAAAGAGGAGCUUAAUUGUG	57-79
AD-53071.1	A-108498.1	ACCCAGCAACUCUCAAGUUUUx	840-860	A-108499.1	AAAACUUGAGAGUUGCUGGGUCU	838-860
AD-53024.1	A-108408.1	GAAUAUGUCACUUGAACUCUAx	393-413	A-108409.1	UUGAGUUCAAGUGACAUUUUCUU	391-413
AD-52977.1	A-108314.1	AAUUAAAGCUCUUCUUUUUUx	60-80	A-108315.1	AUAAAAAGAGGAGCUUAAUUGU	58-80
AD-53064.1	A-108574.1	CAUUUAUUUGAAUAUUUUUUx	1078-1098	A-108575.1	AAAAAGAUUUUCAAUAUAAUUGU	1076-1098
AD-53033.1	A-108458.1	ACUAAUAACUUUAAUUCAAx	483-503	A-108459.1	UUUUGAAUUAAAGUUAGUUAGUUG	481-503
AD-52954.1	A-108322.1	UUUUUUGUCCUCUAGUUUUUUx	77-97	A-108323.1	AAAUAAACUAGAGGAAACAAUAAA	75-97
AD-53098.1	A-108554.1	CAUAGUGAAGCAAUUAAUUx	1017-1037	A-108555.1	UAAUUAGAUUGCUUCACUAUGGA	1015-1037
AD-53092.1	A-108552.1	CCAUAGUGAAGCAAUUAAUUx	1016-1036	A-108553.1	AAUUAGAUUGCUUCACUAUGGAG	1014-1036
AD-53073.1	A-108530.1	GAUCACAAAACUCAAUGAAx	923-943	A-108531.1	UUUCAUUUGAAGUUUUUGUGAUCCA	921-943
AD-53132.1	A-108628.1	AUGGAAAGUUUAUACUCUAUAx	1364-1384	A-108629.1	UUUAAGAGUAUAACCUUCCAUUU	1362-1384
AD-53086.1	A-108550.1	UCCAUAGUGAAGCAACUCAAUx	1015-1035	A-108551.1	AUUAGAUUGCUUCACUAUGGAGU	1013-1035
AD-52961.1	A-108340.1	CUAUGUUAGACGAUGUAAAAx	164-184	A-108341.1	UUUUUACAUCGUCUAACAUAAGCA	162-184

AD-52983.1	A-108316.1	AUUUAGCUCUUCUUUUUUUUUx	61-81	A-108317.1	AAUAAAAAGAGGAGCUUAAUUG	59-81
AD-53027.1	A-108456.1	AACUAAACUAAUUUUUUCAAx	482-502	A-108457.1	UUUGAAUUUAAAGUUUAGUUAGUUGC	480-502
AD-52986.1	A-108364.1	GGCCAAUUUAAUGACAUUUUx	247-267	A-108365.1	AAAUUGUCAUUAAUUUUGGCCCU	245-267
AD-52989.1	A-108318.1	UUUUUUUUUCCUCUAGUUUUx	75-95	A-108319.1	AUAACUAGAGGAACAAUAAAAAG	73-95
AD-52981.1	A-108378.1	ACAUUUUUUGAUCAGUCUUUUx	278-298	A-108379.1	AAAAAGACUGAUCAAAAUAGUUG	276-298
AD-53077.1	A-108500.1	CCGAGCAACUCUCAAGUUUUx	841-861	A-108501.1	AAAAACUUGAGAGUUUGCUGGGUC	839-861
AD-53095.1	A-108506.1	CAGGUAGUCCAUUGGACAUUAAx	884-904	A-108507.1	UUAAUUGUCCAUUGGACUACCUGAU	882-904
AD-52970.1	A-108390.1	ACUGAGAAAGAACUACAUUAAx	345-365	A-108391.1	UUAAUUGUAGUUCUUCUCAGUUC	343-365
AD-53015.1	A-108452.1	GAGCAACUAAACUAAUUUUx	478-498	A-108453.1	AAUUAGUUUAGUUUAGUUUGCUCUU	476-498
AD-52147.1	A-108618.1	AACAACCUAAAUUGGUAAAUx	1282-1302	A-108619.1	AUAUUUACCAUUUAGGUUUUUUU	1280-1302
AD-53103.1	A-108540.1	CCUAGAGAGAAUAUACUCCAUx	999-1019	A-108541.1	AUGGAGUAUAUCUUCUCUAGGCC	997-1019
AD-52969.1	A-108374.1	CAACAUUUUUGAUCAGUCUUUx	276-296	A-108375.1	AAAGACUGAUCAAAAUAGUUUGAG	274-296
AD-53075.1	A-108562.1	ACAACAAACAUUUAUUUGAAUx	1070-1090	A-108563.1	AUUCAAUAUAAUGUUUUUGUUGUCU	1068-1090
AD-52994.1	A-108398.1	ACAUUAAACUACAAGUCAAAx	358-378	A-108399.1	UUUGACUUUGUAGUUUUAUAGUAG	356-378
AD-52960.1	A-108324.1	CUAGUUUUUUCCUCCAGAAUx	88-108	A-108325.1	AAUUCUGGAGGAAAUAAACUAGAG	86-108
AD-53003.1	A-108448.1	AAGAGCAACUAAACUAAUUAAx	476-496	A-108449.1	UUAAGUUAGUUUAGUUUGCUCUUCU	474-496
AD-52995.1	A-108320.1	UUUUAUUUUCCUCUAGUUUUx	76-96	A-108321.1	AAUAAUAGAGGGAACAAUAAAAA	74-96
AD-53037.1	A-108428.1	CUCCUAGAGAAAAAUUUCUx	430-450	A-108429.1	UAGAAUUUUUUUUCUUCUAGGAGGC	428-450
AD-53087.1	A-108566.1	AACAAACAUUUAUUUGAAUx	1072-1092	A-108567.1	AUAUUCAAUAUAAUUGUUUUGUUGU	1070-1092
AD-53076.1	A-108578.1	GGAAUUCACGAAACCAACUx	1105-1125	A-108579.1	AUAGUUGGUUUUCGUGAUUUUCCCA	1103-1125
AD-52975.1	A-108376.1	AACAUUUUUUGAUCAGUCUUUx	277-297	A-108377.1	AAAAGACUGAUCAAAAUAGUUGA	275-297
AD-53138.1	A-108630.1	UGGAAGGUUAUACUCUUAUAAx	1365-1385	A-108631.1	UUUAUAGAGUAUAACCUUCCAUU	1363-1385

AD-53091.1	A-108536.1	GGAGAACUACAAAUAUGGUUUX	948-968	A-108537.1	AAACCAUAUUUGUAGUUCUCCCA	946-968
AD-53124.1	A-108594.1	GAAAACAAAGAUUUGGUGUUUX	1174-1194	A-108595.1	AAACACCAAAUUCUUUGUUUCCG	1172-1194
AD-53125.1	A-108610.1	AGUGGAGAGAAAACACCUAAX	1271-1291	A-108611.1	UUAGGUUGUUUUCUCACACUCA	1269-1291
AD-53036.1	A-108412.1	GUCACUUGAACUCACACUCAAX	399-419	A-108413.1	UUUGAGUUGAGUUCAAGUGACAU	397-419
AD-53061.1	A-108526.1	GAUGGAUCACAAAACUUCUAUX	919-939	A-108527.1	AUUGAAGUUUUUGUGAUCCAUUA	917-939
AD-53093.1	A-108568.1	ACAAACAUUAUAUUUGAAUAUUX	1073-1093	A-108569.1	AAUAUUCAAUAUAAUUGUUUGUUG	1071-1093
AD-53137.1	A-108614.1	UGUGGAGAAAAACACCUAAUUX	1273-1293	A-108615.1	AUUUAGGUUGUUUUCUCCACACU	1271-1293
AD-52999.1	A-108384.1	AUCAGUCUUUUUAUGAUCUAUX	287-307	A-108385.1	AUAGAUCAUAAAAACACUGAUA	285-307
AD-53069.1	A-108560.1	GACAAACAAAUUAUAUUGAAUX	1069-1089	A-108561.1	UUCAAUAUAUUGUUUGUUGUCUU	1067-1089
AD-53034.1	A-108474.1	CAACAGCAUAGUCAAAUAAAAUX	622-642	A-108475.1	UUUUUUUGACUAUGCUGUUGGU	620-642
AD-52976.1	A-108392.1	CUGAGAGAAACUAUAUAUAAAAUX	346-366	A-108393.1	UUUAUAUGUAGUUCUUCUCAGUU	344-366
AD-52996.1	A-108336.1	UGCUAUGUUAGACGAUGUAAAAUX	162-182	A-108337.1	UUUACAUCGUCUAACAUAGCAAA	160-182
AD-53029.1	A-108488.1	AACCCACAGAAAUUUCUCUAUX	680-700	A-108489.1	AUAGAGAAAUUUCUGUGGUUUCU	678-700
AD-53020.1	A-108438.1	CUUCAACAAAAAGUGAAUAUUX	451-471	A-108439.1	AUAUUUCACUUUUUGUUGAAGUA	449-471
AD-53042.1	A-108414.1	UCACUUGAACUCUACUCUAAAAUX	400-420	A-108415.1	UUUUUGAGUUGAGUUCUAAUGACA	398-420
AD-53011.1	A-108482.1	CAUAGUCAAAUAAAAAGAAUAUX	628-648	A-108483.1	UAUUUCUUUUUAUUUGACUAUGCU	626-648
AD-52957.1	A-108370.1	CAAAAAUCUACAAUAUUUUGAUX	268-288	A-108371.1	AUCAAUAUGUUUGAGUUUUUGAA	266-288
AD-53008.1	A-108434.1	UACUUCACAAAAAGUGAAUAUX	449-469	A-108435.1	AUUUCACUUUUUGUUGAAGUAGA	447-469
AD-53065.1	A-108496.1	GACCCAGCAACUCUCAAGUUUX	839-859	A-108497.1	AAACUUGAGAGUUUGCUGGGUCUG	837-859
AD-53115.1	A-108638.1	UUGAAUGAACUGAGGCAAAUUX	1427-1447	A-108639.1	AAUUUGCCUCAGUUCAUUCAAAG	1425-1447
AD-53012.1	A-108404.1	UAUAAAACUACAAAGUCAAAAAUX	361-381	A-108405.1	AUUUUUGACUUGUAGUUUAUAUG	359-381
AD-53004.1	A-108464.1	AAACAAGAUAAUAGCAUCAAAUX	559-579	A-108465.1	UUUGAUGCUAUAUUCUUGUUUUU	557-579

AD-53090.1	A-108520.1	UUAAUUAACAUCGAAUAGAUX	901-921	A-108521.1	AUCUAUUCGAUGUUUGAAUUAUG	899-921
AD-53010.1	A-108466.1	GAUAAUAGCAUCAAAAGACCUUX	565-585	A-108467.1	AAGGUCUUUUGAUGCUAUUAUCUU	563-585
AD-52998.1	A-108368.1	UGACAUUUUUUUAACAAACUCAAX	258-278	A-108369.1	UUAGUUUUUUUGAAAUAGUGCAUU	256-278
AD-52992.1	A-108366.1	AAUUAAUAGACAUUUUUAACAAx	251-271	A-108367.1	UUUGAAAUUAGUCAUUAAUUUGG	249-271
AD-53068.1	A-108544.1	GAAGAUUACUCCAUAGUGAAX	1005-1025	A-108545.1	UUCACUAUGGAGUAUAUCUUCUC	1003-1025
AD-53032.1	A-108442.1	AAUAAUUAAGAGAGCAACUAAX	467-487	A-108443.1	UUAGUUGCUCUUCUAAAUUUUC	465-487
AD-52967.1	A-108342.1	CGAUGUAAAAAUUUUAGCCAAX	174-194	A-108343.1	UUGGCUAAAAUUUUUACAUCGUC	172-194
AD-53096.1	A-108522.1	UUAACAUCGAAUAGAUUGGAUX	905-925	A-108523.1	AUCCAUCUAUUGCAUGUUUGAAUU	903-925
AD-53131.1	A-108612.1	GUGUGGAGAAAAACAACCUAAAX	1272-1292	A-108613.1	UUUAGGUUUUUUUUCUCCACACUC	1270-1292
AD-52963.1	A-108372.1	UCAACAUUUUUGAUCAGUCUUX	275-295	A-108373.1	AAGACUGAUCAAAUUAGUUGAGU	273-295
AD-53089.1	A-108504.1	UCAGGUAGUCCAUUGGACAUUAX	883-903	A-108505.1	UAAUGUCCAUGGACUACCUGAUA	881-903
AD-53044.1	A-108446.1	UUUAGAAGAGCAACUAACUAAX	471-491	A-108447.1	UUAGUUAGUUGCUCUUCUAAAUUA	469-491
AD-52988.1	A-108396.1	UACAUUAAAAACUACAAGUCAAX	357-377	A-108397.1	UUGACUUUGAUGUUUAUAUGUAGU	355-377
AD-53067.1	A-108528.1	GGAUCACAAAACUUCAAUGAAX	922-942	A-108529.1	UUCAUUUGAAGUUUUUGUGAUCCA	920-942
AD-53009.1	A-108450.1	AGAGCAACUAACUAAACUUAAUx	477-497	A-108451.1	AUUAAAGUUAGUUAGUUGCUCUUC	475-497
AD-53022.1	A-108470.1	ACCAACAGCAUAGUCAAAUAAx	620-640	A-108471.1	UUUUUUGACUAUGCUGUUGGUUU	618-640
AD-53016.1	A-108468.1	AACCAACAGCAUAGUCAAAUAX	619-639	A-108469.1	UAUUUGACUAUGCUGUUGGUUUA	617-639
AD-53007.1	A-108418.1	GAACUACACUCAAAACUUGAAX	406-426	A-108419.1	UUCAAAGUUUUGAGUUUGAGUUCAA	404-426
AD-53148.1	A-108634.1	UACUCUAUAAAAUCAACCAAAx	1375-1395	A-108635.1	UUUGGUUGAUUUUAUAAGAGUAUA	1373-1395
AD-53040.1	A-108476.1	CAGCAUAGUCAAAUAAAAGAAx	625-645	A-108477.1	UUUUUUUAUUUGACUAUGCUGUU	623-645
AD-53041.1	A-108492.1	GAAAUAAAGAAUUGUAAAACAUX	748-768	A-108493.1	AUGUUUUUACAUUUUCUUAUUUCAU	746-768
AD-53039.1	A-108460.1	CUAACUAACUUAUUUCAAAAUx	484-504	A-108461.1	AUUUUGAAUUAAGUUAGUUAGUU	482-504

AD-53139.1	A-108646.1	AUGAACUGAGGCAAAUUUAAAX	1431-1451	A-108647.1	UUAAAAUUUGCCUCAGUUAUUC	1429-1451
AD-53144.1	A-108648.1	UGAACUGAGGCAAAUUUAAAX	1432-1452	A-108649.1	UUUUAAAAUUUGCCUCAGUUAUUC	1430-1452
AD-53142.1	A-108616.1	AAACAACCUAAAUGGUAAAUAX	1281-1301	A-108617.1	UAUUUACCAUUUAGGUUGUUUUC	1279-1301
AD-53108.1	A-108620.1	ACAACCUAAUUGGUAAAUAX	1283-1303	A-108621.1	UAUAUUUACCAUUUAGGUUGUUU	1281-1303
AD-53079.1	A-108532.1	AACGUGGGAGAAACUACAAAUAX	942-962	A-108533.1	UAUUUGUAGUUCUCCACCGUUUC	940-962
AD-53133.1	A-108644.1	AAUGAACUGAGGCAAAUUUAAAX	1430-1450	A-108645.1	UUAAAAUUUGCCUCAGUUAUUA	1428-1450
AD-53104.1	A-108556.1	GUUGGAAAGACUGGAAAGACAAX	1053-1073	A-108557.1	UUGUCUUUCCAGUCUCCAAACUC	1051-1073
AD-53088.1	A-108582.1	UGGCAAUUGUCCCAUUGCAAUx	1149-1169	A-108583.1	AUUUGCAUUGGGGACAUUGCCAGU	1147-1169
AD-53101.1	A-108508.1	GGUAGUCCAUUGGACAUUAAUUX	886-906	A-108509.1	AAUUAAUGUCCAUUGGACUACCUG	884-906
AD-53000.1	A-108400.1	CAUAUAAACUACAAGUCAAAAAX	359-379	A-108401.1	UUUUAGACUUGUAGUUUAUUAUGUA	357-379
AD-53112.1	A-108590.1	AAUCCCGGAAACAAAGAUUUX	1167-1187	A-108591.1	AAAUCUUUGUUUUUCCGGGAUUGC	1165-1187
AD-53107.1	A-108604.1	CUACUUUGGGAUCACAAAAGCAAX	1196-1216	A-108605.1	UUGCUUUUGUGAUCCCAAGUAGAA	1194-1216
AD-53121.1	A-108640.1	UGAAUGAACUCAGGCAAAUUUX	1428-1448	A-108641.1	AAAAUUUGCCUCAGUUCAUUCAAA	1426-1448
AD-53046.1	A-108478.1	AGCAUAGUCAAAUAAAAAGAAAX	626-646	A-108479.1	UUUCUUUUUAUUUGACUAGUCUGU	624-646
AD-53038.1	A-108444.1	AUUUAGAAAGAGCAACUAAACUAX	470-490	A-108445.1	UAGUUAGUUGCUCUUAUAAAUU	468-490
AD-53140.1	A-108662.1	AGGCAAAUUUAAAAAGGCAAUAX	1439-1459	A-108663.1	UAUUGCCUUUUUAAAAUUUGCCUCA	1437-1459
AD-52987.1	A-108380.1	CAUAUUUGAUCAGUCUUUUUUUAX	279-299	A-108381.1	UAAAAAGACUGAUCAAAUAUUGUU	277-299
AD-53130.1	A-108596.1	AAAACAAAGAUUUUGGUGUUUUUX	1175-1195	A-108597.1	AAAAACCAAAUCUUUUGUUUUUCC	1173-1195
AD-53106.1	A-108588.1	CAAUCCCGGAAACAAAGAUUUX	1166-1186	A-108589.1	AAUCUUUGUUUUUCCGGGAUUGCA	1164-1186
AD-53081.1	A-108564.1	CAACAAACAUUAUAUUUGAAUAX	1071-1091	A-108565.1	UAUUCAAUAUAUUGUUUUUGUUGUC	1069-1091
AD-53118.1	A-108592.1	GGAAAAACAAAGAUUUUGGUGUUX	1173-1193	A-108593.1	AACACCAAAUCUUUUGUUUUUCCGG	1171-1193
AD-53136.1	A-108598.1	ACAAAGAUUUUGGUGUUUUUCUAX	1178-1198	A-108599.1	UAGAAAAACACCAAAUCUUUUGUUU	1176-1198

AD-53127.1	A-108642.1	GAUAAACUGAGGCAAAUUUAX	1429-1449	A-108643.1	UAAUUUGCCUCAGUUCAUUCAA	1427-1449
AD-53066.1	A-108512.1	CCAUGGACAUUAAUUCAACAUX	892-912	A-108513.1	AUGUUGAAUUUAAUGUCCAUUGGAC	890-912
AD-53013.1	A-108420.1	AACUCAACUCACAAACUUGAAAX	407-427	A-108421.1	UUUCAAGUUUUUGAGUUAGUUCA	405-427
AD-52991.1	A-108350.1	CAGUUGGGACAUGGUCUUAAAX	205-225	A-108351.1	UUUAAGACCAUGUCCCAACUGAA	203-225
AD-53099.1	A-108570.1	AACAUUUAUUUGAAUAUUCUUX	1076-1096	A-108571.1	AAGAAUAUUCAAAUAUAUAGUUUG	1074-1096
AD-52958.1	A-108386.1	ACCAGUGAAAUCAAAAGAAAX	316-336	A-108387.1	UUCUUCUUUGAUUUACACUGGUUU	314-336
AD-53097.1	A-108538.1	GUUGGGCCUAGAGAGAAUAUAX	993-1013	A-108539.1	UAUAUCUUUCUCUAGGCCCAACCA	991-1013
AD-52966.1	A-108326.1	CUCCAGAGCCAAAUUCAAGAUx	140-160	A-108327.1	AUCUUGAUUUUUGGCUCUGGAGAU	138-160
AD-53145.1	A-108664.1	GGCAAUUUUAAAAGGCAAUAX	1440-1460	A-108665.1	UUUUUGCCUUUUUAAUUUUGCCUC	1438-1460
AD-53113.1	A-108606.1	UACUUGGGAUCACAAAGCAAAx	1197-1217	A-108607.1	UUUGCUUUUGUGAUCCCAAGUAGA	1195-1217
AD-52993.1	A-108382.1	GAUCAGUCUUUUUAUGAUUAX	286-306	A-108383.1	UAGAUCAUAAAAGACUGAUCAA	284-306
AD-53031.1	A-108426.1	GAAAGCCUCCUAGAGAAAAAX	424-444	A-108427.1	UUUUUCUUUCUAGGAGGCUUUCAA	422-444
AD-53017.1	A-108484.1	AGUCAAAUAAAAGAAAAUAGAAX	631-651	A-108485.1	UUCUAAUUUCUUUUUAUUUGACUUAU	629-651
AD-53143.1	A-108632.1	AUACUCUAAAAAUCAACCAAX	1374-1394	A-108633.1	UUGGUUGAUUUUUUAUAGAGUAUAA	1372-1394
AD-53149.1	A-108650.1	GAACUGAGGCAAAUUUAAAAAX	1433-1453	A-108651.1	UUUUUAAAAUUUGCCUCAGUUCAU	1431-1453_G21A
AD-53059.1	A-108494.1	AGACCCAGCAACUCUCAAGUUX	838-858	A-108495.1	AACUUGAGAGUUGCUGGGUCUGA	836-858
AD-53006.1	A-108402.1	AUAUAAACUACAAGUCAAAAAAX	360-380	A-108403.1	UUUUUGACUUGUAGUUUAUAUUGU	358-380
AD-53025.1	A-108424.1	UGAAAGCCUCCUAGAGAAAAAX	423-443	A-108425.1	UUUUUCUUUCUAGGAGGCUUUCACAG	421-443
AD-53085.1	A-108534.1	GGGAGAAACUACAAAAUAGGUUX	947-967	A-108535.1	AACCAUAAUUUGUAGUUCUCCAC	945-967
AD-52984.1	A-108332.1	AGAUUUGCUAUGUUAAGACGAUX	157-177	A-108333.1	AUCGUCUAAACAUAAGCAAAUUCUUG	155-177
AD-53023.1	A-108486.1	GAACCCACAGAAAAUUUCUCUAX	679-699	A-108487.1	UAGAGAAAUUUCUGGGGUUCUU	677-699

AD-53014.1	A-108436.1	ACUUAACAAAAAGUGAAUAx	450-470	A-108437.1	UAUUUCACUUUUUGUAGAAGUAG	448-470
AD-53060.1	A-108510.1	AGUCCAUGGACAUUAAUUCAAx	889-909	A-108511.1	UUGAAUUAAUGUCCAUGGACUAC	887-909
AD-53110.1	A-10852.1	AACUGAGGCAAAUUUAAAAGAx	1434-1454	A-108653.1	UCUUUUAAAAUUUGCCUCAGUUA	1432-1454_G21A
AD-52980.1	A-108362.1	GGCCAAAUAUAGACAUUAUx	246-266	A-108363.1	AAUAUGUCAUUAUUUUGGCCUU	244-266
AD-53109.1	A-108636.1	AUCCAUCCAACAGAUUCAGAAx	1402-1422	A-108637.1	UUCUGAAUCUGUUGGAUGGAUCA	1400-1422
AD-53141.1	A-108600.1	AAGAUUUGGUGUUUUCUUAUx	1181-1201	A-108601.1	AAGUAGAAAACACACCAAAUCUUUG	1179-1201
AD-53126.1	A-108626.1	GUCUAAAAUGGAAGGUUAUAx	1356-1376	A-108627.1	UAUAACCUUCCAUUUUUGAGACUU	1354-1376
AD-53116.1	A-108654.1	ACUGAGGCAAAUUUAAAAGGAX	1435-1455	A-108655.1	UCCUUUUAAAAUUUGCCUCAGUUC	1433-1455_C21A
AD-52997.1	A-108352.1	GGGACAUUGGUCUUAAGACUUAx	210-230	A-108353.1	AAGUCUUUAAGACCAUGUCCCAA	208-230
AD-53120.1	A-108624.1	AUGGUAAAUAUACAAAACCAAx	1292-1312	A-108625.1	UUGGUUUUGUUAUAUUUACCAUUU	1290-1312
AD-53070.1	A-108576.1	GGGAAUACACGAAACCAACUAx	1104-1124	A-108577.1	UAGUUGGUUUCGUGAUUUUCCCAA	1102-1124
AD-53028.1	A-108472.1	CCAACAGCAUAGUCAAUAUAAx	621-641	A-108473.1	UUUAUUUGACUAUGCUGUUGGUU	619-641
AD-53146.1	A-108602.1	UUUUCUACUUGGGAUCACAAAx	1192-1212	A-108603.1	UUUGUAUCCCAAGUAGAAAAACA	1190-1212
AD-52982.1	A-108394.1	AGAAUAUAUAUAACUACAAX	352-372	A-108395.1	UUGUAGUUUAUAUGUAGUUCUUC	350-372
AD-53111.1	A-108668.1	AGAGUAUGUGUAAAAAUCUGUx	1915-1935	A-108669.1	ACAGAUUUUUUACACAUACUCUGU	1913-1935
AD-53045.1	A-108462.1	AAAACAAGAUAAUAGCAUCAAx	558-578	A-108463.1	UUUGAUGCUAAUAUCUUGUUIUUUC	556-578
AD-53123.1	A-108672.1	AGUAUGUGUAAAAAUCUGUAx	1917-1937	A-108673.1	UUACAGAUUUUUUACACAUACUCU	1915-1937
AD-53018.1	A-108406.1	AGUCAAAAUAAGAGGUAAAx	372-392	A-108407.1	UUUACCCUUCUAAUUUUUGACUUG	370-392
AD-52956.1	A-108354.1	GGACAUUGGUCUUAAAAGACUUUx	211-231	A-108355.1	AAAGUCUUUAAAGACCAUGUCCCA	209-231
AD-53134.1	A-108660.1	GAGGCAAAUUUAAAAGGCAUAx	1438-1458	A-108661.1	AUUGCCUUUUAAAAUUUGCCUCAG	1436-1458
AD-52968.1	A-108358.1	GUCUUAAGACUUAUUGUCCAUAx	218-238	A-108359.1	UAUGGACAAAGUCUUUAAAGACCA	216-238

AD-53122.1	A-108656.1	CUGAGGCAAAUUUUAAAAGGCAX	1436-1456	A-108657.1	UGCCUUUUAAAAUUUGCCUCAGUU	1434-1456
AD-53100.1	A-108586.1	GCAAUCCCGGAAACAAAGAUX	1165-1185	A-108587.1	AUCUUUGUUUUUCCGGGAUUGCAU	1163-1185
AD-53128.1	A-108658.1	UGAGGCAAAUUUUAAAAGGCAAX	1437-1457	A-108659.1	UUGCCUUUUAAAAUUUGCCUCAGU	1435-1457
AD-53043.1	A-108430.1	UCUACUUCACAAAAAGUGAAX	447-467	A-108431.1	UUCACUUUUUGUUUGAAGUAGAAU	445-467
AD-53135.1	A-108676.1	UAUGUGUAAAAAUCUGUAAUAX	1919-1939	A-108677.1	UAUUACAGAUUUUUUACACAUACU	1917-1939
AD-53094.1	A-108584.1	AAUGCAAUCCCGGAAAAACAAAX	1162-1182	A-108585.1	UUUGUUUUUCCGGGAUUUGCAUUGG	1160-1182
AD-53019.1	A-108422.1	CUUGAAAAGCCUCCUAGAAGAAX	421-441	A-108423.1	UUCUUCUJAGGAGGCUUUCAGUU	419-441
AD-53129.1	A-108674.1	GUAUGUGUAAAAAUCUGUAAUX	1918-1938	A-108675.1	AUUACAGAUUUUUUACACAUACUC	1916-1938
AD-53150.1	A-108666.1	CAGAGUAUGUGUAAAAAUCUUX	1914-1934	A-108667.1	AAGAUUUUUUACACAUACUCUGUG	1912-1934_G21U
AD-53117.1	A-108670.1	GAGUAUGUGUAAAAAUCUGUAX	1916-1936	A-108671.1	UACAGAUUUUUUACACAUACUCUG	1914-1936
AD-52985.1	A-108348.1	UCAGUUUGGGACAUUGGUCUUAAX	204-224	A-108349.1	UUAAGACCAUGUCCCAACUGAAG	202-224
AD-52962.1	A-108356.1	GGUCUUAAAAGACUUUUGUCCAUX	217-237	A-108357.1	AUGGACAAAAGUCUUUAAAGACCAU	215-237
AD-52974.1	A-108360.1	UCUUAAAAGACUUUUGUCCAUAAX	219-239	A-108361.1	UUUUGGACAAAAGUCUUUAAAGACC	217-239
AD-52979.1	A-108346.1	UUCAGUUUGGGACAUUGGUCUUAAX	203-223	A-108347.1	UAAGACCAUGUCCCAACUGAAGG	201-223

The symbol “x” indicates that the sequence contains a GalNAc conjugate.

Table 8. Modified sense and antisense strand sequences of ANGPTL3 GalNac-conjugated dsRNAs

Duplex ID	Sense OligoName	Sense Sequence	Antisense OligoName	Antisense Sequence
AD-53063.1	A-108558.1	AfaAfgAfcAfaCfaAfaCfaUfaUfaUfl96	A-108559.1	aAfuAfuAfaUfgUfuUfuGfuCfuUfusCfsc
AD-52965.1	A-108310.1	AfcAfaUfaAfaGfcUfcCfuUfcUfuUfuUfl96	A-108311.1	aAfaAfaGfaAfgGfagcUfuAfaUfuGfusGfsa
AD-53030.1	A-108410.1	UfgUfcAfcUfuGfaAfaUfcAfaCfuCfaAfl96	A-108411.1	uUfgAfgUfuGfaGfuucAfaGfuGfaCfasUfsa
AD-52953.1	A-108306.1	UfcAfcAfaUfaAfaGfcUfcCfuUfcUfuUfl96	A-108307.1	aAfaGfaAfgGfaGfcuuAfaUfuGfuGfasAfcsc
AD-53001.1	A-108416.1	CfuUfgAfaCfuCfaAfaUfcAfaCfaCfuUfl96	A-108417.1	aAfgUfuUfuGfaGfuugAfgUfuCfaAfgsUfsg
AD-53080.1	A-108548.1	CfuCfaUfaUfgUfgAfaGfcAfaUfcUfaAfl96	A-108549.1	uUfaGfaUfuGfcUfucaCfuAfuGfgAfgsUfsa
AD-52971.1	A-108312.1	CfaAfuUfaAfgCfuCfcUfuCfuUfuUfaAfl96	A-108313.1	uAfaAfaAfgAfaGfgagCfuUfaAfuUfgsUfsg
AD-53071.1	A-108498.1	AfcCfcAfgCfaAfcUfcUfcAfaGfuUfuUfl96	A-108499.1	aAfaAfcUfuGfaGfaguUfgCfuGfgGfusCfsu
AD-53024.1	A-108408.1	GfaAfuAfuGfuCfaCfuUfgAfaCfuCfaAfl96	A-108409.1	uUfgAfgUfuCfaAfgugAfcAfuAfuUfcsUfsu
AD-52977.1	A-108314.1	AfaUfuAfaGfcUfcCfuUfcUfuUfaUfl96	A-108315.1	aUfaAfaAfaGfaAfggaGfcUfuAfaUfusGfsu
AD-53064.1	A-108574.1	CfaUfuAfuAfuUfgAfaUfaUfuCfuUfuUfl96	A-108575.1	aAfaAfgAfaUfaUfucaAfuAfaUfagsUfsu
AD-53033.1	A-108458.1	AfcUfaAfcUfaAfcUfuAfaUfuCfaAfaAfl96	A-108459.1	uUfuUfgAfaUfaAfaUfuGfuUfaGfusUfsg
AD-52954.1	A-108322.1	UfuAfuUfgUfuCfcUfcUfaGfuUfaUfuUfl96	A-108323.1	aAfaUfaAfcUfaGfaggAfaCfaAfaAfasAfsa
AD-53098.1	A-108554.1	CfaUfaGfuGfaAfgCfaAfuCfuAfaUfaUfl96	A-108555.1	uAfaUfuAfgAfuUfgcuUfcAfcUfaUfsgGfsa
AD-53092.1	A-108552.1	CfcAfuAfgUfgAfaGfcAfaUfcUfaAfuUfl96	A-108553.1	aAfuUfaGfaUfuGfcuuCfaCfuAfuGfsgAfg
AD-53073.1	A-108530.1	GfaUfcAfaAfaAfaCfuUfcAfaUfgAfaAfl96	A-108531.1	uUfuCfaUfuGfaAfguuUfuGfuGfaUfcsCfsa
AD-53132.1	A-108628.1	AfuGfgAfaGfgUfuAfaUfcUfuUfaUfaAfl96	A-108629.1	uUfaUfaGfaGfuAfuuaaCfcUfuCfcAfusUfsu

AD-53086.1	A-108550.1	UfcCfaUfaGfuGfAfaGfCfaAfuCfuAfaUfl96	A-108551.1	aUfuAfgAfuUfgCfuucAfcUfaUfgGfasGfsu
AD-52961.1	A-108340.1	CfuAfuGfuUfaGfAfcGfAfuGfuAfaAfaAfl96	A-108341.1	uUfuUfuAfcAfuCfugucUfaAfaAfgsCfsa
AD-52983.1	A-108316.1	AfuUfaAfgCfuCfcUfuCfuUfuUfuAfuUfl96	A-108317.1	aAfuAfaAfaAfgAfggAfgCfuUfaAfuUfsg
AD-53027.1	A-108456.1	AfaCfuAfaCfuAfaCfuUfaAfuUfcAfaAfl96	A-108457.1	uUfuGfaAfuUfaAfguuAfgUfuAfgUfusGfsc
AD-52986.1	A-108364.1	GfgCfcAfaAfuUfaAfaGfaCfaUfaUfuUfl96	A-108365.1	aAfaUfaUfgUfcAfuuaAfuUfuGfgCfcsCfsu
AD-52989.1	A-108318.1	UfuUfuAfuUfgUfuCfcUfcUfaGfuUfaUfl96	A-108319.1	aUfaAfcUfaGfaGfgaaCfaAfuAfaAfasAfg
AD-52981.1	A-108378.1	AfcAfuAfuUfuGfaUfaAfgUfcUfuUfuUfl96	A-108379.1	aAfaAfaGfaCfuGfaucAfaAfuAfuGfusUfsg
AD-53077.1	A-108500.1	CfcCfaGfcAfaCfuCfuCfaAfgUfuUfuUfl96	A-108501.1	aAfaAfaCfuUfgAfgagUfuGfcUfgGfgsUfsc
AD-53095.1	A-108506.1	CfaGfgUfaGfuCfcAfaGfgAfcAfuUfaAfl96	A-108507.1	uUfaAfuGfuCfcAfuaggAfcUfaCfcUfgsAfsu
AD-52970.1	A-108390.1	AfcUfgAfgAfaGfaAfcUfaCfaUfaUfaAfl96	A-108391.1	uUfaUfaUfgUfaGfuucUfuCfuCfaGfusUfsc
AD-53015.1	A-108452.1	GfaGfcAfaCfuAfaCfuAfaCfuUfaAfuUfl96	A-108453.1	aAfuUfaAfgUfuAfguuAfgUfuGfcUfcsUfsu
AD-53147.1	A-108618.1	AfaCfaAfcCfuAfaAfaGfgUfaAfaUfaUfl96	A-108619.1	aUfaUfuUfaCfcAfuuuAfgGfuUfgUfusUfsu
AD-53103.1	A-108540.1	CfcUfaGfaGfaAfgAfuAfuAfcUfcCfaUfl96	A-108541.1	aUfgGfaGfuAfuAfuUfcUfcUfaGfgsCfsc
AD-52969.1	A-108374.1	CfaAfcAfuAfuUfuUfgGfaUfcAfgUfcUfuUfl96	A-108375.1	aAfaGfaCfuGfaUfcaaAfuAfuGfuUfgsAfsu
AD-53075.1	A-108562.1	AfcAfaCfaAfaCfuAfuAfuUfgAfaUfl96	A-108563.1	aUfuCfaAfuAfuAfaugUfuUfgUfuGfusCfsu
AD-52994.1	A-108398.1	AfcAfuAfuAfaAfcUfuAfaCfaAfgUfcAfaAfl96	A-108399.1	uUfuGfaCfuUfgUfaguUfuAfuAfuGfusAfsu
AD-52960.1	A-108324.1	CfuAfgUfuAfuUfuUfcUfcUfcCfaGfaAfuUfl96	A-108325.1	aAfuUfcUfgGfaGfgaaAfuAfaCfuAfgsAfsu
AD-53003.1	A-108448.1	AfaGfaGfcAfaCfuAfaCfuAfaCfuUfaAfl96	A-108449.1	uUfaAfgUfuAfgUfuagUfuGfcUfcUfusCfsu
AD-52995.1	A-108320.1	UfuUfaUfuGfuUfcCfuCfuAfgUfuAfuUfl96	A-108321.1	aAfuAfaCfuAfgAfggAfaAfaUfaAfasAfsa
AD-53037.1	A-108428.1	CfuCfcUfaGfaAfgAfaAfaAfuCfuAfl96	A-108429.1	uAfgAfaUfuUfuUfuUfcUfaGfgAfgsGfsc
AD-53087.1	A-108566.1	AfaCfaAfaCfaUfuAfuAfuUfgAfaUfaUfl96	A-108567.1	aUfaUfuCfaAfaAfuAfuUfgUfuUfgUfusGfsu

AD-53076.1	A-108578.1	GfgAfaAfuCfaCfGfAfaAfcCfaAfcUfaUfl96	A-108579.1	aUfaGfuUfgGfuUfucgUfgAfuUfuCfcsCfsa
AD-52975.1	A-108376.1	AfaCfaUfaUfuUfgAfuCfaGfuCfuUfuUfl96	A-108377.1	aAfaAfgAfcUfgAfuAfaUfaUfgUfusGfsa
AD-53138.1	A-108630.1	UfgGfaAfgGfuUfaUfaCfuCfuAfaAfl96	A-108631.1	uUfuAfuAfgAfgUfaUfaAfcCfuUfcCfasUfsu
AD-53091.1	A-108536.1	GfgAfgAfaCfuAfcAfaAfuAfuGfuUfuUfl96	A-108537.1	aAfaCfcAfuAfuUfuguAfgUfuCfuCfcsCfsa
AD-53124.1	A-108594.1	GfaAfaAfcAfaAfgAfuUfuGfgUfgUfuUfl96	A-108595.1	aAfaCfaCfcAfaAfcuUfuGfuUfuUfcsCfsg
AD-53125.1	A-108610.1	AfgUfgUfgGfaGfaAfaAfcAfaCfcUfaAfl96	A-108611.1	uUfaGfgUfuGfuUfuUfuUfcCfaCfaCfusCfsa
AD-53036.1	A-108412.1	GfuCfaCfuUfgAfaCfuCfaAfcUfaAfl96	A-108413.1	uUfuGfaGfuUfgAfguuCfaAfgUfgAfcAfsu
AD-53061.1	A-108526.1	GfaUfgGfaUfcAfcAfaAfaCfuUfaAfl96	A-108527.1	aUfuGfaAfgUfuUfuguGfaUfcCfaUfcsUfsa
AD-53093.1	A-108568.1	AfcAfaAfcAfuUfaUfaUfuGfaAfuUfl96	A-108569.1	aAfuAfuUfcAfaUfaUfaAfuUfuGfusUfsg
AD-53137.1	A-108614.1	UfgUfgGfaGfaAfaAfcAfaCfcUfaAfl96	A-108615.1	aUfuUfaGfgUfuGfuUfuUfcCfaCfasCfsu
AD-52999.1	A-108384.1	AfuCfaGfuCfuUfuUfuAfuGfaUfcUfaUfl96	A-108385.1	aUfaGfaUfcAfuAfaAfaAfgAfcUfgAfcUfsa
AD-53069.1	A-108560.1	GfaCfaAfcAfaAfcAfuUfaUfuGfaAfl96	A-108561.1	uUfcAfaUfaUfaAfuUfuGfuUfgUfcsUfsu
AD-53034.1	A-108474.1	CfaAfcAfgCfaUfaGfuCfaAfaUfaAfaAfl96	A-108475.1	uUfuUfaUfuUfgAfcuaUfgCfuGfuUfsgGfsu
AD-52976.1	A-108392.1	CfuGfaGfaAfgAfaCfuAfcAfuAfaAfl96	A-108393.1	uUfuAfuAfuGfuAfguuCfuUfcUfcAfgsUfsu
AD-52996.1	A-108336.1	UfgCfuAfuGfuUfaAfgCfaCfuGfuAfaAfl96	A-108337.1	uUfuAfcAfuCfGufcuAfcAfuAfgCfasAfsa
AD-53029.1	A-108488.1	AfaCfcCfaCfaGfaAfaUfuUfcUfcUfaUfl96	A-108489.1	aUfaGfaGfaAfaUfuUfcUfgGfgUfusCfsu
AD-53020.1	A-108438.1	CfuUfcAfaCfaAfaAfaGfuGfaAfaUfaUfl96	A-108439.1	aUfaUfuUfcAfcUfuUfuUfgUfuGfaAfgsUfsa
AD-53042.1	A-108414.1	UfcAfcUfuGfaAfcUfcAfaCfuCfaAfaAfl96	A-108415.1	uUfuUfgAfgUfuGfaguUfcAfaGfuGfasCfsa
AD-53011.1	A-108482.1	CfaUfaGfuCfaAfaUfaAfaAfgAfaAfl96	A-108483.1	uAfuUfuCfuUfuUfaUfuUfgAfcUfaUfsgCfsu
AD-52957.1	A-108370.1	CfaAfaAfaCfuCfaAfaCfuAfuUfuGfaUfl96	A-108371.1	aUfcAfaAfaAfuGfuUfgAfuUfuUfsgAfsa
AD-53008.1	A-108434.1	UfaCfuUfcAfaCfaAfaAfaGfuGfaAfaUfl96	A-108435.1	aUfuUfcAfcUfuUfuUfuUfgAfaAfgUfasGfsa

AD-53065.1	A-108496.1	GfaCfcCfaGfcAfaCfuCfuCfaAfgUfuUfl96	A-108497.1	aAfaCfuUfgAfgAfguuGfcUfgGfgUfcsUfsg
AD-53115.1	A-108638.1	UfuGfaAfuGfaAfcUfgAfgGfcAfaAfuUfl96	A-108639.1	aAfuUfuGfcCfuCfaguUfcAfuUfcAfasAfg
AD-53012.1	A-108404.1	UfaUfaAfaCfuAfcAfaGfuCfaAfaAfuUfl96	A-108405.1	aUfuUfuUfgAfcUfuguAfgUfuUfaUfasUfsg
AD-53004.1	A-108464.1	AfaAfcAfaGfaUfaAfaUfgCfaUfcAfaAfl96	A-108465.1	uUfuGfaUfgCfuAfuuaUfcUfuGfuUfusUfsu
AD-53021.1	A-108454.1	CfaAfcUfaAfcUfaAfcUfuAfaUfuCfaAfl96	A-108455.1	uUfgAfaUfaAfaGfuuaGfuUfaGfuUfgsCfsu
AD-52955.1	A-108338.1	GfcUfaUfgUfuAfgAfcGfaUfgUfaAfaAfl96	A-108339.1	uUfuUfaCfaUfcGfucuAfaCfaUfaGfcsAfsa
AD-53119.1	A-108608.1	AfcUfuGfgGfaUfcAfcAfaAfgCfaAfaAfl96	A-108609.1	uUfuUfgCfuUfuGfugaUfcCfcAfaGfusAfg
AD-52990.1	A-108334.1	UfuGfcUfaUfgUfuUfaAfgAfcGfaUfgUfaAfl96	A-108335.1	uUfaCfaUfcGfuCfuuaCfaUfaGfcAfasAfsu
AD-52964.1	A-108388.1	AfaCfuGfaGfaAfgAfcAfaCfuAfaAfuAfl96	A-108389.1	uAfuAfuGfuAfgUfucuUfcUfcAfgUfusCfsc
AD-52973.1	A-108344.1	GfaUfgUfaAfaAfaUfuUfuAfgCfcAfaUfl96	A-108345.1	aUfuGfgCfuAfaAfaUfuUfaCfaUfcsGfsu
AD-53074.1	A-108546.1	AfcUfcCfaUfaGfuGfaAfgCfaAfuCfuAfl96	A-108547.1	uAfgAfuUfgCfuUfcacUfaUfgGfaGfusAfsu
AD-53026.1	A-108440.1	UfuCfaAfcAfaAfaAfgUfgAfaAfuAfuUfl96	A-108441.1	aAfuAfuUfuCfaCfuuuUfuGfuUfgAfasGfsu
AD-53062.1	A-108542.1	CfuAfgAfgAfaGfaUfaUfaCfuCfcAfuAfl96	A-108543.1	uAfuGfgAfgUfaUfaucUfuCfuAfgsGfsc
AD-53114.1	A-108622.1	CfaAfcCfuAfaAfuUfgUfaAfaUfaAfl96	A-108623.1	uUfaUfaUfuUfaCfcuUfuAfgGfuUfgsUfsu
AD-53082.1	A-108580.1	GfaAfaUfcAfcGfaAfaCfcAfaCfuAfuAfl96	A-108581.1	uAfuAfgUfuGfgUfuucGfuGfaUfuUfcsCfsc
AD-53035.1	A-108490.1	CfcAfcAfgAfaAfuUfuCfuCfuAfuCfuUfl96	A-108491.1	aAfgAfuAfgAfgAfaauUfuCfuGfuGfgsGfsu
AD-52978.1	A-108330.1	AfaAfuCfaAfgAfuUfuGfcUfaUfgUfuAfl96	A-108331.1	uAfaCfaUfaGfcAfaauCfuUfgAfuUfusUfsg
AD-53084.1	A-108518.1	AfcAfuUfaAfuUfcAfaCfaUfcGfaAfuAfl96	A-108519.1	uAfuUfcGfaUfgUfugaAfuUfaAfuGfusCfsc
AD-52972.1	A-108328.1	CfcAfgAfgCfcAfaAfaUfcAfaGfaUfuUfl96	A-108329.1	aAfaUfcUfuGfaUfuuuGfgCfuCfuGfgsAfg
AD-53002.1	A-108432.1	CfuAfcUfuCfaAfcAfaAfaAfgUfgAfaAfl96	A-108433.1	uUfuCfaCfuUfuUfuguUfgAfaGfuAfgsAfsa
AD-53078.1	A-108516.1	GfaCfaUfuAfaUfuUfcAfaAfuCfaAfaUfl96	A-108517.1	aUfuCfgAfuGfuUfgaaUfuAfaUfgUfcsCfsa

AD-53072.1	A-108514.1	GfgAfcAfuUfaAfuUfcAfaCfaUfcGfaAfl96	A-108515.1	uUfcGfaUfgUfuGfaauUfaAfuGfuCfcsAfsu
AD-53005.1	A-108480.1	GfcAfuAfgUfcAfaAfuAfaAfaGfaAfaUfl96	A-108481.1	aUfuUfcUfuUfuAfuuuGfaCfuAfuGfcsUfsg
AD-53083.1	A-108502.1	CfuCfuCfaAfgUfuUfuUfcAfuGfuCfuAfl96	A-108503.1	uAfgAfcAfuGfaAfaaaCfuUfgAfgAfgsUfsu
AD-53102.1	A-108524.1	AfuCfGfaUfaGfaUfgGfaUfcAfcAfaAfl96	A-108525.1	uUfuGfuGfaUfcCfaucUfaUfuCfGfaUfsGfsu
AD-53105.1	A-108572.1	AfcAfuUfaUfaUfuGfaAfuAfuUfcUfuUfl96	A-108573.1	aAfaGfaAfuAfuUfcaaUfaUfaAfuGfusUfsu
AD-53090.1	A-108520.1	UfuAfaUfuCfaAfcAfuCfGfaUfaUfaGfaUfl96	A-108521.1	aUfcUfaUfuCfGfaUfuUfgAfaUfuAfasUfsg
AD-53010.1	A-108466.1	GfaUfaAfuAfgCfaUfcAfaAfaAfgAfcCfuUfl96	A-108467.1	aAfgGfuCfuUfuGfaugCfuAfuUfaUfcsUfsu
AD-52998.1	A-108368.1	UfgAfcAfuAfuUfuCfaAfaAfaCfuCfaAfl96	A-108369.1	uUfgAfgUfuUfuUfgeaaAfuAfuGfuCfasUfsu
AD-52992.1	A-108366.1	AfaAfuUfaAfuGfaAfcUfaUfuUfcAfaAfl96	A-108367.1	uUfuGfaAfaUfaUfgucAfuUfaAfuUfusGfsg
AD-53068.1	A-108544.1	GfaAfgAfuAfuAfcUfcCfaUfaGfuGfaAfl96	A-108545.1	uUfcAfcUfaUfgGfaguAfuAfuCfuUfcsUfsc
AD-53032.1	A-108442.1	AfaUfaUfuUfaGfaAfaAfgAfcAfaUfaAfl96	A-108443.1	uUfaGfuUfgCfuCfuucUfaAfaUfaUfusUfsc
AD-52967.1	A-108342.1	CfgAfuGfuAfaAfaAfuUfuUfaGfcCfaAfl96	A-108343.1	uUfgGfcUfaAfaAfuuuUfuAfcAfuCfcsUfsc
AD-53096.1	A-108522.1	UfuCfaAfcAfuCfGfaAfaUfaGfaUfgGfaUfl96	A-108523.1	aUfcCfaUfcUfaUfugAfuGfuUfgAfasUfsu
AD-53131.1	A-108612.1	GfuGfuGfgAfgAfaAfaCfaAfcCfuAfaAfl96	A-108613.1	uUfuAfgGfuUfgUfuuuCfuCfcAfcAfcUfsc
AD-52963.1	A-108372.1	UfcAfaCfaUfaUfuUfgAfuCfaGfuCfuUfl96	A-108373.1	aAfgAfcUfgAfuCfaaaUfaUfgUfuGfasGfsu
AD-53089.1	A-108504.1	UfcAfgGfuAfgUfcCfaUfgGfaCfaUfaAfl96	A-108505.1	uAfaUfgUfcCfaUfggaCfuAfcCfuGfasUfsc
AD-53044.1	A-108446.1	UfuUfaGfaAfgAfcCfaAfcUfaAfcUfaAfl96	A-108447.1	uUfaGfuUfaGfuUfgcuCfuUfcUfaAfasUfsc
AD-52988.1	A-108396.1	UfaCfaUfaUfaAfaAfcUfaAfaGfuCfaAfl96	A-108397.1	uUfgAfcUfuGfuAfguuUfaUfaUfgUfasGfsu
AD-53067.1	A-108528.1	GfgAfuCfaCfaAfaAfcUfuCfaAfuGfaAfl96	A-108529.1	uUfcAfuUfgAfaGfuuuUfgUfgAfuCfcsAfsu
AD-53009.1	A-108450.1	AfgAfgCfaAfcUfaAfcUfaAfcUfuAfaUfl96	A-108451.1	aUfuAfaGfuUfaGfuuaGfuUfgCfuCfusUfsc
AD-53022.1	A-108470.1	AfcCfaAfcAfgCfaUfaGfuCfaAfaUfaAfl96	A-108471.1	uUfaUfuUfgAfcUfaugCfuGfuUfgGfusUfsu

AD-53016.1	A-108468.1	AfaCfaCfaCfaGfCfaAfaUfgUfcAfaAfaAfl96	A-108469.1	uAfuUfuGfaCfuAfuGfcUfgUfuGfgUfusUfsa
AD-53007.1	A-108418.1	GfaAfcUfcAfaCfuUfcAfaAfaAfcUfuGfaAfl96	A-108419.1	uUfcAfaGfuUfuUfgagUfuGfaGfuUfcsAfsa
AD-53148.1	A-108634.1	UfaCfuCfuAfuAfaAfaUfcAfaCfaAfaAfl96	A-108635.1	uUfuGfgUfuGfaUfuuuAfuAfgAfgUfasUfsa
AD-53040.1	A-108476.1	CfaGfaAfuAfgUfcAfaAfaAfaAfaGfaAfl96	A-108477.1	uUfcUfuUfuAfuUfugaCfuAfuGfcUfgsUfsu
AD-53041.1	A-108492.1	GfaAfaUfaAfgAfaAfaUfgUfaAfaCfaAfl96	A-108493.1	aUfgUfuUfuAfcAfuuuCfuUfaUfuUfcsAfsu
AD-53039.1	A-108460.1	CfuAfaCfuAfaCfuUfaAfuUfcAfaAfaAfl96	A-108461.1	aUfuUfuGfaAfuUfaagUfuAfgUfuAfgsUfsu
AD-53139.1	A-108646.1	AfuGfaAfcUfgAfgGfCfaAfaUfuAfaAfl96	A-108647.1	uUfuAfaAfuUfuGfcuCfaGfuUfcAfuUfsc
AD-53144.1	A-108648.1	UfgAfaCfuGfaGfCfaAfaUfuUfaAfaAfl96	A-108649.1	uUfuUfaAfaUfuUfgccUfcAfgUfuCfasUfsu
AD-53142.1	A-108616.1	AfaAfaCfcUfaAfaUfgGfuAfaAfaAfl96	A-108617.1	uAfuUfuAfcCfaUfuuaGfgUfuGfuUfusUfsc
AD-53108.1	A-108620.1	AfcAfaCfcUfaAfaUfgGfuAfaAfaAfl96	A-108621.1	uAfuAfuUfuAfcCfaUfuGfgUfuGfusUfsu
AD-53079.1	A-108532.1	AfaCfuUfgGfgAfgAfaCfuAfaAfaAfl96	A-108533.1	uAfuUfuGfuAfgUfucuCfcCfaCfgUfusUfsc
AD-53133.1	A-108644.1	AfaUfgAfaCfuGfaGfgCfaAfaUfuUfaAfl96	A-108645.1	uUfaAfaUfuUfgCfcuAfgUfuCfaUfusCfsa
AD-53104.1	A-108556.1	GfuUfgGfaAfgAfcUfgGfaAfaGfaCfaAfl96	A-108557.1	uUfgUfcUfuUfcCfaguCfuUfcCfaAfcUfsc
AD-53088.1	A-108582.1	UfgGfaAfaUfgUfcCfcCfaAfaGfaCfaAfl96	A-108583.1	aUfuGfcAfuUfgGfggaCfaUfuGfcCfasGfsu
AD-53101.1	A-108508.1	GfgUfaGfuCfcAfuUfgGfaAfaAfaUfuUfl96	A-108509.1	aAfuUfaAfuGfuCfcuGfgAfcUfaCfcsUfsg
AD-53000.1	A-108400.1	CfaUfaUfaCfuUfaAfaGfuCfaAfaAfl96	A-108401.1	uUfuUfgAfcUfuGfuagUfuUfaUfaUfgsUfsa
AD-53112.1	A-108590.1	AfaUfcCfcGfgAfaAfaCfaAfaGfaUfuUfl96	A-108591.1	aAfaUfcUfuUfgUfuuuCfcGfgGfaUfusGfsc
AD-53107.1	A-108604.1	CfuAfcUfuGfgGfaUfcAfaAfaAfgCfaAfl96	A-108605.1	uUfgCfuUfuGfuGfaucCfcAfaGfuAfgsAfsa
AD-53121.1	A-108640.1	UfgAfaUfgAfaCfuUfgGfaGfgCfaAfaUfuUfl96	A-108641.1	aAfaUfuUfgCfcUfcagUfuCfaUfuCfasAfsa
AD-53046.1	A-108478.1	AfgCfaUfaGfuCfaAfaUfaAfaAfgAfaAfl96	A-108479.1	uUfuCfuUfuUfaUfuugAfcUfaUfgCfusGfsu
AD-53038.1	A-108444.1	AfuUfuAfgAfaGfaGfCfaCfaCfaAfl96	A-108445.1	uAfgUfuAfgUfuGfcuUfuCfuAfaAfuAfsu

AD-53140.1	A-108662.1	AfgGfcAfaAfuUfuUfAfaAfaGfgCfaAfuAfl96	A-108663.1	uAfuUfgCfcUfuUfuuaAfuUfuGfcCfusCfsa
AD-52987.1	A-108380.1	CfaUfaUfuUfgAfuUfCfaGfcUfuUfuAfl96	A-108381.1	uAfaAfaAfgAfcUfgauCfaAfaUfaUfgsUfsu
AD-53130.1	A-108596.1	AfaAfaCfaAfaGfaAfuUfuUfgGfuGfuUfuUfl96	A-108597.1	aAfaAfcAfcCfaAfaucUfuUfgUfuUfusCfsc
AD-53106.1	A-108588.1	CfaAfuCfcGfgGfaAfaAfcAfaAfgAfuUfl96	A-108589.1	aAfuCfuUfuGfuUfuucCfgGfgAfuUfgsCfsa
AD-53081.1	A-108564.1	CfaAfcAfaAfcAfuUfaUfaUfuGfaAfuAfl96	A-108565.1	uAfuUfcAfaUfaUfaauGfuUfuGfuUfgsUfsc
AD-53118.1	A-108592.1	GfgAfaAfaCfaAfaGfaUfuUfgGfuGfuUfl96	A-108593.1	aAfcAfcCfaAfaUfcuuUfgUfuUfuCfcsGfsg
AD-53136.1	A-108598.1	AfcAfaAfgAfuUfuUfgUfgUfuUfuCfuAfl96	A-108599.1	uAfgAfaAfaCfaCfaaAfuCfuUfuGfusUfsu
AD-53127.1	A-108642.1	GfaAfuGfaAfcUfgAfgGfcAfaAfuUfuAfl96	A-108643.1	uAfaAfuUfuGfcCfucaGfuUfcAfuUfcsAfsa
AD-53066.1	A-108512.1	CfcAfuGfgAfcAfuUfaAfuUfcAfaCfaUfl96	A-108513.1	aUfgUfuGfaAfuUfaauGfuCfcAfuGfgsAfsc
AD-53013.1	A-108420.1	AfaCfuCfaAfcUfcAfaAfaCfuUfgAfaAfl96	A-108421.1	uUfuCfaAfgUfuUfugaGfuUfgAfgUfusCfsa
AD-52991.1	A-108350.1	CfaGfuUfgGfgAfcAfuGfgUfcUfuAfaAfl96	A-108351.1	uUfuAfaGfaCfaCfauguCfcCfaAfcUfgsAfsa
AD-53099.1	A-108570.1	AfaCfaUfuAfuAfuUfuUfgAfaUfaUfuCfuUfl96	A-108571.1	aAfgAfaUfaUfuCfaaAfuAfaUfgUfusUfsg
AD-52958.1	A-108386.1	AfcCfaGfuGfaAfaUfcAfaAfgAfaGfaAfl96	A-108387.1	uUfcUfuCfuUfuGfaauUfcAfcUfgGfusUfsu
AD-53097.1	A-108538.1	GfuUfgGfgCfcUfAfGfaGfaAfgAfuAfuAfl96	A-108539.1	uAfuAfuCfuUfcUfuaGfgCfcCfaAfcCfsa
AD-52966.1	A-108326.1	CfuCfcAfgAfgCfcAfaAfaUfcAfaGfaUfl96	A-108327.1	aUfcUfuGfaUfuUfuggCfuCfuGfgAfgsAfsu
AD-53145.1	A-108664.1	GfgCfaAfaUfuUfAfAfaAfgGfcAfaUfaAfl96	A-108665.1	uUfaUfuGfcCfuUfuuaAfaUfuUfgCfcsUfsc
AD-53113.1	A-108606.1	UfaCfuUfgGfgAfuUfcAfaAfaGfcAfaAfl96	A-108607.1	uUfuGfcUfuUfgUfgauCfcCfaAfgUfasGfsa
AD-52993.1	A-108382.1	GfaUfcAfgUfcUfuUfuUfaUfgAfuCfuAfl96	A-108383.1	uAfgAfuCfaUfaAfaaGfaCfuGfaUfcsAfsa
AD-53031.1	A-108426.1	GfaAfaGfcCfuCfcUfaGfaAfgAfaAfl96	A-108427.1	uUfuUfuCfuUfcUfaggAfgGfcUfuUfcsAfsa
AD-53017.1	A-108484.1	AfgUfcAfaAfuAfaAfaGfaAfaUfaGfaAfl96	A-108485.1	uUfcUfaUfuUfcUfuuuAfuUfuGfaCfusAfsu
AD-53143.1	A-108632.1	AfuAfcUfcUfaUfaAfaAfuCfaAfcCfaAfl96	A-108633.1	uUfgGfuUfgAfuUfuuaUfaGfaGfuAfusAfsa

AD-53149.1	A-108650.1	GfaAfcUfgAfgGfCfaAfaUfuAfaAfaAfl96	A-108651.1	uUfuUfuAfaAfaUfuUfgcCfuCfaGfuUfcsAfsu
AD-53059.1	A-108494.1	AfgAfcCfcAfgCfaAfaUfcUfcAfaGfuUfl96	A-108495.1	aAfcUfuGfaGfaGfuUfgGfuCfuGfuCfusGfsa
AD-53006.1	A-108402.1	AfuAfaAfaAfcUfaAfaAfgUfcAfaAfaAfl96	A-108403.1	uUfuUfuGfaCfuUfguaGfuUfuAfaAfsuGfsu
AD-53025.1	A-108424.1	UfgAfaAfgCfcUfcCfuAfgAfaGfaAfaAfl96	A-108425.1	uUfuUfcUfuCfuAfggaGfgCfuUfuCfasAfsfsg
AD-53085.1	A-108534.1	GfgGfaGfaAfcUfaAfaAfaUfaUfgGfuUfl96	A-108535.1	aAfcCfaUfaUfuUfguaGfuUfcUfcCfcsAfcsc
AD-52984.1	A-108332.1	AfgAfuUfuGfcUfaAfaAfaUfaUfgAfaAfaAfl96	A-108333.1	aUfcGfuCfuAfaAfaAfaGfaAfaAfuCfusUfsg
AD-53023.1	A-108486.1	GfaAfcCfcAfaAfaAfaAfaUfuCfuCfuAfl96	A-108487.1	uAfgAfgAfaAfaUfuUfuGfuGfgGfuUfcsUfsu
AD-53014.1	A-108436.1	AfcUfuCfaAfaAfaAfaAfaUfgAfaAfaAfl96	A-108437.1	uAfuUfuCfaCfuUfuuuGfuUfgAfaGfusAfsfsg
AD-53060.1	A-108510.1	AfgUfcCfaUfgGfaAfaUfuAfaUfuCfaAfl96	A-108511.1	uUfgAfaUfuAfaUfgucCfaUfgGfaCfusAfcsc
AD-53110.1	A-108652.1	AfaCfuGfaGfgCfaAfaUfuUfaAfaAfgAfl96	A-108653.1	uCfuUfuUfaAfaUfuugCfuUfcAfgUfusCfsa
AD-52980.1	A-108362.1	GfgGfcCfaAfaUfuAfaUfgAfaAfaUfuAfl96	A-108363.1	aAfuAfuGfuCfaUfuuaUfuUfgGfcCfcsUfsu
AD-53109.1	A-108636.1	AfuCfcAfuCfcAfaAfaGfaUfuCfaGfaAfl96	A-108637.1	uUfcUfgAfaUfcUfguuGfgAfuGfgAfuCfsa
AD-53141.1	A-108600.1	AfaGfaUfuUfgGfuUfgUfuUfcUfaCfuUfl96	A-108601.1	aAfgUfaGfaAfaAfaAfaAfaUfcUfusUfsg
AD-53126.1	A-108626.1	GfuCfuCfaAfaAfaUfgGfaAfaGfuUfuAfl96	A-108627.1	uAfuAfaCfcUfuCfaUfuUfgAfgAfcUfsu
AD-53116.1	A-108654.1	AfcUfgAfgGfcAfaAfaUfuAfaAfaGfgAfl96	A-108655.1	uCfcUfuUfuAfaAfaAfaAfaAfaGfusUfsc
AD-52997.1	A-108352.1	GfgGfaCfaUfgGfuUfcUfaAfaGfaCfuUfl96	A-108353.1	aAfgUfcUfuUfaAfgacCfaUfgUfcCfcsAfsa
AD-53120.1	A-108624.1	AfuGfgUfaAfaUfaAfaAfaAfaAfaAfl96	A-108625.1	uUfgGfuUfuGfuUfaAfaUfuUfaCfaAfuUfsu
AD-53070.1	A-108576.1	GfgGfaAfaUfcAfcGfaAfaCfaCfaCfuAfl96	A-108577.1	uAfgUfuGfgUfuUfcguGfaUfuUfcCfcsAfsa
AD-53028.1	A-108472.1	CfcAfaCfaGfcAfaUfaAfaAfaAfaAfl96	A-108473.1	uUfuAfuUfuGfaCfuauGfcUfgUfuGfgsUfsu
AD-53146.1	A-108602.1	UfuUfuCfuAfcUfuUfgGfaUfcAfaAfaAfl96	A-108603.1	uUfuGfuGfaUfcCfaaGfaAfaAfasCfsa
AD-52982.1	A-108394.1	AfgAfaCfuAfaAfaUfaAfaAfaAfaAfl96	A-108395.1	uUfgUfaGfuUfuAfaAfaAfaAfaAfuCfusUfsc

AD-53111.1	A-108668.1	AfgAfgUfaUfgUfgUfaAfaAfaUfcUfgUfl96	A-108669.1	aCfaGfaUfuUfuUfacaCfaUfaCfuCfusGfsu
AD-53045.1	A-108462.1	AfaAfaCfaAfgAfaUfaGfcAfuCfaAfl96	A-108463.1	uUfgAfuGfcUfaUfuauCfuUfgUfuUfusUfsc
AD-53123.1	A-108672.1	AfgUfaUfgUfgUfaAfaAfaUfcUfgUfaAfl96	A-108673.1	uUfaCfaGfaUfuUfuuaCfaCfaUfaCfusCfsu
AD-53018.1	A-108406.1	AfgUfaCfaAfaUfgAfaAfgAfgGfuAfaAfl96	A-108407.1	uUfaAfcCfuCfuUfcauUfuUfuGfaCfusUfsg
AD-52956.1	A-108354.1	GfgAfaCfuGfgUfcUfuAfaAfgAfcUfuUfl96	A-108355.1	aAfaGfuCfuUfuUfaAfaCfaCfuGfuCfcsCfsa
AD-53134.1	A-108660.1	GfaGfgCfaAfaUfuUfaAfaAfgGfcAfaUfl96	A-108661.1	aUfuGfcCfuUfuUfaaaUfuUfgCfcUfcsAfsf
AD-52968.1	A-108358.1	GfuCfuUfaAfaGfaCfuUfuGfuCfcAfuAfl96	A-108359.1	uAfuGfgAfaAfaAfgucUfuUfaAfgAfcCfsa
AD-53122.1	A-108656.1	CfuGfaGfgCfaAfaUfuUfaAfaAfgGfcAfl96	A-108657.1	uGfcCfuUfuUfaAfaauUfgCfcUfaAfgsUfsu
AD-53100.1	A-108586.1	GfcAfaUfcCfcGfgAfaAfaCfaAfaGfaUfl96	A-108587.1	aUfuUfuUfgUfuUfuuccGfgGfaUfuGfcsAfsu
AD-53128.1	A-108658.1	UfgAfgGfcAfaAfuUfuUfaAfaGfgCfaAfl96	A-108659.1	uUfgCfcUfuUfuUfaaaUfuGfcCfuCfasGfsu
AD-53043.1	A-108430.1	UfcUfaCfuUfcAfaCfaAfaAfaGfuGfaAfl96	A-108431.1	uUfaCfcUfuUfuUfguuGfaAfgUfaGfasAfsu
AD-53135.1	A-108676.1	UfaUfgUfgUfaAfaAfaUfcUfgUfaAfuAfl96	A-108677.1	uAfuUfaCfaGfaUfuuuUfaCfaCfaUfasCfsu
AD-53094.1	A-108584.1	AfaUfgCfaAfuCfcCfgGfaAfaAfaAfl96	A-108585.1	uUfuGfuUfuUfcCfaggAfuUfgCfaUfusGfsg
AD-53019.1	A-108422.1	CfuUfgAfaAfgCfcUfcCfuAfaAfaGfaAfl96	A-108423.1	uUfcUfuCfuAfgGfaggCfuUfuCfaAfgsUfsu
AD-53129.1	A-108674.1	GfuAfuGfuGfuAfaAfaAfaCfuGfuAfaUfl96	A-108675.1	aUfuAfaAfgAfuUfuuuAfaAfaAfaAfcUfsc
AD-53150.1	A-108666.1	CfaGfaGfuAfuGfuUfgUfaAfaAfaCfuUfl96	A-108667.1	aAfgAfuUfuUfuAfaCfaAfaAfaUfcsUfsg
AD-53117.1	A-108670.1	GfaGfuAfuGfuGfuUfaAfaAfaCfuGfuAfl96	A-108671.1	uAfaAfgAfuUfuUfuacAfaAfaUfcsUfsg
AD-52985.1	A-108348.1	UfcAfgUfuGfgGfaCfaUfgGfuCfuUfaAfl96	A-108349.1	uUfaAfgAfaCfaUfgucCfaCfaCfuGfasAfsf
AD-52962.1	A-108356.1	GfgUfcUfuAfaAfaAfaUfuUfgUfcCfaUfl96	A-108357.1	aUfgGfaCfaAfaGfuUfuAfaGfaCfcsAfsu

AD-52974.1	A-108360.1	UfcUfuAfaAfgAfCfuUfgUfcCfaUfaAfL96	A-108361.1	uUfaUfgGfaCfaAfaguCfuUfuAfaGfasCfsc
AD-52979.1	A-108346.1	UfuCfaGfuUfgGfGfAfcAfuGfgUfcUfuAfL96	A-108347.1	uAfaGfaCfcAfuGfuccCfaAfcUfgAfasGfsg

Lowercase nucleotides (a, u, g, c) are 2'-O-methyl nucleotides; Nf (e.g., Af) is a 2'-fluoro nucleotide; s is a phosphothiorate linkage; L96 indicates a GalNAc ligand.

Table 9. Unmodified Sense and antisense strand sequences of ANGPTL3 dsRNAs without GalNal conjugation

These sequences are the same as the sequences listed in Table 7 except that they do not contain GalNal conjugation.

Duplex Name	Sense OligoName	Sense Sequence	Antisense OligoName	Antisense Sequence	Position in NM_014495.2
AD-52637.1	A-108817.1	UCACAAUUUAGCUCUCCUUCUUU	A-108307.2	AAAGAAAGGAGCUUAAUUUGUGAAC	54-76
AD-52638.1	A-108825.1	UUUUUUGUCCUCUAGUUAUUU	A-108323.2	AAAUAAACUAGAGGAAACAAUAAAA	75-97
AD-52639.1	A-108833.1	GCUAUGUUAAGACGAUGUAAAA	A-108339.2	UUUUAACAUCGUCUAAACAUAGCAA	161-183
AD-52640.1	A-108841.1	GGACAUGGUCUUAAAGACUUU	A-108355.2	AAAGUCUUUAAAGACCAUGUCCCA	209-231
AD-52641.1	A-108849.1	CAAAAACUCAACAUUUUUGAU	A-108371.2	AUCAAAUUAUGUUGAGUUUUUGAA	266-288
AD-52642.1	A-108857.1	ACCAGUGAAAUCAAAGAGAA	A-108387.2	UUUUUUUUUGAUUUUACUGGUUU	314-336
AD-52643.1	A-108818.1	CACAAUUUAGCUCUCCUUCUUU	A-108309.2	AAAAGAAGGAGGCUUAAUUUGUGAA	55-77
AD-52645.1	A-108834.1	CUAUGUUAAGACGAUGUAAAA	A-108341.2	UUUUUACAUCGUCUAAACAUAGCA	162-184
AD-52647.1	A-108850.1	UCAACAUUUUUGAUCAGUCUU	A-108373.2	AAGACUGAUCAAAUAUUGUUGAGU	273-295
AD-52648.1	A-108858.1	AACUGAGAAGAAACUACAUUA	A-108389.2	UAUAUGUAUUUUCUUCACAGUCC	342-364
AD-52649.1	A-108819.1	ACAAUUUAGCUCUCCUUCUUUU	A-108311.2	AAAAAGAAGGAGGCUUAAUUUGUGA	56-78
AD-52650.1	A-108827.1	CUCCAGAGCCAAAUAUCAAGAU	A-108327.2	AUCUUGAUUUUUGGCUCUCGGAGAU	138-160
AD-52651.1	A-108835.1	CGAUGUAAAAUUUUAGCCAA	A-108343.2	UUUGCUAAAAUUUUUACAUCGUC	172-194
AD-52652.1	A-108843.1	GUCUUAAGACUUUUGUCCAU	A-108359.2	UAUGGACAAAAGUCUUUAAAGACCA	216-238
AD-52653.1	A-108851.1	CAACAUUUUUGAUCAGUCUUU	A-108375.2	AAAGACUGAUCAAAUAUUGUUGAG	274-296
AD-52654.1	A-108859.1	ACUGAGAAGAACUACAUUA	A-108391.2	UUUAUUGUAUUUUCUUCACAGUUC	343-365

AD-52656.1	A-108828.1	CCAGAGCCAAAAUCAAGAUUU	A-108329.2	AAAUUUGAUUUUUGGCUCUGGAG	140-162
AD-52657.1	A-108836.1	GAUGUAAAAUUUUAGCCAAU	A-108345.2	AUUGGCUAAAAUUUUUACAUCGU	173-195
AD-52658.1	A-108844.1	UCUUAAAGACUUUGUCCAUAA	A-108361.2	UUUUGGACAAAGUCUUUAAAGACC	217-239
AD-52659.1	A-108852.1	AACAUUUUUGAUCAGUCUUUU	A-108377.2	AAAAGACUGAUCAAAUUAGUUUGA	275-297
AD-52660.1	A-108860.1	CUGAGAAGAACUACAUUAAA	A-108393.2	UUUUAUUGUAGUUUCUUCAGUU	344-366
AD-52661.1	A-108821.1	AAUUAAGCUCUUCUUUUUAU	A-108315.2	AUAAAAAGAGGAGGCUUAAUUGU	58-80
AD-52662.1	A-108829.1	AAUCAAAGAUUUGCUAUGUUA	A-108331.2	UAACAUAGCAAAUUCUUGAUUUUG	149-171
AD-52663.1	A-108837.1	UUCAGUUGGGACAUGGUCUUA	A-108347.2	UAAAGACAUGUCCCAACUGAAGG	201-223
AD-52664.1	A-108845.1	GGGCCAAAUAUUGACAUAUU	A-108363.2	AAUUGUCAUUAAUUUUGGCCUU	244-266
AD-52665.1	A-108853.1	ACAUUUUUGAUCAGUCUUUUU	A-108379.2	AAAAAGACUGAUCAAAUAUGUUG	276-298
AD-52666.1	A-108861.1	AGAACUACAUUAAACUACAA	A-108395.2	UUGUAGUUUAUUGAUGUUCUUC	350-372
AD-52667.1	A-108822.1	AUUUAGCUCUUCUUUUUAUU	A-108317.2	AAUAAAAAGAGGAGGCUUAAUUG	59-81
AD-52668.1	A-108830.1	AGAUUUGCUAUGUUAGACGAU	A-108333.2	AUCGUCUAACAUAGCAAAUCUUG	155-177
AD-52669.1	A-108838.1	UCAGUUGGGACAUGGUCUAAA	A-108349.2	UUAAAGACCAUGUCCCAACUGAAG	202-224
AD-52670.1	A-108846.1	GGCCAAAUAUUGACAUAUUU	A-108365.2	AAAUUGUCAUUAAUUUUGGCCCU	245-267
AD-52671.1	A-108854.1	CAUUAUUUGAUCAGUCUUUUUA	A-108381.2	UAAAAAGACUGAUCAAAUAUGUU	277-299
AD-52672.1	A-108862.1	UACAUUAAACUACAAGUCA	A-108397.2	UUGACUUGUAGUUUAUUGUAGU	355-377
AD-52673.1	A-108823.1	UUUUUUUGUUCUUCUAGUUUAU	A-108319.2	AUAACUAGAGGAAACAAUAAAAAG	73-95
AD-52674.1	A-108831.1	UUGCUAUGUUUAGACGAUGUAA	A-108335.2	UUACAUUGUCUAACAUAGCAAAU	159-181
AD-52675.1	A-108839.1	CAGUUGGGACAUGGUCUUAAA	A-108351.2	UUUAAAGACCAUGUCCCAACUGAA	203-225
AD-52676.1	A-108847.1	AAAUUAAUGACAUAUUUCAA	A-108367.2	UUUGAAAUUUGUCAUUAAUUUGG	249-271

AD-52677.1	A-108855.1	GAUCAGUCUUUUUAUGAUCUA	A-108383.2	UAGAUCAUAAAAAGACUGAUCAA	284-306
AD-52678.1	A-108863.1	ACAUUAAACUACAAGUCAAA	A-108399.2	UUUGACUUUGUAGUUUUUAUAGUAG	356-378
AD-52679.1	A-108824.1	UUUAUUUGUUCUUCUAGUUUU	A-108321.2	AAUAAACUAGAGGAACAUAUAAAA	74-96
AD-52680.1	A-108832.1	UGCUAUGUUUAGACGAUGUAAA	A-108337.2	UUUACAUCGUCUACAACAUAGCAAA	160-182
AD-52681.1	A-108840.1	GGGACAUGGUCUUUAAAGACUU	A-108353.2	AAGUCUUUAAAGACCAUGUCCCAA	208-230
AD-52682.1	A-108848.1	UGACAUUUUUUAAAAACUCA	A-108369.2	UUUGAGUUUUUGAAAAUUGUCAUU	256-278
AD-52683.1	A-108856.1	AUCAGUCUUUUUAUGAUCUAU	A-108385.2	AUAGAUCAUAAAAAGACUGAUCA	285-307
AD-52684.1	A-108864.1	CAUAUAAACUACAAGUCAAAA	A-108401.2	UUUUGACUUUGUAGUUUUUAUUGUA	357-379
AD-52685.1	A-108872.1	CUUGAACUCAACUAAAAACUU	A-108417.2	AAGUUUUUGAGUUUGAGUUUCAAAGUG	401-423
AD-52686.1	A-108880.1	CUACUUCAACAAAAAGUGAAA	A-108433.2	UUUCACUUUUUUGUUUGAAGUAGAA	446-468
AD-52687.1	A-108888.1	AAGAGCAACUAAACUUAUAA	A-108449.2	UUAAGUUAGUUAGUUUGCUCUUCU	474-496
AD-52688.1	A-108896.1	AAACAAGAUAAUAGCAUCAAA	A-108465.2	UUUGAUGCUAUUAUCUUGUUUUUU	557-579
AD-52689.1	A-108904.1	GCAUAGUCAAAUAAAAGAAU	A-108481.2	AUUUCUUUUUUUUUGACUAGCUG	625-647
AD-52690.1	A-108865.1	AUAUAAACUACAAGUCAAAAA	A-108403.2	UUUUUGACUUUGUAGUUUUUAUUGU	358-380
AD-52691.1	A-108873.1	GAACUCAACUCAAAAAACUUGAA	A-108419.2	UUCAAGUUUUUGAGUUUGAGUUCAA	404-426
AD-52692.1	A-108881.1	UACUUCAACAAAAAGUGAAAU	A-108435.2	AUUUCACUUUUUUGUUGAAGUAGA	447-469
AD-52693.1	A-108889.1	AGAGCAACUAAACUAAUUAU	A-108451.2	AUUAAGUUAGUUAGUUUGCUCUUC	475-497
AD-52694.1	A-108897.1	GAUAUAGCAUCAAAAGACCUU	A-108467.2	AAGGUCUUUGAUGCUAUUAUCUU	563-585
AD-52695.1	A-108905.1	CAUAGUCAAAUAAAAGAAUA	A-108483.2	UAUUUCUUUUUUUUGACUAGCU	626-648
AD-52696.1	A-108866.1	UAUAAACUACAAGUCAAAAAU	A-108405.2	AUUUUUGACUUGUAGUUUUUAUUG	359-381
AD-52697.1	A-108874.1	AACUCAACUCAAAACUUGAAA	A-108421.2	UUUCAAGUUUUUGAGUUUGAGUUCA	405-427

AD-52698.1	A-108882.1	ACUUCACAAAAAGUGAAUA	A-108437.2	UAUUUCACUUUUUUUGAAGUAG	448-470
AD-52699.1	A-108890.1	GAGCAACUAACUAACUUAUU	A-108453.2	AAUUAAAGUUAGUUAGUUGCUUU	476-498
AD-52700.1	A-108898.1	AACCAACAGCAUAGUCAAAUA	A-108469.2	UAUUUGACUAUGCUGUUGGUUA	617-639
AD-52701.1	A-108906.1	AGUCAAAUAAAAAGAAUAGAA	A-108485.2	UUCUAUUUUUUUUUUUUGACUAU	629-651
AD-52702.1	A-108867.1	AGUCAAAAUUGAAGAGGUAAA	A-108407.2	UUUACCUUUAUUUUUUUGACUUG	370-392
AD-52703.1	A-108875.1	CUUGAAAGCCUCCUAGAAGAA	A-108423.2	UUCUUUCUAGGAGGCUUUCAAGUU	419-441
AD-52704.1	A-108883.1	CUUCAACAAAAAGUGAAUAU	A-108439.2	AUAUUUCACUUUUUUUGUAAAGUA	449-471
AD-52705.1	A-108891.1	CAACUAACUAACUUAUUUCAA	A-108455.2	UUGAAUUAAAGUUAGUUAGUUGCU	479-501
AD-52706.1	A-108899.1	ACCAACAGCAUAGUCAAAUAA	A-108471.2	UUUUUUUGACUAUGCUGUUGGUUU	618-640
AD-52707.1	A-108907.1	GAACCCACAGAAAUUUCUCUA	A-108487.2	UAGAGAAUUUUUCUGUGGGUUCUU	677-699
AD-52708.1	A-108868.1	GAAUUGUCACUUGAACUCAA	A-108409.2	UUUGAUUCAAAGUGACAUUUUCUU	391-413
AD-52709.1	A-108876.1	UGAAAGCCUCCUAGAAGAAAA	A-108425.2	UUUUUCUUUAGGAGGCUUUCAAG	421-443
AD-52710.1	A-108884.1	UUCAACAAAAAGUGAAUAUU	A-108441.2	AAUAAUUUACUUUUUUUGUAAAGU	450-472
AD-52711.1	A-108892.1	AACUAACUAACUUAUUUCAA	A-108457.2	UUUGAAUUAAAGUUAGUUAGUUGC	480-502
AD-52712.1	A-108900.1	CCAACAGCAUAGUCAAAUAAA	A-108473.2	UUUAAUUUGACUAUGCUGUUGGUU	619-641
AD-52713.1	A-108908.1	AACCCACAGAAAUUUCUCUAU	A-108489.2	AUAGAGAAAUUUUCUGUGGGUUCU	678-700
AD-52714.1	A-108869.1	UGUCACUUUGAACUCUAAUCUAA	A-108411.2	UUGAGUUUGAGUUUCAAGUGACAU	396-418
AD-52715.1	A-108877.1	GAAAGCCUCCUAGAAGAAAA	A-108427.2	UUUUUCUUUAGGAGGCUUUUCAA	422-444
AD-52716.1	A-108885.1	AAUAAUUUAGAAGAGCAACUAA	A-108443.2	UUAGUUUGCUCUUCUAAAAUUUUC	465-487
AD-52717.1	A-108893.1	ACUAACUAACUUAUUUCAA	A-108459.2	UUUUAGAAUUAAAGUUAGUUAGUUG	481-503
AD-52718.1	A-108901.1	CAACAGCAUAGUCAAAUAAA	A-108475.2	UUUUUUUUGACUAUGCUGUUGGU	620-642

AD-52719.1	A-108909.1	CCACAGAAUUUUCUCUAUCUU	A-108491.2	AAGAUAGAGAAUUUUCUGUGGGU	681-703
AD-52720.1	A-108870.1	GUCACUUGAACUCUACUCAA	A-108413.2	UUUGAGUUAGAUUCAAAGUGACAU	397-419
AD-52721.1	A-108878.1	CUCUAGAAGAAAAUUUCUA	A-108429.2	UAGAAUUUUUUUUUUUUUAGGAGGC	428-450
AD-52722.1	A-108886.1	AUUUAGAAGAGCAACUAACUA	A-108445.2	UAGUUAGUUGCUCUUCUAAAAUUAU	468-490
AD-52723.1	A-108894.1	CUAACUAACUUAUUUCAAUUU	A-108461.2	AUUUUGAAUUAAAGUUAGUUAGUU	482-504
AD-52724.1	A-108902.1	CAGCAUAGUCAAAAUAAAAAGAA	A-108477.2	UUUUUUUUUUUUUUUUUUUUUUUU	623-645
AD-52725.1	A-108910.1	GAAUAAGAAAAUUGAAAAACAU	A-108493.2	AUGUUUUACAUUUUUUUUUUUUUUUU	746-768
AD-52726.1	A-108871.1	UCACUUGAACUCUACUCAA	A-108415.2	UUUUGAGUUAGUUUCAAAGUGACA	398-420
AD-52727.1	A-108879.1	UCUACUUCACAAAAAGUGAA	A-108431.2	UUCACUUUUUUUUUUUUUUUUUUUU	445-467
AD-52728.1	A-108887.1	UUUAGAAGAGCAACUAAACUAA	A-108447.2	UUAGUUAGUUUGCUCUUUCAAUAU	469-491
AD-52729.1	A-108895.1	AAAACAAGAUAAUAGCAUCA	A-108463.2	UUGAUGCUAAUUAUCUUUUUUUUUU	556-578
AD-52730.1	A-108903.1	AGCAUAGUCAAAAUAAAAAGAA	A-108479.2	UUUCUUUUUUUUUUUUUUUUUUUUUU	624-646
AD-52731.1	A-108958.1	AGACCCAGCAACUCUCAAGUU	A-108495.2	AACUUGAGAGUUUGCUGGGGUCUGA	836-858
AD-52732.1	A-108966.1	AGUCCAUUGGACAUUAAUUCAA	A-108511.2	UUGAAUUAAUUGUCCAUUGGACUAC	887-909
AD-52733.1	A-108974.1	GAUGGAUCACAAAAACUUCAAU	A-108527.2	AUUGAAGUUUUUUUGUGAUCCAUCA	917-939
AD-52734.1	A-108982.1	CUAGAGAAGAUUAUACUCCAU	A-108543.2	UAUGGAGUAUAUCUUUCUAGGC	998-1020
AD-52735.1	A-108990.1	AAAGACAACAAACAUUAUAUU	A-108559.2	AAUAUAUUGUUUUUUUUUUUUUUUU	1064-1086
AD-52736.1	A-108998.1	CAUUUAUUUGAAUAUUUUUUUU	A-108575.2	AAAAGAAUAUUCAAUAUAAUUGUU	1076-1098
AD-52737.1	A-108959.1	GACCCAGCAACUCUCAAGUUU	A-108497.2	AAACUUGAGAGUUUGCUGGGGUCUG	837-859
AD-52739.1	A-108975.1	GGAUACAAAAACUUCAAUGAA	A-108529.2	UUCAUUUGAAGUUUUUGUGAUCCAU	920-942
AD-52740.1	A-108983.1	GAAGAUUAUACUCCAUAGUGAA	A-108545.2	UUCACUAUGGAGUAUAUCUUCUC	1003-1025

AD-52741.1	A-108991.1	GACAACAAACAUUAUAUUGAA	A-108561.2	UUCAAUAUAAUGUUUUGUUGUCUU	1067-1089
AD-52742.1	A-108999.1	GGGAAUACACGAAACCAACUA	A-108577.2	UAGUUUGUUUUCGUGAUUUUCCCAA	1102-1124
AD-52743.1	A-108960.1	ACCCAGCAACUCUCAAGUUUU	A-108499.2	AAAACUUAGAGAGUUGCUGGGUCU	838-860
AD-52744.1	A-108968.1	GGACAUUAUUUUAACAUCGAA	A-108515.2	UUCGAUUGUUGAAUUAUUGUCCAU	894-916
AD-52745.1	A-108976.1	GAUCACAAAACUUCAAUGAAA	A-108531.2	UUUCAUUGAAGUUUUUGUGAUCCA	921-943
AD-52746.1	A-108984.1	ACUCCAUAGUGAAGCAACUUA	A-108547.2	UAGAUUGCUUCACUAUUGGAGUAU	1011-1033
AD-52747.1	A-108992.1	ACAACAAACAUUAUUAUUGAAU	A-108563.2	AUUCAAUAUAAUGUUUUGUUGUCU	1068-1090
AD-52748.1	A-109000.1	GGAAUACACGAAACCAACUAU	A-108579.2	AUAGUUGGUUUUCGUGAUUUUCCCA	1103-1125
AD-52749.1	A-108961.1	CCCAGCAACUCUCAAGUUUUU	A-108501.2	AAAAACUUAGAGAGUUGCUGGGUC	839-861
AD-52750.1	A-108969.1	GACAUUAUUUAACAUCGAAU	A-108517.2	AUUCGAUUGUUGAAUUAUUGUCCA	895-917
AD-52751.1	A-108977.1	AACGUGGGAGAACUACAAUA	A-108533.2	UAUUUGUAGUUUCCACCGUUUC	940-962
AD-52752.1	A-108985.1	CUCCAUAGUGAAGCAAUUUA	A-108549.2	UUAGAUUGCUUCACUAUUGGAGUA	1012-1034
AD-52753.1	A-108993.1	CAACAAACAUUAUUAUUGAAUA	A-108565.2	UAUUCAAUAUAAUGUUUUGUUGUC	1069-1091
AD-52754.1	A-109001.1	GAAUACACGAAACCAACUAUA	A-108581.2	UAUAGUUGGUUUUCGUGAUUUUCCC	1104-1126
AD-52755.1	A-108962.1	CUUCUAGUUUUUCAUGUCUA	A-108503.2	UAGACAUGAAAAACUUGAGAGUU	847-869
AD-52756.1	A-108970.1	ACAUUAUUUUAACAUCGAAUA	A-108519.2	UAUUCGAUUGUUGAAUUAUUGUCC	896-918
AD-52757.1	A-108978.1	GGGAGAACUACAAUAUUGGUU	A-108535.2	AACCAUUAUUUGUAGUUUCUCCAC	945-967
AD-52758.1	A-108986.1	UCCAUAGUGAAGCAAUUUAU	A-108551.2	AUUAGAUUGCUUCACUAUUGGAGU	1013-1035
AD-52759.1	A-108994.1	AACAACAUUAUUAUUGAAUAU	A-108567.2	AUAUCAAUAUAAUGUUUUGUUGU	1070-1092
AD-52760.1	A-109002.1	UGGCAUUGUCCCCCAUUGCAU	A-108583.2	AUUGCAUUGGGGACAUUGCCAGU	1147-1169
AD-52761.1	A-108963.1	UCAGGUAGUCCUUGGACAUUA	A-108505.2	UAAUGUCCAUUGGACUACCUGUA	881-903

AD-52762.1	A-108971.1	UUAAUUAACAUCGAAUAGAU	A-108521.2	AUCUAUUCGAUUGAAUUAUUG	899-921
AD-52763.1	A-108979.1	GGAGAAACUACAAAUAGGUUU	A-108537.2	AAACCAUAAUUUGUAGUUUCUCCA	946-968
AD-52764.1	A-108987.1	CCAUAGUGAAGCAAUCUAAUU	A-108553.2	AAUUAGAUUGCUUCACUAUGGAG	1014-1036
AD-52765.1	A-108995.1	ACAAACAUUAUAUUGAAUAUU	A-108569.2	AAUAUCAAUAUAAUUGUUUGUUG	1071-1093
AD-52766.1	A-109003.1	AAUGCAAUCCCGGAAACAAA	A-108585.2	UUUGUUUUCCGGGAUUGCAUUGG	1160-1182
AD-52767.1	A-108964.1	CAGGUAGUCCAUUGGACAUUAA	A-108507.2	UUAAUGUCCAUGGACUACCUGAU	882-904
AD-52768.1	A-108972.1	UUCAACAUCGAAUAGAUUGAU	A-108523.2	AUCCAUCUAUUCGAUUGUUGAAUU	903-925
AD-52769.1	A-108980.1	GUUGGGCCUAGAGAAGAUUAU	A-108539.2	UAUAUCUUCUCUAGGCCCAACCA	991-1013
AD-52770.1	A-108988.1	CAUAGUGAAGCAAUCUAAUUA	A-108555.2	UAAUUAGAUUGCUUCACUAUGGA	1015-1037
AD-52771.1	A-108996.1	AACAUUAUAUUGAAUAUUCUU	A-108571.2	AAGAAUAUCAAUAUAAUUGUUUG	1074-1096
AD-52772.1	A-109004.1	GCAAUCCCGGAAACAAAGAU	A-108587.2	AUCUUUGUUUUCCGGGAUUGCAU	1163-1185
AD-52773.1	A-108965.1	GGUAGUCCAUUGGACAUUAAUU	A-108509.2	AAUUAUUGUCCAUGGACUACCUUG	884-906
AD-52774.1	A-108973.1	AUCGAAUAGAUGGAUCACAAA	A-108525.2	UUUGUGAUCCAUCUAUUCGAUGU	909-931
AD-52775.1	A-108981.1	CCUAGAGAAGAUUAUCUCCAU	A-108541.2	AUGGAGUAUAUUCUUCUAGGCC	997-1019
AD-52776.1	A-108989.1	GUUGGAAGACUGGAAAGACAA	A-108557.2	UUUGUCUUUCCAGUCUCCCAACUC	1051-1073
AD-52777.1	A-108997.1	ACAUUAUAUUGAAUAUUCUUU	A-108573.2	AAAGAAUAUCAAUAUAAUUGUUU	1075-1097
AD-52778.1	A-109005.1	CAAUCCCGGAAACAAAGAUU	A-108589.2	AAUCUUUGUUUUCCGGGAUUGCA	1164-1186
AD-52779.1	A-109013.1	CUACUUGGGAUCACAAAGCAA	A-108605.2	UUUCUUUGUGAUCCCAAGUAGAA	1194-1216
AD-52780.1	A-109021.1	ACAACCUAAUUGGUAAUAUA	A-108621.2	UAUAUUUACCAUUUAGGUUGUUU	1281-1303
AD-52781.1	A-109029.1	AUCCAUCCAACAGAUUCAGAA	A-108637.2	UUCUGAAUCUGUUGGAUGGAUCA	1400-1422

AD-52782.1	A-109037.1	AACUGAGGCAAAUUUAAAAAGA	A-108653.2	UCUUUUAAAAUUUGCCUCAGUUA	1432-1454_G21A
AD-52783.1	A-109045.1	AGAGUAUGUGUAAAAAUCUGU	A-108669.2	ACAGAUUUUUACACAUACUCUGU	1913-1935
AD-52784.1	A-109006.1	AAUCCCGAAAAACAAGAUUU	A-108591.2	AAAUUUUGUUUUCCGGGAUUGC	1165-1187
AD-52785.1	A-109014.1	UACUUGGGGAUCACAAAGCAAA	A-108607.2	UUUGCUUUUGUGAUCCCAAGUAGA	1195-1217
AD-52786.1	A-109022.1	CAACCUAAAUGGUAAAAUAAA	A-108623.2	UUUAUUUUACCAUUUJAGGUUUU	1282-1304
AD-52787.1	A-109030.1	UUGAUUGAACUGAGGCAAAUU	A-108639.2	AAUUUGCCUCAGUUCAUUCAAAAG	1425-1447
AD-52788.1	A-109038.1	ACUGAGGCAAAUUUAAAAAGGA	A-108655.2	UCCUUUUAAAAUUUGCCUCAGUUC	1433-1455_C21A
AD-52789.1	A-109046.1	GAGUAUGUGUAAAAAUCUGUA	A-108671.2	UACAGAUUUUUUACACAUACUCUG	1914-1936
AD-52791.1	A-109015.1	ACUUGGGAUCACAAAGCAAAA	A-108609.2	UUUUUGCUUUUGUGAUCCCAAGUAG	1196-1218
AD-52792.1	A-109023.1	AUGGUAAAUAUAAACAAACCAA	A-108625.2	UUGGUUUUGUUUAUUUUACCAUUU	1290-1312
AD-52793.1	A-109031.1	UGAAUGAACUGAGGGCAAAUUU	A-108641.2	AAUUUGCCUCAGUUCAUUCAAA	1426-1448
AD-52794.1	A-109039.1	CUGAGGCAAAUUUAAAAAGGCA	A-108657.2	UGCCUUUUAAAAUUUGCCUCAGUU	1434-1456
AD-52795.1	A-109047.1	AGUAUGUGUAAAAAUCUGUAA	A-108673.2	UUACAGAUUUUUACACAUACUCU	1915-1937
AD-52796.1	A-109008.1	GAAAAAAGAUUUUGGUGUUU	A-108595.2	AAACACCAAAUCUUUGUUUUCCG	1172-1194
AD-52797.1	A-109016.1	AGUGUGGAGAAAAACAACCUAA	A-108611.2	UUAGGUUGUUUUUCUCCACACUCA	1269-1291
AD-52798.1	A-109024.1	GUUCAAAAUGGAAGGUUAUA	A-108627.2	UAUAACCUUCCAUUUUUGAGACUU	1354-1376
AD-52799.1	A-109032.1	GAAUGAACUGAGGCAAAUUUA	A-108643.2	UAAUUUGCCUCAGUUCAUUCAA	1427-1449
AD-52800.1	A-109040.1	UGAGGCAAAUUUAAAAAGGCAA	A-108659.2	UUGCCUUUUAAAAUUUGCCUCAGU	1435-1457
AD-52801.1	A-109048.1	GUUUGUGUAAAAAUCUGUAAU	A-108675.2	AUUACAGAUUUUUACACAUACUC	1916-1938

AD-52802.1	A-109009.1	AAAAAAGAUUUUGGUGUUUU	A-108597.2	AAAACACCAAAUUCUUUGUUUCC	1173-1195
AD-52803.1	A-109017.1	GUGUGGAGAAAAACAACCUAAA	A-108613.2	UUUAGGUUGUUUUUCUCCACACUC	1270-1292
AD-52804.1	A-109025.1	AUGGAAGGUUAUACUCUAUAA	A-108629.2	UUUAAGAGUAUAACCUCCAUUU	1362-1384
AD-52805.1	A-109033.1	AAUGAACUGAGGCAAAUUUAA	A-108645.2	UUAAAAUUUGCCUCAGUUCAUUA	1428-1450
AD-52806.1	A-109041.1	GAGGCAAAUUUAAAAGGCAAU	A-108661.2	AUUGCCUUUUAAAUUUGCCUCAG	1436-1458
AD-52807.1	A-109049.1	UAUGUGUAAAAAUUCUGUAUA	A-108677.2	UAUUACAGAUUUUUUACACAUACU	1917-1939
AD-52808.1	A-109010.1	ACAAAGAUUUUGGUGUUUUCUA	A-108599.2	UAGAAAAACCAAAUUCUUUGUUU	1176-1198
AD-52809.1	A-109018.1	UGUGGAGAAAAACAACCUAAAA	A-108615.2	AUUUAGGUUGUUUUUCUCCACACU	1271-1293
AD-52810.1	A-109026.1	UGGAAGGUUAUACUCUAUAAA	A-108631.2	UUUAUAGAGUAUAACCUUCCAUU	1363-1385
AD-52811.1	A-109034.1	AUGAACUGAGGCAAAUUUAAA	A-108647.2	UUUAAAAUUUGCCUCAGUUCAUUC	1429-1451
AD-52812.1	A-109042.1	AGGCAAAUUUAAAAGGCAUA	A-108663.2	UAUUGCCUUUUAAAUUUGCCUCA	1437-1459
AD-52813.1	A-109011.1	AAGAUUUUGGUGUUUUCUACUU	A-108601.2	AAGUAGAAAAACACCAAAUUCUUG	1179-1201
AD-52814.1	A-109019.1	AAACAACCUAAAUGGUAAAUA	A-108617.2	UAUUUACCAUUUJAGGUUGUUUUC	1279-1301
AD-52815.1	A-109027.1	AUACUCUAUAAAUAUCAAACCA	A-108633.2	UUGGUUGAUUUUUUAUAGAGUAUAA	1372-1394
AD-52816.1	A-109035.1	UGAACUGAGGCAAAUUUAAAA	A-108649.2	UUUUAAAAUUUGCCUCAGUUCAUU	1430-1452
AD-52817.1	A-109043.1	GGCAAAUUUAAAAGGCAAUAA	A-108665.2	UUUUUGCCUUUUAAAUUUGCCUC	1438-1460
AD-52818.1	A-109012.1	UUUUCUACUUGGGAUCACAAA	A-108603.2	UUUGUGAUCCCAAGUAGAAAAACA	1190-1212
AD-52819.1	A-109020.1	AACAACCUAAAUGGUAAAUAU	A-108619.2	AUAUUUACCAUUUJAGGUUGUUUU	1280-1302
AD-52820.1	A-109028.1	UACUCUAUAAAUAUCAAACCA	A-108635.2	UUUGGUUGAUUUUUUAUAGAGUAUA	1373-1395

AD-52821.1	A-109036.1	GAACUGAGGC AAAUUUAAAAA	A-108651.2	UUUUUUUUUUUGCCUCAGUUUCAU	1431- 1453_G21A
AD-52822.1	A-109044.1	CAGAGUAUGUGUAAAAAUCUU	A-108667.2	AAGAUUUUUUACACAUACUCUGUG	1912- 1934_G21U

Table 10. Modified Sense and antisense strand sequences of ANGPTL3 dsRNAs without GalNal conjugation

These sequences are the same as the sequences listed in Table 8 except that they do not contain GalNal conjugation.

Duplex Name	Sense Oligo Name	Sense Sequence	Antisense Oligo Name	Antisense Oligo Sequence
AD-52637.1	A-108817.1	UfcAfcAfaUfuAfaGfcUfcCfuUfcUfuUf	A-108307.2	aAfaGfaAfgGfaGfcuuAfaUfuGfuGfasAfsc
AD-52638.1	A-108825.1	UfuAfuUfgUfuCfcUfcUfaGfuUfaUfuUf	A-108323.2	aAfaUfaAfcUfaGfaggAfaCfaAfuAfasAfsa
AD-52639.1	A-108833.1	GfcUfaUfgUfuAfgAfcGfaUfgUfaAfaAf	A-108339.2	uUfuUfaCfaUfcGfucuAfaCfaUfaGfcsAfsa
AD-52640.1	A-108841.1	GfgAfcAfuGfgUfcCfuUfaAfaAfgAfuUfuUf	A-108355.2	aAfaGfuCfuUfuAfaGfaCfcAfuGfuCfcsCfsa
AD-52641.1	A-108849.1	CfaAfaAfaCfuCfaAfcAfaAfuUfuGfaUf	A-108371.2	aUfcAfaAfuAfuGfuugAfgUfuUfuUfgsAfsa
AD-52642.1	A-108857.1	AfcCfaGfuGfaAfaUfcAfaAfgAfaGfaAf	A-108387.2	uUfcUfuCfuUfuGfauuUfcAfcUfgGfusUfsu
AD-52643.1	A-108818.1	CfaCfaAfuUfaAfcGfcUfcCfuCfuUfuUf	A-108309.2	aAfaAfgAfaGfgAfgcuUfaAfuUfgUfgsAfsa
AD-52645.1	A-108834.1	CfuAfuGfuUfaGfaCfcGfaAfuGfuAfaAfaAf	A-108341.2	uUfuUfuAfcAfuCfugUfaAfcAfuAfgsCfsa
AD-52647.1	A-108850.1	UfcAfaCfaUfaUfuUfgAfuCfaGfuCfuUf	A-108373.2	aAfgAfcUfgAfuCfaaaUfaUfgUfuGfasGfsu
AD-52648.1	A-108858.1	AfaCfuGfaGfaAfaGfaCfuAfcAfaAfuAf	A-108389.2	uAfuAfuGfuAfgUfcuUfcUfcAfgUfusCfsc
AD-52649.1	A-108819.1	AfcAfaUfuAfaGfcUfcCfuUfcUfuUfuUf	A-108311.2	aAfaAfaGfaAfgGfagcUfaUfaUfuGfusGfsa
AD-52650.1	A-108827.1	CfuCfcAfgAfgCfcAfaAfaUfcAfaGfaUf	A-108327.2	aUfcUfuGfaUfuUfuggCfuCfuGfgAfgsAfsu
AD-52651.1	A-108835.1	CfgAfuGfuAfaAfaAfuUfuUfaGfcCfaAf	A-108343.2	uUfgGfcUfaAfaAfuuuUfuAfcAfuCfcsUfsc
AD-52652.1	A-108843.1	GfuCfuUfaAfaGfaCfuUfuGfuCfcAfuAf	A-108359.2	uAfuGfgAfcAfaAfgucUfuUfaAfgAfcCfsa
AD-52653.1	A-108851.1	CfaAfcAfuAfuUfuGfaUfcAfgUfcUfuUf	A-108375.2	aAfaGfaCfuGfaUfaaaAfaUfuGfuUfgsAfsa
AD-52654.1	A-108859.1	AfcUfgAfgAfaGfaAfcUfaCfaUfaUfaAf	A-108391.2	uUfaUfaUfgUfaGfuucUfuCfuCfaGfusUfsc

AD-52656.1	A-108828.1	CfcAfgAfgCfcAfAfAfAfUfcAfaGfaUfuUf	A-108329.2	aAfaUfcUfuGfaUfuuuGfgCfuCfuGfgsAfg
AD-52657.1	A-108836.1	GfaUfgUfaAfaAfaUfuUfuUfaGfcAfaUf	A-108345.2	aUfuGfgCfuAfaAfaUfuUfuUfaCfaUfcsGfsu
AD-52658.1	A-108844.1	UfcUfuAfaAfgAfcUfuUfgUfcCfaUfaAf	A-108361.2	uUfaUfgGfaCfaAfaguCfuUfuAfaGfasCfsc
AD-52659.1	A-108852.1	AfaCfaUfaUfuUfgGfaUfuCfaGfuCfuUfuUf	A-108377.2	aAfaAfgAfcUfgAfucaAfaUfaUfgUfusGfsa
AD-52660.1	A-108860.1	CfuGfaGfaAfgAfcUfuAfcAfuAfaUfaAf	A-108393.2	uUfuAfuAfuGfuAfguuCfuUfcUfcsAfgsUfsu
AD-52661.1	A-108821.1	AfaUfuAfaGfcUfcCfuUfcUfuUfuUfaUf	A-108315.2	aUfaAfaAfaGfaAfggaGfcUfuAfaUfusGfsu
AD-52662.1	A-108829.1	AfaAfuCfaAfgAfuUfuGfuUfaUfgUfuUf	A-108331.2	uAfaCfaUfaGfcAfaauCfuUfgAfuUfusUfsg
AD-52663.1	A-108837.1	UfuCfaGfuUfgGfgAfcAfuGfgUfcUfuUf	A-108347.2	uAfaGfaCfcAfuGfuuccCfaAfcUfgAfasGfsg
AD-52664.1	A-108845.1	GfgGfcCfaAfaUfuAfaUfgAfcAfuAfuUf	A-108363.2	aAfuAfuGfuCfaUfuuaUfuUfgGfcCfcsUfsu
AD-52665.1	A-108853.1	AfcAfuAfuUfuGfaUfcAfuUfgAfcAfuUfuUf	A-108379.2	aAfaAfaGfaCfuGfaucAfaAfuAfuGfusUfsg
AD-52666.1	A-108861.1	AfgAfaCfuAfcAfuAfaUfaAfcUfaCfaAf	A-108395.2	uUfgUfaGfuUfuAfuauGfuAfgUfuCfusUfsc
AD-52667.1	A-108822.1	AfuUfaAfgCfuCfcUfuCfuUfuUfuUfuUf	A-108317.2	aAfuAfaAfaAfgAfggAfgCfuUfaAfusUfsg
AD-52668.1	A-108830.1	AfgAfuUfuGfcUfaUfgUfuAfgAfcGfaUf	A-108333.2	aUfcGfuCfuAfaCfauaGfcAfaAfuCfusUfsg
AD-52669.1	A-108838.1	UfcAfgUfuGfgAfcCfaUfgGfuCfuUfaAf	A-108349.2	uUfaAfgAfcCfaUfgucCfcAfaCfuGfasAfg
AD-52670.1	A-108846.1	GfgCfcAfaAfuUfaAfuGfaCfaUfaUfuUf	A-108365.2	aAfaUfaUfgUfcAfuuaAfuUfuGfgCfcsCfsu
AD-52671.1	A-108854.1	CfaUfaUfuUfgAfuUfcAfuCfuUfuUfaUf	A-108381.2	uAfaAfaAfgAfcUfgauCfaAfaUfaUfgsUfsu
AD-52672.1	A-108862.1	UfaCfaUfaUfaAfaUfcAfuAfaGfuCfaAf	A-108397.2	uUfgAfcUfuGfuAfguuUfaUfaUfgUfasGfsu
AD-52673.1	A-108823.1	UfuUfuAfuUfgUfuCfcUfcUfaGfuUfaUf	A-108319.2	aUfaAfcUfaGfaGfgaaCfaAfaAfaAfasAfg
AD-52674.1	A-108831.1	UfuGfcUfaUfgUfuAfgAfcGfaUfgUfaAf	A-108335.2	uUfaCfaUfcGfuCfuuaCfaUfaGfcAfasAfsu
AD-52675.1	A-108839.1	CfaGfuUfgGfgAfcAfuGfgUfcUfuAfaAf	A-108351.2	uUfuAfaGfaCfcAfuguCfcCfaAfcUfgsAfsa
AD-52676.1	A-108847.1	AfaAfuUfaAfuGfaCfaUfaUfuUfcAfaAf	A-108367.2	uUfuGfaAfaUfaUfgucAfuUfaAfuUfusGfsg

AD-52677.1	A-108855.1	GfaUfcAfgUfcUfuUfuUfaUfgAfuCfuAf	A-108383.2	uAfgAfuCfaUfaAfaaaGfaCfuGfaUfcsAfsa
AD-52678.1	A-108863.1	AfcAfuAfuAfaAfcUfaCfaAfgUfcAfaAf	A-108399.2	uUfuGfaCfuUfgUfaguUfuAfuAfuGfusAfsG
AD-52679.1	A-108824.1	UfuUfaUfuGfuUfcCfuCfuAfgUfuUfuUf	A-108321.2	aAfuAfaCfuAfgAfggaAfcAfaUfaAfasAfsa
AD-52680.1	A-108832.1	UfgCfuAfuGfuUfaAfgCfaCfaGfuGfuAfaAf	A-108337.2	uUfuAfcAfuCfuUfcuaAfcAfuAfgCfasAfsa
AD-52681.1	A-108840.1	GfgGfaCfaUfgGfuCfuUfaAfaGfaCfuUf	A-108353.2	aAfgUfcUfuUfaAfgacCfaUfgUfcCfcsAfsa
AD-52682.1	A-108848.1	UfgAfcAfuAfuUfuCfaAfaAfaCfuCfaAf	A-108369.2	uUfgAfgUfuUfuUfgaaAfuAfuGfuCfasUfsu
AD-52683.1	A-108856.1	AfuCfaGfuCfuUfuUfuAfuGfaUfcUfaUf	A-108385.2	aUfaGfaUfcAfuAfaaaAfgAfcUfgAfusCfsa
AD-52684.1	A-108864.1	CfaUfaUfaAfaCfuAfaCfaGfuCfaAfaAf	A-108401.2	uUfuUfgAfcUfuGfuagUfuUfaUfaUfgsUfsa
AD-52685.1	A-108872.1	CfuUfgAfaCfuCfaAfcUfaCfaAfaCfuUf	A-108417.2	aAfgUfuUfuGfaGfuugAfgUfuCfaAfgsUfsG
AD-52686.1	A-108880.1	CfuAfcUfuCfaAfcAfaAfaAfgUfgAfaAf	A-108433.2	uUfuCfaCfuUfuUfuguUfgAfaGfuAfgsAfsa
AD-52687.1	A-108888.1	AfaGfaGfcAfaCfuAfaCfuAfaCfuUfaAf	A-108449.2	uUfaAfgUfuAfgUfuagUfuGfcUfcUfusCfsu
AD-52688.1	A-108896.1	AfaAfcAfaGfaUfaAfuAfgCfaUfcAfaAf	A-108465.2	uUfuGfaUfgCfuAfuuaUfcUfuGfuUfusUfsu
AD-52689.1	A-108904.1	GfcAfuAfgUfcAfaAfaAfaAfaGfaAfaUf	A-108481.2	aUfuUfcUfuUfuAfuuuGfaCfuAfuGfcsUfsG
AD-52690.1	A-108865.1	AfuAfuAfaAfcUfaAfaAfgUfcAfaAfaAf	A-108403.2	uUfuUfuGfaCfuUfguaGfuUfuAfuAfusGfsu
AD-52691.1	A-108873.1	GfaAfcUfcAfaCfuCfaAfaAfcUfuGfaAf	A-108419.2	uUfcAfaGfuUfuUfgagUfuGfaGfuUfcsAfsa
AD-52692.1	A-108881.1	UfaCfuUfcAfaCfaAfaAfaGfuGfaAfaUf	A-108435.2	aUfuUfcAfcUfuUfuugUfuGfaAfgUfasGfsa
AD-52693.1	A-108889.1	AfgAfgCfaAfcUfaAfaCfuAfaUfuAfaUf	A-108451.2	aUfuAfaGfuUfaGfuuaGfuUfgCfuCfusUfsc
AD-52694.1	A-108897.1	GfaUfaAfuAfgCfaUfcAfaAfgAfcCfuUf	A-108467.2	aAfgGfuCfuUfuGfaugCfuAfuUfaUfcsUfsu
AD-52695.1	A-108905.1	CfaUfaGfuCfaAfaUfaAfaAfgAfaAfuAf	A-108483.2	uAfuUfuCfuUfuUfaaUfgAfcUfaUfgsCfsu
AD-52696.1	A-108866.1	UfaUfaAfaCfuAfcAfaGfuCfaAfaAfaUf	A-108405.2	aUfuUfuUfgAfcUfcUfuAfgUfuUfaUfasUfsG
AD-52697.1	A-108874.1	AfaCfuCfaAfcUfcAfaAfaCfuUfgAfaAf	A-108421.2	uUfuCfaAfgUfuUfugaGfuUfgAfgUfusCfsa

AD-52698.1	A-108882.1	AfcUfuCfaAfcAfaAfaAfgUfgAfaAfuAf	A-108437.2	uAfuUfuCfaCfuUfuuuGfuUfgAfaGfusAfsG
AD-52699.1	A-108890.1	GfaGfcAfaCfuAfaCfuAfaCfuUfaAfuUf	A-108453.2	aAfuUfaAfgUfuAfguuAfgUfuGfcUfcsUfsu
AD-52700.1	A-108898.1	AfaCfcAfaCfaGfcAfaUfgUfcAfaAfuAf	A-108469.2	uAfuUfuGfaCfuAfgcUfgUfuGfgUfusUfsa
AD-52701.1	A-108906.1	AfgUfcAfaAfuAfaAfaGfaAfaUfaGfaAf	A-108485.2	uUfcUfaUfuUfcUfuuuAfuUfuGfaCfusAfsu
AD-52702.1	A-108867.1	AfgUfcAfaAfaAfuUfgAfaAfgGfuAfaAf	A-108407.2	uUfuAfcCfuCfuUfcauUfuUfuGfaCfusUfsg
AD-52703.1	A-108875.1	CfuUfgAfaAfgCfcUfcCfuAfgAfaGfaAf	A-108423.2	uUfcUfuCfuAfgGfaggCfuUfuCfaAfgsUfsu
AD-52704.1	A-108883.1	CfuUfcAfaCfaAfaAfaGfuGfaAfaUfaUf	A-108439.2	aUfaUfuUfcAfcUfuuuUfgUfuGfaAfgsUfsa
AD-52705.1	A-108891.1	CfaAfcUfaAfcUfaAfaCfuAfaUfuCfaAf	A-108455.2	uUfgAfaUfuAfaGfuuaGfuUfaGfuUfgsCfsu
AD-52706.1	A-108899.1	AfcCfaAfcAfgCfaUfaGfuCfaAfaUfaAf	A-108471.2	uUfaUfuUfgAfcUfaugCfuGfuUfgGfusUfsu
AD-52707.1	A-108907.1	GfaAfcCfcAfcAfgAfaAfuUfuCfuCfuAf	A-108487.2	uAfgAfgAfaAfuUfucuGfuGfgGfuUfcsUfsu
AD-52708.1	A-108868.1	GfaAfuAfuGfuCfaCfcUfgAfaCfuCfaAf	A-108409.2	uUfgAfgUfuCfaAfgugAfcAfuAfuUfcsUfsu
AD-52709.1	A-108876.1	UfgAfaAfgCfcUfcCfuAfgAfaGfaAfaAf	A-108425.2	uUfuUfcUfuCfuAfggaGfgCfuUfuCfasAfsG
AD-52710.1	A-108884.1	UfuCfaAfcAfaAfaAfgUfgAfaAfuAfuUf	A-108441.2	aAfuAfuUfuCfaCfuuuUfuGfuUfgAfasGfsu
AD-52711.1	A-108892.1	AfaCfuAfaCfuAfaCfuUfaAfuUfcAfaAf	A-108457.2	uUfuGfaAfuUfaAfguuAfgUfuUfcsUfsc
AD-52712.1	A-108900.1	CfcAfaCfaGfcAfuUfgUfcAfaAfuAfaAf	A-108473.2	uUfuAfuUfuGfaCfuauGfcUfgUfuGfgsUfsu
AD-52713.1	A-108908.1	AfaCfcCfaCfaGfaAfaUfuUfcUfuUfaUf	A-108489.2	aUfaGfaGfaAfaUfuucUfgUfgUfusCfsu
AD-52714.1	A-108869.1	UfgUfcAfcUfuGfaAfaUfcAfaCfuCfaAf	A-108411.2	uUfgAfgUfuGfaGfuucAfaGfuGfaCfasUfsa
AD-52715.1	A-108877.1	GfaAfaGfcCfuCfcUfaGfaAfgAfaAfaAf	A-108427.2	uUfuUfuCfuUfclUfaggAfgGfcUfuUfcsAfsa
AD-52716.1	A-108885.1	AfaUfaUfuUfaGfaAfgAfgCfaAfcUfaAf	A-108443.2	uUfaGfuUfgCfuCfuucUfaAfaUfaUfusUfsc
AD-52717.1	A-108893.1	AfcUfaAfcUfaAfcUfuAfaUfuCfaAfaAf	A-108459.2	uUfuUfgAfaUfaAfaUfaGfuUfaGfusUfsg
AD-52718.1	A-108901.1	CfaAfcAfgCfaUfaAfgGfuCfaAfaUfaAfaAf	A-108475.2	uUfuUfaUfuUfgAfcuaUfgCfuGfuUfgsGfsu

AD-52719.1	A-108909.1	CfcAfcAfgAfaAfuUfuUfcuAfuCfuUf	A-108491.2	aAfgAfuAfgAfgAfaaUfuCfuGfuGfgsGfsu
AD-52720.1	A-108870.1	GfuCfaCfuUfgAfaAfcuAfaAfaAf	A-108413.2	uUfuGfaGfuUfgAfguuCfaAfgUfgAfcAfsu
AD-52721.1	A-108878.1	CfuCfcUfaGfaGfaAfaAfaUfuCfuAf	A-108429.2	uAfgAfaUfuUfuUfucuUfcUfaGfgAfgsGfsc
AD-52722.1	A-108886.1	AfuUfuAfgAfaGfaGfaAfaCfuAfaCfuAf	A-108445.2	uAfgUfuAfgUfuGfucUfuCfuAfaAfsu
AD-52723.1	A-108894.1	CfuAfaCfuAfaCfuUfaAfuUfaAfaAf	A-108461.2	aUfuUfuGfaAfaUfaaagUfuAfgUfuAfgsUfsu
AD-52724.1	A-108902.1	CfaGfcAfuAfgUfcAfaAfaAfaGfaAf	A-108477.2	uUfcUfuUfuAfuUfugaCfuAfuGfcUfgsUfsu
AD-52725.1	A-108910.1	GfaAfaUfaAfgAfaAfuGfuAfaAfaCfaUf	A-108493.2	aUfgUfuUfuAfaAfuUfuUfaUfuUfcsAfsu
AD-52726.1	A-108871.1	UfcAfcUfuGfaAfcUfaAfaCfuCfaAfaAf	A-108415.2	uUfuUfgAfgUfuGfaguUfcAfaGfuGfasCfsa
AD-52727.1	A-108879.1	UfcUfaCfuUfcAfaAfaAfaGfuGfaAf	A-108431.2	uUfcAfcUfuUfuUfguuGfaAfgUfaGfasAfsu
AD-52728.1	A-108887.1	UfuUfaGfaAfgAfgCfaAfcUfaAfaUfaAf	A-108447.2	uUfaGfuUfaGfuUfgcuCfuUfcUfaAfasUfsa
AD-52729.1	A-108895.1	AfaAfaCfaAfgAfuAfaUfaGfaAfuCfaAf	A-108463.2	uUfgAfuGfcUfaUfaUfuUfgUfuUfcsUfsc
AD-52730.1	A-108903.1	AfgCfaUfaGfuCfaAfaUfaAfaAfgAfaAf	A-108479.2	uUfuCfuUfuUfaUfuugAfcUfaUfgCfusGfsu
AD-52731.1	A-108958.1	AfgAfcCfcAfgCfaAfcUfcUfaAfaGfuUf	A-108495.2	aAfcUfuGfaGfaGfuugCfuGfgGfuCfusGfsa
AD-52732.1	A-108966.1	AfgUfcCfaUfgGfaAfcUfaUfaUfaCfaAf	A-108511.2	uUfgAfaUfaAfaUfgucCfaUfgGfaCfusAfcsc
AD-52733.1	A-108974.1	GfaUfgGfaUfcAfaAfaAfaCfuUfaAfaUf	A-108527.2	aUfuGfaAfgUfuUfuguGfaUfcCfaUfcsUfsa
AD-52734.1	A-108982.1	CfuAfgAfgAfaGfaUfaUfaCfuCfaAfaAf	A-108543.2	uAfuGfgAfgUfaUfaucUfuCfuAfgsGfsc
AD-52735.1	A-108990.1	AfaAfgAfcAfaCfaAfaCfaUfuAfuUfuUf	A-108559.2	aAfuAfaAfaUfgUfuugUfuGfuCfuUfusCfsc
AD-52736.1	A-108998.1	CfaUfuAfuUfgGfaAfaUfaUfuCfuUfuUf	A-108575.2	aAfaAfgAfaUfaUfucaAfaAfuAfaUfgsUfsu
AD-52737.1	A-108959.1	GfaCfcCfaGfcAfaAfcUfuCfaAfgUfuUf	A-108497.2	aAfaCfuUfgAfgAfguuGfcUfgGfgUfcsUfsg
AD-52739.1	A-108975.1	GfgAfuCfaCfaAfaAfaUfuCfaAfaGfaAf	A-108529.2	uUfcAfuUfgAfaGfuuuUfgUfgAfuCfcsAfsu
AD-52740.1	A-108983.1	GfaAfgAfuAfaAfcUfcCfaUfaGfuGfaAf	A-108545.2	uUfcAfcUfaUfgGfaguAfaUfuCfuUfcsUfsc

AD-52741.1	A-108991.1	GfaCfaAfcAfaAfcAfaUfaUfaUfuGfaAf	A-108561.2	uUfcAfaUfaUfaAfuGfuUfuGfuUfgUfcsUfsu
AD-52742.1	A-108999.1	GfgGfaAfaUfaCfGfaAfaCfaCfaCfuAf	A-108577.2	uAfgUfuGfgUfuUfcguGfaUfuUfcCfcsAfsa
AD-52743.1	A-108960.1	AfcCfcAfgCfaAfcUfcUfaCfaGfuUfuUf	A-108499.2	aAfaAfcUfuGfaGfaguUfgCfuGfgGfusCfsu
AD-52744.1	A-108968.1	GfgAfcAfuUfaAfuUfaCfaCfaUfcGfaAf	A-108515.2	uUfcGfaUfgUfuGfaaUfaAfuGfuCfcsAfsu
AD-52745.1	A-108976.1	GfaUfcAfcAfaAfcUfcUfaCfaUfgAfaAf	A-108531.2	uUfuCfaUfuGfaAfguuUfuGfuGfaUfcsCfsa
AD-52746.1	A-108984.1	AfcUfcCfaUfaGfUfgfaAfcGfaAfuCfuAf	A-108547.2	uAfgAfuUfgCfuUfcacUfaUfgGfaGfusAfsu
AD-52747.1	A-108992.1	AfcAfaCfaAfaCfaUfaAfuUfgAfaUf	A-108563.2	aUfuCfaAfuAfaAfaugUfuUfgUfuGfusCfsu
AD-52748.1	A-109000.1	GfgAfaAfuCfaCfGfaAfaAfcCfaAfcUfaUf	A-108579.2	aUfaGfuUfgGfuUfucgUfgAfuUfuCfcsCfsa
AD-52749.1	A-108961.1	CfcCfaGfcAfaCfuUfcUfaAfcAfgUfuUfuUf	A-108501.2	aAfaAfaCfuUfgAfgagUfuGfcUfgGfgsUfsc
AD-52750.1	A-108969.1	GfaCfaUfaUfaUfuUfcUfaAfcAfuCfaAfaUf	A-108517.2	aUfuCfgAfuGfuUfgaaUfuAfaUfgUfcsCfsa
AD-52751.1	A-108977.1	AfaCfgUfgGfgAfgAfaCfaCfaAfaAfuAf	A-108533.2	uAfuUfuGfuAfgUfucuCfcCfaCfgUfusUfsc
AD-52752.1	A-108985.1	CfuCfcAfuAfgUfgAfaGfcAfaUfcUfaAf	A-108549.2	uUfaGfaUfuGfcUfucaCfuAfuGfgAfgsUfsa
AD-52753.1	A-108993.1	CfaAfcAfaAfcAfuUfuUfaUfuGfaAfuAf	A-108565.2	uAfuUfcAfaUfaUfaaGfuUfuGfuUfgsUfsc
AD-52754.1	A-109001.1	GfaAfaUfcAfcGfaAfaCfaCfaCfuAfuAf	A-108581.2	uAfuAfgUfuGfgUfuucGfuGfaUfuUfcsCfsc
AD-52755.1	A-108962.1	CfuCfuCfaAfgUfuUfuUfcAfuGfuCfuAf	A-108503.2	uAfgAfcAfuGfaAfaaaCfuUfgAfgAfgsUfsu
AD-52756.1	A-108970.1	AfcAfuUfaAfuUfcAfaCfaUfcGfaAfuAf	A-108519.2	uAfuUfcGfaUfgUfugaAfuUfaAfuGfusCfsc
AD-52757.1	A-108978.1	GfgGfaGfaAfcUfaAfcAfaUfaUfgGfuUf	A-108535.2	aAfcCfaUfaUfuUfguaGfuUfcCfcsAfsa
AD-52758.1	A-108986.1	UfcCfaUfaGfuGfaAfgCfaAfuCfuAfaUf	A-108551.2	aUfuAfgAfuUfgCfuucAfcUfaUfgGfasGfsu
AD-52759.1	A-108994.1	AfaCfaAfaCfaUfuAfuAfuUfgAfaUfaUf	A-108567.2	aUfaUfuCfaAfaAfaaaUfgUfuUfgUfusGfsu
AD-52760.1	A-109002.1	UfgGfcAfaUfgUfcCfcCfaAfuGfcAfaUf	A-108583.2	aUfuGfcAfuUfgGfggaCfaUfuGfcCfasGfsu
AD-52761.1	A-108963.1	UfcAfgGfuAfgUfcCfaUfgGfaCfaUfuAf	A-108505.2	uAfaUfgUfcCfaUfggaCfuAfcCfuGfasUfsa

AD-52762.1	A-108971.1	UfuAfaUfuCfaAfcAfaUfcGfaUfaGfaUf	A-108521.2	aUfcUfaUfuCfGafugUfGfAfaUfuAfasUfsg
AD-52763.1	A-108979.1	GfgAfgAfaCfuAfcAfaAfuAfuGfgUfuUf	A-108537.2	aAfaCfaAfaUfuUfuguAfgUfuCfuCfcsCfsa
AD-52764.1	A-108987.1	CfcAfuAfgUfgAfaGfcAfaUfcUfaAfuUf	A-108553.2	aAfuUfaGfaUfuGfcuuCfaCfuAfuGfsgAfsa
AD-52765.1	A-108995.1	AfcAfaAfcAfuUfaUfaUfuGfaAfaUfuUf	A-108569.2	aAfuAfuUfcAfaUfaUfaUfuGfuUfuGfusUfsg
AD-52766.1	A-109003.1	AfaUfgCfaAfuCfcCfcGfaAfaAfaAfaAf	A-108585.2	uUfuGfuUfuUfcCfGggAfuUfgCfaUfusGfsg
AD-52767.1	A-108964.1	CfaGfgUfaGfuCfcAfaUfgAfcAfuUfaAf	A-108507.2	uUfaAfuGfuCfcAfuGgAfcUfaCfcUfsgAfsu
AD-52768.1	A-108972.1	UfuCfaAfcAfuCfGfAfaUfaGfaUfgGfaUf	A-108523.2	aUfcCfaUfcUfaUfucgAfuGfuUfgAfasUfsu
AD-52769.1	A-108980.1	GfuUfgGfgCfcUfaAfcGfaGfaAfaAfuUf	A-108539.2	uAfuAfuCfuUfcUfuaGfgCfcCfaAfcCfsa
AD-52770.1	A-108988.1	CfaUfaGfuGfaAfcGfCfaAfuCfuAfaUfuUf	A-108555.2	uAfaUfaAfgAfuUfgcuUfcAfcUfaUfsgGfsa
AD-52771.1	A-108996.1	AfaCfaUfuAfuUfuUfgAfaUfaUfuCfuUf	A-108571.2	aAfgAfaUfaUfuCfaauAfaUfaUfgUfusUfsg
AD-52772.1	A-109004.1	GfcAfaUfcCfcGfAfaAfaCfaAfaGfaUf	A-108587.2	aUfcUfuUfgUfuUfuccGfgGfaUfuGfcsAfsu
AD-52773.1	A-108965.1	GfgUfaGfuCfcAfuUfgfgAfcAfuUfaAfuUf	A-108509.2	aAfuUfaAfuGfuCfcauGfgAfcUfaCfcsUfsg
AD-52774.1	A-108973.1	AfuCfgAfaUfaGfaUfgGfaUfcAfcAfaAf	A-108525.2	uUfuGfuGfaUfcCfaucUfaUfuCfgAfusGfsu
AD-52775.1	A-108981.1	CfcUfaGfaGfaAfcGfAfuAfuAfuUfcCfaUf	A-108541.2	aUfgGfaGfuAfuAfuUfuUfcUfaGfsgCfsc
AD-52776.1	A-108989.1	GfuUfgGfaAfgAfcUfgGfaAfaGfaCfaAf	A-108557.2	uUfgUfcUfuUfcCfaguCfuUfcCfaAfcUfsc
AD-52777.1	A-108997.1	AfcAfuUfaUfaUfuUfgGfaAfuAfuUfcUfuUf	A-108573.2	aAfaGfaAfuAfuUfcaaUfaUfaAfuGfusUfsu
AD-52778.1	A-109005.1	CfaAfuCfcCfgGfAfaAfaAfcAfaAfgAfuUf	A-108589.2	aAfuCfuUfuGfuUfuucCfgGfgAfuUfsgCfsa
AD-52779.1	A-109013.1	CfuAfcUfuGfgGfAfuUfcAfcAfaAfgCfaAf	A-108605.2	uUfgCfuUfuGfuGfaucCfcAfaGfuAfgAfsa
AD-52780.1	A-109021.1	AfcAfaCfcUfaAfaUfuUfgGfuAfaAfuAfuUf	A-108621.2	uAfuAfuUfuAfcCfaauUfaGfgUfuGfusUfsu
AD-52781.1	A-109029.1	AfuCfcAfuCfcAfaUfcGfaUfuCfaGfaAf	A-108637.2	uUfcUfgAfaUfcUfguuGfgAfuGfgAfsCfsa
AD-52782.1	A-109037.1	AfaCfuGfaGfgCfaAfaUfuUfaAfaAfgAf	A-108653.2	uCfuUfuUfaAfaUfuugCfcUfcAfgUfusCfsa

AD-52783.1	A-109045.1	AfgAfgUfaUfgUfgUfaAfaAfaUfcUfgUf	A-108669.2	aCfaGfaUfuUfuUfacaCfaUfaCfuCfusGfsu
AD-52784.1	A-109006.1	AfaUfcCfcGfgAfAfaCfaAfaGfaUfuUf	A-108591.2	aAfaUfcUfuUfgUfuuuCfcGfgGfaUfusGfsc
AD-52785.1	A-109014.1	UfaCfuUfgGfgAfUfcfaCfaAfaGfcAfaAf	A-108607.2	uUfuGfcUfuUfgUfgauCfcCfaAfgUfasGfsa
AD-52786.1	A-109022.1	CfaAfcCfuAfaAfuUfgUfaAfaUfaUfaAf	A-108623.2	uUfaUfaUfuUfaCfcauUfuAfgGfuUfgsUfsu
AD-52787.1	A-109030.1	UfuGfaAfuGfaAfcUfgAfgGfcAfaAfuUf	A-108639.2	aAfuUfuGfcCfuCfaguUfcAfuUfcAfasAfsf
AD-52788.1	A-109038.1	AfcUfgAfgGfcAfAfaUfuAfaAfaGfgAf	A-108655.2	uCfcUfuUfuAfaAfuuuGfcCfuCfaGfusUfsc
AD-52789.1	A-109046.1	GfaGfuAfuGfuGfuAfaAfaAfuCfuGfuAf	A-108671.2	uAfcAfgAfuUfuUfuacAfcAfuAfcUfcsUfsg
AD-52791.1	A-109015.1	AfcUfuGfgGfaUfcAfaAfaAfgCfaAfaAf	A-108609.2	uUfuUfgCfuUfuGfugaUfcCfcAfaGfusAfsf
AD-52792.1	A-109023.1	AfuGfgUfaAfaUfaUfaAfaAfaAfcCfaAf	A-108625.2	uUfgGfuUfuGfuUfaUfaUfaCfcAfusUfsu
AD-52793.1	A-109031.1	UfgAfaUfgAfaCfuGfaGfgCfaAfaUfuUf	A-108641.2	aAfaUfuUfgCfcUfcagUfuCfaUfuCfasAfsa
AD-52794.1	A-109039.1	CfuGfaGfgCfaAfaUfuUfaAfaAfgGfcAf	A-108657.2	uGfcCfuUfuUfaAfaUfuUfgCfcUfcAfgsUfsu
AD-52795.1	A-109047.1	AfgUfaUfgUfgUfaAfaAfaUfcUfgUfaAf	A-108673.2	uUfaCfaGfaUfuUfuuaCfaCfaUfaCfusCfsu
AD-52796.1	A-109008.1	GfaAfaAfcAfaAfgAfuUfuGfgUfgUfuUf	A-108595.2	aAfaCfaCfcAfaAfuUfuGfuUfuUfcsCfsg
AD-52797.1	A-109016.1	AfgUfgUfgGfaGfaAfaAfaCfaCfcUfaAf	A-108611.2	uUfaGfgUfuGfuUfuucUfcCfaCfaCfusCfsa
AD-52798.1	A-109024.1	GfuCfuCfaAfaAfUfgGfaAfaGfgUfuAfuAf	A-108627.2	uAfaAfaCfcUfuCfcauUfuUfgAfgAfcUfsu
AD-52799.1	A-109032.1	GfaAfuGfaAfaCfuGfaAfaAfaUfuUfuAf	A-108643.2	uAfaAfuUfuGfcCfucaGfuUfcAfuUfcsAfsa
AD-52800.1	A-109040.1	UfgAfgGfcAfaAfuUfuAfaAfaGfgCfaAf	A-108659.2	uUfgCfcUfuUfuAfaUfuGfcCfuCfasGfsu
AD-52801.1	A-109048.1	GfuAfuGfuGfaAfaAfaCfuGfuAfaUf	A-108675.2	aUfuAfcAfgAfuUfuuuAfcAfaAfcUfsc
AD-52802.1	A-109009.1	AfaAfaCfaAfaGfaUfuUfgGfuGfuUfuUf	A-108597.2	aAfaAfcAfcCfaAfaucUfuUfgUfuUfusCfsc
AD-52803.1	A-109017.1	GfuGfuGfgAfgAfaAfaCfaAfcCfuAfaAf	A-108613.2	uUfuAfgGfuUfgUfuuuCfuCfcAfcAfcUfsc
AD-52804.1	A-109025.1	AfuGfgAfaGfgUfuAfaUfcUfaUfaAf	A-108629.2	uUfaUfaGfaGfuAfaaaCfcUfuCfcAfusUfsu

AD-52805.1	A-109033.1	AfaUfgAfaCfuGfAfgGfCfaAfaUfuUfaAf	A-108645.2	uUfaAfaUfuUfgCfcucAfgUfuCfaUfusCfsa
AD-52806.1	A-109041.1	GfaGfgCfaAfaUfuUfaAfaAfgGfcAfaUf	A-108661.2	aUfuGfcCfuUfuUfaaaUfuUfgCfcUfcsAfsa
AD-52807.1	A-109049.1	UfaUfgUfgUfaAfaAfaUfcUfgUfaAfaUf	A-108677.2	uAfuUfaCfaGfaUfuuuUfaCfaCfaUfasCfsu
AD-52808.1	A-109010.1	AfcAfaAfgAfuUfuUfgUfgUfuUfuCfuAf	A-108599.2	uAfgAfaAfaCfaCfaaAfuCfuUfuGfusUfsu
AD-52809.1	A-109018.1	UfgUfgGfaGfaAfaAfaCfaCfuUfaAfaUf	A-108615.2	aUfuUfaGfgUfuGfuuuUfcUfcCfaCfasCfsu
AD-52810.1	A-109026.1	UfgGfaAfgGfuUfaUfaCfuCfuAfaAfaUf	A-108631.2	uUfuAfaAfaAfgAfgUfaaAfcCfuUfcCfasUfsu
AD-52811.1	A-109034.1	AfuGfaAfcUfgAfgGfcAfaAfaUfuAfaUf	A-108647.2	uUfuAfaAfaUfuGfcuCfaGfuUfcAfuUfsc
AD-52812.1	A-109042.1	AfgGfcAfaAfuUfuUfaAfaAfgGfcAfaUf	A-108663.2	uAfuUfgCfcUfuUfuuaAfuUfuGfcCfusCfsa
AD-52813.1	A-109011.1	AfaGfaUfuUfgGfuUfgUfuUfcUfaCfuUf	A-108601.2	aAfgUfaGfaAfaAfcacCfaAfaUfcUfusUfsg
AD-52814.1	A-109019.1	AfaAfcAfaCfcUfaAfaUfgGfuAfaAfuAf	A-108617.2	uAfuUfuAfcCfaUfuuaGfgUfuGfuUfusUfsc
AD-52815.1	A-109027.1	AfuAfcUfcUfaUfaAfaAfuCfaAfcCfaAf	A-108633.2	uUfgGfuUfgAfuUfuuaUfaGfaGfuAfuAfsa
AD-52816.1	A-109035.1	UfgAfaCfuGfaGfcGfaAfaUfuUfaAfaUf	A-108649.2	uUfuUfaAfaUfuUfgccUfcAfgUfuCfasUfsu
AD-52817.1	A-109043.1	GfgCfaAfaUfuUfaAfaAfgGfcAfaUfaUf	A-108665.2	uUfaUfuGfcCfuUfuuaAfaUfuUfgCfcUfsc
AD-52818.1	A-109012.1	UfuUfuCfuAfcUfuUfgGfgGfaUfcAfaAfaUf	A-108603.2	uUfuGfuGfaUfcCfaaGfuAfgAfaAfasCfsa
AD-52819.1	A-109020.1	AfaCfaAfcCfuAfaAfaUfgGfgUfaAfaUf	A-108619.2	aUfaUfuUfaCfaAfuuuAfgGfuUfgUfusUfsu
AD-52820.1	A-109028.1	UfaCfuCfuAfaAfaAfaUfcAfaCfcAfaAf	A-108635.2	uUfuGfgUfuGfaUfuuuAfuAfgAfgUfasUfsa
AD-52821.1	A-109036.1	GfaAfcUfgAfgGfcAfaAfaUfuAfaAfaUf	A-108651.2	uUfuUfuAfaAfuUfugCfuCfaGfuUfcsAfsu
AD-52822.1	A-109044.1	CfaGfaGfuAfuGfuUfgGfuAfaAfaAfuCfuUf	A-108667.2	aAfgAfuUfuUfuAfcacAfuAfcUfcUfgsUfsg

Table 11. Results of single dose screen using ANGPTL3 GaNAc-conjugated dsRNA

Modified siRNAs were tested by transfection in Hep3b cells and by free-uptake in primary cynomolgus monkey (PCH) cells at the above-stated doses.

DUPLICATE ID	10nM (RNAimax)	0.1nM (RNAimax)	500nM PCH Celsis (FU)	100nM PCH Celsis (FU)	10nM PCH Celsis (FU)	STDEV 10nM (RNAimax)	STDEV 0.1nM (RNAimax)	STDEV 500nM (FU)	STDEV 100nM (FU)	STDEV 10nM (FU)
AD1955/naïve FU	0.93	0.93	1.01	0.91	1.17	0.02	0.08	0.09	0.00	0.07
AD1955/naïve FU	1.02	1.09	1.07	1.07	0.92	0.06	0.04	0.02	0.00	0.03
AD1955/naïve FU	1.06	0.99	0.93	1.02	0.93	0.03	0.00	0.09	0.01	0.02
AD1955/naïve FU	1.05	0.90	1.05	1.03	1.03	0.04	0.02	0.01	0.05	0.01
AD1955/naïve FU	1.06	1.08	0.90	0.97	1.03	0.02	0.01	0.02	0.04	0.09
AD1955/naïve FU	0.90	1.03	1.05	1.00	0.94	0.04	0.03	0.01	0.04	0.05
AD-45165 (TTR)	0.91	0.98	1.06	0.98	0.96	0.05	0.01	0.05	0.00	0.00
AD-52953.1	0.06	0.34	0.15	0.17	0.46	0.00	0.01	0.00	0.01	0.01
AD-52954.1	0.09	0.39	0.17	0.20	0.55	0.00	0.01	0.00	0.01	0.00
AD-52955.1	0.11	0.59	0.38	0.41	0.75	0.01	0.04	0.02	0.01	0.12
AD-52956.1	0.31	0.94	0.79	0.94	1.17	0.01	0.00	0.02	0.06	0.02
AD-52957.1	0.13	0.61	0.35	0.38	0.73	0.01	0.00	0.01	0.00	0.04
AD-52958.1	0.19	0.74	0.66	0.71	0.97	0.01	0.01	0.02	0.07	0.06

AD-52960.1	0.14	0.59	0.31	0.32	0.55	0.01	0.01	0.00	0.02	0.02
AD-52961.1	0.05	0.66	0.27	0.24	0.49	0.00	0.00	0.00	0.02	0.02
AD-52962.1	0.83	0.89	1.03	1.02	1.26	0.02	0.05	0.07	0.07	0.07
AD-52963.1	0.07	0.72	0.46	0.56	0.91	0.00	0.00	0.00	0.00	0.06
AD-52964.1	0.13	0.73	0.41	0.47	0.68	0.01	0.03	0.02	0.03	0.01
AD-52965.1	0.07	0.44	0.16	0.18	0.43	0.00	0.01	0.00	0.01	0.01
AD-52966.1	0.12	0.76	0.67	0.72	0.96	0.00	0.02	0.05	0.01	0.01
AD-52967.1	0.10	0.75	0.44	0.58	0.89	0.01	0.04	0.02	0.03	0.04
AD-52968.1	1.01	0.96	0.87	0.91	1.15	0.00	0.01	0.09	0.03	0.02
AD-52969.1	0.04	0.46	0.22	0.29	0.59	0.00	0.00	0.01	0.02	0.04
AD-52970.1	0.06	0.45	0.27	0.30	0.51	0.00	0.00	0.01	0.02	0.00
AD-52971.1	0.08	0.55	0.20	0.22	0.45	0.00	0.00	0.01	0.02	0.05
AD-52972.1	0.10	0.73	0.41	0.49	0.81	0.00	0.01	0.01	0.02	0.01
AD-52973.1	0.11	0.73	0.36	0.46	0.75	0.01	0.01	0.03	0.02	0.02
AD-52974.1	1.00	0.95	1.00	1.09	1.27	0.01	0.01	0.08	0.05	0.06
AD-52975.1	0.07	0.54	0.25	0.34	0.66	0.00	0.01	0.01	0.01	0.03
AD-52976.1	0.17	0.59	0.35	0.41	0.65	0.00	0.02	0.04	0.01	0.01
AD-52977.1	0.07	0.45	0.16	0.25	0.50	0.01	0.02	0.00	0.02	0.03
AD-52978.1	0.10	0.72	0.39	0.53	0.77	0.00	0.02	0.00	0.08	0.03
AD-52979.1	0.54	0.92	0.99	1.12	1.28	0.01	0.02	0.02	0.04	0.05
AD-52980.1	0.29	0.85	0.67	0.85	1.03	0.01	0.01	0.05	0.05	0.04

AD-52981.1	0.07	0.44	0.20	0.26	0.59	0.01	0.02	0.00	0.00	0.03
AD-52982.1	0.28	0.87	0.67	0.99	1.14	0.01	0.01	0.04	0.00	0.01
AD-52983.1	0.06	0.40	0.14	0.40	0.46	0.00	0.00	0.01	0.05	0.02
AD-52984.1	0.29	0.87	0.66	0.74	1.09	0.01	0.02	0.01	0.00	0.00
AD-52985.1	0.72	0.87	0.89	1.18	1.22	0.03	0.00	0.05	0.03	0.16
AD-52986.1	0.08	0.47	0.24	0.30	0.48	0.00	0.02	0.02	0.00	0.06
AD-52987.1	0.16	0.83	0.42	0.73	1.09	0.00	0.00	0.01	0.02	0.02
AD-52988.1	0.11	0.73	0.42	0.60	0.96	0.01	0.04	0.00	0.00	0.10
AD-52989.1	0.05	0.48	0.15	0.42	0.46	0.00	0.02	0.00	0.02	0.00
AD-52990.1	0.14	0.86	0.33	0.45	0.77	0.00	0.01	0.00	0.02	0.05
AD-52991.1	0.16	0.86	0.58	0.69	1.05	0.00	0.00	0.02	0.00	0.02
AD-52992.1	0.08	0.65	0.42	0.56	0.90	0.00	0.01	0.02	0.01	0.00
AD-52993.1	0.13	0.87	0.53	0.76	1.08	0.02	0.03	0.04	0.04	0.00
AD-52994.1	0.10	0.52	0.28	0.33	0.53	0.01	0.00	0.02	0.00	0.01
AD-52995.1	0.06	0.56	0.19	0.41	0.60	0.00	0.01	0.04	0.02	0.05
AD-52996.1	0.09	0.68	0.26	0.47	0.68	0.00	0.03	0.01	0.04	0.01
AD-52997.1	0.59	1.03	0.87	0.51	1.25	0.05	0.01	0.00	0.01	0.01
AD-52998.1	0.09	0.79	0.44	0.55	0.85	0.00	0.00	0.04	0.03	0.10
AD-52999.1	0.08	0.57	0.17	0.36	0.84	0.01	0.00	0.01	0.02	0.00
AD-53000.1	0.38	0.94	0.58	0.67	0.85	0.01	0.02	0.03	0.03	0.02
AD-53001.1	0.05	0.48	0.21	0.18	0.40	0.00	0.00	0.01	0.00	0.05

AD-53002.1	0.07	0.65	0.43	0.48	0.80	0.00	0.05	0.04	0.01	0.02
AD-53003.1	0.05	0.46	0.31	0.34	0.56	0.01	0.01	0.00	0.02	0.05
AD-53004.1	0.05	0.36	0.29	0.66	0.57	0.00	0.01	0.03	0.35	0.02
AD-53005.1	0.05	0.72	0.32	0.58	0.83	0.01	0.00	0.01	0.29	0.00
AD-53006.1	0.21	0.82	0.66	0.77	1.03	0.01	0.00	0.02	0.07	0.02
AD-53007.1	0.12	0.76	0.55	0.73	0.74	0.01	0.00	0.00	0.08	0.20
AD-53008.1	0.07	0.68	0.28	0.36	0.84	0.00	0.02	0.01	0.05	0.03
AD-53009.1	0.10	0.61	0.48	0.60	0.91	0.00	0.02	0.01	0.01	0.06
AD-53010.1	0.05	0.58	0.47	0.54	0.84	0.00	0.02	0.00	0.02	0.03
AD-53011.1	0.07	0.65	0.29	0.34	0.84	0.00	0.03	0.07	0.01	0.04
AD-53012.1	0.06	0.55	0.36	0.45	0.70	0.00	0.03	0.02	0.02	0.00
AD-53013.1	0.11	0.85	0.59	0.70	1.01	0.00	0.00	0.03	0.03	0.02
AD-53014.1	0.16	0.78	0.61	0.78	1.11	0.00	0.02	0.01	0.05	0.00
AD-53015.1	0.03	0.35	0.25	0.37	0.46	0.01	0.01	0.01	0.00	0.01
AD-53016.1	0.03	0.56	0.40	0.58	1.01	0.00	0.01	0.02	0.06	0.09
AD-53017.1	0.07	0.71	0.64	0.78	0.98	0.00	0.01	0.01	0.05	0.00
AD-53018.1	0.30	0.96	0.75	0.97	1.14	0.00	0.02	0.02	0.03	0.05
AD-53019.1	0.27	0.99	0.77	1.05	1.31	0.00	0.01	0.01	0.04	0.00
AD-53020.1	0.04	0.64	0.32	0.45	0.69	0.00	0.00	0.03	0.02	0.03
AD-53021.1	0.04	0.68	0.36	0.48	0.70	0.01	0.01	0.02	0.07	0.00
AD-53022.1	0.05	0.76	0.36	0.59	1.04	0.01	0.01	0.02	0.03	0.06

AD-53023.1	0.10	0.83	0.69	0.84	0.97	0.01	0.01	0.06	0.02	0.01
AD-53024.1	0.09	0.44	0.23	0.23	0.44	0.00	0.00	0.03	0.01	0.02
AD-53025.1	0.09	0.87	0.58	0.80	1.09	0.00	0.03	0.01	0.04	0.04
AD-53026.1	0.05	0.60	0.35	0.46	0.77	0.01	0.01	0.02	0.05	0.03
AD-53027.1	0.02	0.32	0.26	0.30	0.45	0.00	0.01	0.02	0.03	0.02
AD-53028.1	0.19	0.82	0.77	0.95	1.04	0.01	0.04	0.05	0.01	0.03
AD-53029.1	0.02	0.52	0.32	0.41	0.72	0.00	0.00	0.01	0.02	0.07
AD-53030.1	0.09	0.42	0.15	0.16	0.46	0.00	0.00	0.00	0.00	0.02
AD-53031.1	0.12	0.79	0.63	0.73	1.04	0.02	0.05	0.02	0.04	0.03
AD-53032.1	0.12	0.71	0.41	0.59	0.90	0.01	0.00	0.02	0.04	0.00
AD-53033.1	0.02	0.48	0.20	0.21	0.51	0.00	0.02	0.02	0.01	0.00
AD-53034.1	0.04	0.52	0.31	0.36	0.71	0.00	0.01	0.07	0.02	0.01
AD-53035.1	0.02	0.63	0.34	0.50	0.85	0.00	0.02	0.03	0.00	0.03
AD-53036.1	0.10	0.57	0.31	0.35	0.65	0.01	0.01	0.03	0.03	0.01
AD-53037.1	0.08	0.47	0.27	0.36	0.60	0.00	0.02	0.01	0.03	0.01
AD-53038.1	0.05	0.85	0.48	0.63	1.08	0.00	0.05	0.00	0.02	0.05
AD-53039.1	0.08	0.82	0.45	0.64	0.97	0.00	0.01	0.01	0.03	0.00
AD-53040.1	0.05	0.79	0.46	0.62	0.97	0.01	0.01	0.01	0.05	0.06
AD-53041.1	0.06	0.72	0.59	0.61	0.86	0.00	0.01	0.05	0.06	0.03
AD-53042.1	0.08	0.85	0.30	0.35	0.81	0.01	0.00	0.00	0.03	0.03
AD-53043.1	0.63	1.00	0.92	1.04	1.07	0.03	0.00	0.06	0.03	0.07

AD-53044.1	0.05	0.91	0.35	0.61	0.97	0.01	0.01	0.01	0.01	0.04	0.02
AD-53045.1	0.20	1.00	0.85	1.00	0.98	0.00	0.03	0.04	0.01	0.04	0.04
AD-53046.1	0.07	0.70	0.44	0.62	1.12	0.00	0.01	0.03	0.00	0.00	0.09
AD-53059.1	0.35	1.04	0.75	0.85	0.86	0.01	0.01	0.03	0.02	0.02	0.04
AD-53060.1	0.34	0.85	0.72	0.96	0.82	0.00	0.01	0.02	0.01	0.01	0.02
AD-53061.1	0.17	0.94	0.36	0.37	0.59	0.00	0.00	0.02	0.00	0.00	0.02
AD-53062.1	0.09	0.76	0.43	0.47	0.69	0.01	0.01	0.01	0.03	0.01	0.01
AD-53063.1	0.06	0.48	0.18	0.16	0.25	0.00	0.01	0.01	0.01	0.01	0.02
AD-53064.1	0.07	0.59	0.22	0.22	0.48	0.01	0.02	0.01	0.02	0.02	0.06
AD-53065.1	0.08	0.97	0.45	0.39	0.64	0.01	0.01	0.02	0.01	0.01	0.01
AD-53066.1	0.12	0.99	0.73	0.67	0.88	0.01	0.03	0.01	0.01	0.01	0.05
AD-53067.1	0.12	1.08	0.59	0.60	0.79	0.00	0.12	0.01	0.01	0.01	0.03
AD-53068.1	0.09	0.98	0.46	0.59	0.83	0.00	0.03	0.04	0.07	0.05	0.05
AD-53069.1	0.04	0.69	0.35	0.43	0.59	0.00	0.01	0.01	0.04	0.01	0.01
AD-53070.1	0.17	1.12	0.88	0.83	0.98	0.00	0.01	0.04	0.00	0.01	0.01
AD-53071.1	0.07	0.70	0.23	0.23	0.43	0.00	0.00	0.02	0.00	0.01	0.01
AD-53072.1	0.10	0.90	0.49	0.48	0.75	0.01	0.05	0.00	0.01	0.02	0.02
AD-53073.1	0.07	0.63	0.27	0.30	0.43	0.00	0.00	0.01	0.01	0.01	0.00
AD-53074.1	0.07	0.88	0.46	0.49	0.62	0.01	0.08	0.01	0.06	0.03	0.03
AD-53075.1	0.05	0.76	0.29	0.35	0.50	0.01	0.01	0.00	0.02	0.03	0.03
AD-53076.1	0.09	0.80	0.31	0.40	0.54	0.01	0.01	0.02	0.05	0.02	0.02

AD-53077.1	0.07	0.96	0.29	0.28	0.49	0.00	0.03	0.00	0.01	0.01
AD-53078.1	0.16	0.95	0.51	0.51	0.70	0.00	0.04	0.01	0.01	0.06
AD-53079.1	0.08	0.96	0.59	0.67	0.83	0.00	0.02	0.01	0.03	0.01
AD-53080.1	0.04	0.63	0.20	0.22	0.43	0.00	0.01	0.00	0.01	0.01
AD-53081.1	0.16	1.02	0.63	0.75	0.87	0.00	0.09	0.00	0.02	0.05
AD-53082.1	0.06	0.94	0.50	0.52	0.66	0.01	0.06	0.02	0.03	0.03
AD-53083.1	0.14	0.87	0.48	0.50	0.80	0.01	0.02	0.04	0.06	0.01
AD-53084.1	0.12	0.95	0.50	0.47	0.72	0.01	0.03	0.04	0.00	0.00
AD-53085.1	0.27	1.02	0.68	0.81	0.99	0.01	0.01	0.01	0.05	0.02
AD-53086.1	0.05	0.60	0.26	0.25	0.48	0.00	0.01	0.03	0.00	0.01
AD-53087.1	0.05	0.56	0.32	0.39	0.53	0.00	0.01	0.01	0.03	0.02
AD-53088.1	0.09	0.89	0.53	0.69	0.87	0.00	0.01	0.02	0.04	0.02
AD-53089.1	0.29	0.97	0.58	0.57	0.78	0.01	0.00	0.02	0.02	0.02
AD-53090.1	0.13	0.86	0.56	0.55	0.73	0.00	0.01	0.01	0.03	0.00
AD-53091.1	0.12	0.82	0.27	0.35	0.66	0.00	0.03	0.03	0.01	0.07
AD-53092.1	0.05	0.66	0.26	0.29	0.42	0.00	0.01	0.02	0.04	0.02
AD-53093.1	0.08	0.68	0.36	0.44	0.55	0.00	0.02	0.03	0.04	0.10
AD-53094.1	0.32	1.00	1.05	0.92	1.11	0.02	0.01	0.01	0.00	0.03
AD-53095.1	0.14	0.77	0.29	0.29	0.49	0.00	0.02	0.00	0.01	0.01
AD-53096.1	0.30	0.96	0.61	0.57	0.73	0.03	0.01	0.02	0.02	0.01
AD-53097.1	0.37	0.97	0.67	0.82	0.86	0.01	0.01	0.01	0.02	0.01

AD-53098.1	0.06	0.65	0.22	0.30	0.43	0.00	0.03	0.03	0.00	0.01
AD-53099.1	0.34	0.99	0.61	0.81	0.91	0.00	0.00	0.04	0.02	0.06
AD-53100.1	0.31	1.04	0.95	1.03	1.00	0.02	0.01	0.06	0.02	0.17
AD-53101.1	0.46	0.93	0.63	0.69	0.78	0.00	0.01	0.04	0.03	0.04
AD-53102.1	0.23	0.80	0.60	0.55	0.66	0.00	0.03	0.01	0.02	0.03
AD-53103.1	0.05	0.61	0.27	0.32	0.50	0.01	0.02	0.00	0.01	0.00
AD-53104.1	0.13	0.80	0.64	0.68	0.77	0.00	0.02	0.03	0.01	0.05
AD-53105.1	0.15	0.77	0.43	0.65	0.77	0.01	0.03	0.02	0.02	0.05
AD-53106.1	0.16	0.87	0.72	0.70	0.83	0.01	0.02	0.00	0.00	0.04
AD-53107.1	0.19	0.95	0.62	0.65	0.90	0.00	0.02	0.01	0.03	0.04
AD-53108.1	0.22	0.94	0.60	0.68	0.81	0.00	0.01	0.00	0.03	0.04
AD-53109.1	0.16	1.01	0.82	0.78	0.96	0.01	0.08	0.04	0.01	0.07
AD-53110.1	0.10	0.86	0.79	0.77	0.94	0.00	0.05	0.03	0.01	0.05
AD-53111.1	0.22	0.78	0.94	0.85	1.04	0.01	0.01	0.01	0.01	0.07
AD-53112.1	0.09	0.96	0.64	0.65	0.86	0.01	0.02	0.07	0.07	0.00
AD-53113.1	0.10	0.97	0.71	0.77	0.88	0.01	0.05	0.01	0.02	0.01
AD-53114.1	0.19	0.83	0.48	0.52	0.66	0.01	0.01	0.02	0.01	0.00
AD-53115.1	0.10	0.59	0.42	0.44	0.66	0.01	0.03	0.04	0.00	0.02
AD-53116.1	0.11	0.87	0.82	0.85	0.95	0.00	0.05	0.05	0.05	0.05
AD-53117.1	0.52	0.64	1.21	1.00	1.08	0.01	0.03	0.09	0.04	0.07
AD-53118.1	0.19	1.04	0.60	0.72	0.94	0.00	0.07	0.02	0.05	0.06

AD-53119.1	0.06	0.77	0.44	0.47	0.64	0.01	0.03	0.00	0.01	0.01
AD-53120.1	0.10	0.97	0.78	0.89	1.01	0.01	0.04	0.05	0.01	0.04
AD-53121.1	0.23	0.80	0.58	0.69	0.90	0.01	0.02	0.04	0.02	0.06
AD-53122.1	0.09	0.80	0.90	0.94	1.09	0.01	0.07	0.02	0.04	0.10
AD-53123.1	0.27	0.74	0.95	0.93	0.97	0.00	0.01	0.03	0.01	0.08
AD-53124.1	0.08	0.81	0.33	0.34	0.61	0.01	0.02	0.00	0.01	0.01
AD-53125.1	0.08	0.82	0.34	0.38	0.58	0.00	0.02	0.00	0.01	0.07
AD-53126.1	0.15	0.95	0.70	0.86	1.06	0.01	0.04	0.05	0.02	0.00
AD-53127.1	0.21	0.81	0.62	0.75	0.91	0.02	0.04	0.01	0.03	0.00
AD-53128.1	0.08	0.79	0.80	1.14	1.09	0.00	0.06	0.04	0.01	0.03
AD-53129.1	0.48	0.78	1.05	1.00	1.10	0.00	0.01	0.06	0.01	0.03
AD-53130.1	0.25	1.08	0.63	0.72	0.88	0.01	0.02	0.00	0.01	0.00
AD-53131.1	0.14	0.96	0.54	0.57	0.81	0.02	0.02	0.05	0.01	0.04
AD-53132.1	0.03	0.54	0.24	0.27	0.49	0.00	0.02	0.02	0.00	0.01
AD-53133.1	0.12	0.76	0.50	0.67	0.93	0.00	0.03	0.01	0.01	0.06
AD-53134.1	0.28	0.86	1.14	0.81	0.97	0.01	0.04	0.05	0.02	0.04
AD-53135.1	0.47	0.74	1.03	0.94	1.09	0.01	0.03	0.04	0.07	0.04
AD-53136.1	0.09	0.99	0.64	0.69	0.94	0.01	0.05	0.01	0.05	0.02
AD-53137.1	0.08	0.75	0.39	0.39	0.59	0.01	0.03	0.00	0.00	0.00
AD-53138.1	0.04	0.71	0.33	0.34	0.60	0.00	0.02	0.00	0.03	0.00
AD-53139.1	0.11	0.76	0.55	0.66	0.84	0.01	0.01	0.06	0.01	0.02

AD-53140.1	0.09	0.71	0.64	0.71	0.86	0.00	0.04	0.01	0.02	0.02
AD-53141.1	0.24	1.09	0.77	0.91	0.93	0.00	0.01	0.00	0.06	0.00
AD-53142.1	0.13	0.95	0.55	0.70	0.82	0.01	0.03	0.03	0.04	0.02
AD-53143.1	0.13	0.91	0.67	0.83	0.94	0.01	0.00	0.03	0.03	0.07
AD-53144.1	0.10	0.72	0.54	0.69	0.84	0.01	0.03	0.01	0.03	0.00
AD-53145.1	0.08	0.72	0.70	0.78	0.88	0.01	0.03	0.01	0.08	0.02
AD-53146.1	0.83	1.07	0.85	0.96	0.98	0.01	0.06	0.00	0.05	0.00
AD-53147.1	0.08	0.56	0.27	0.34	0.47	0.00	0.01	0.01	0.01	0.01
AD-53148.1	0.06	0.81	0.61	0.68	0.74	0.01	0.00	0.03	0.06	0.05
AD-53149.1	0.23	0.86	0.71	0.83	0.92	0.01	0.02	0.06	0.02	0.03
AD-53150.1	0.41	0.70	1.03	1.09	1.03	0.03	0.06	0.03	0.04	0.01

Table 12. Dose response screen results for ANGPTL3 GalNac-conjugated dsRNA sequences

A subset of active siRNAs from the single dose screen (refer to data in Table 11) was tested in a dose response experiment by free uptake in PCH cells. A subset of these
5 active siRNAs was also tested in dose response in Hep3B cells by transfection.

	IC ₅₀ (nM)	
	Free uptake	Transfection (RNAiMax)
AD-53063.1	1.60	0.03
AD-53001.1	2.27	0.01
AD-53015.1	2.90	0.02
AD-52953.1	2.94	0.03
AD-52986.1	3.30	0.03
AD-53024.1	3.42	0.02
AD-53033.1	3.42	0.02
AD-53027.1	3.84	0.01
AD-53030.1	3.90	0.03
AD-53080.1	4.08	0.04
AD-53073.1	4.20	0.05
AD-52965.1	4.63	ND
AD-53092.1	5.37	ND
AD-53132.1	5.54	ND
AD-52983.1	5.55	ND
AD-52954.1	5.67	ND
AD-52961.1	6.37	ND
AD-52994.1	6.43	ND
AD-53098.1	6.58	ND
AD-52970.1	6.71	ND
AD-53075.1	6.74	ND
AD-53086.1	7.08	ND
AD-52971.1	7.50	ND
AD-53064.1	8.33	ND

AD-53147.1	8.34	ND
AD-52969.1	8.86	ND
AD-53077.1	8.98	ND
AD-52981.1	9.44	ND
AD-52977.1	10.45	ND
AD-53071.1	11.19	ND
AD-52960.1	13.03	ND
AD-53095.1	21.31	ND
AD-53103.1	21.92	ND

Table 13. Results of single dose screen using sequences listed in Table 10.

Duplex	10nM	0.1nM	0.025nM	STDEV 10nM	STDEV 0.1nM	STDEV 0.025nM
AD-52719.1	0.01	0.60	0.35	0.000	0.093	0.002
AD-52717.1	0.02	0.31	0.32	0.001	0.014	0.008
AD-52713.1	0.02	0.37	0.36	0.001	0.011	0.007
AD-52711.1	0.03	0.22	0.23	0.005	0.011	0.009
AD-52718.1	0.03	0.31	0.39	0.000	0.025	0.023
AD-52687.1	0.03	0.37	0.38	0.005	0.020	0.002
AD-52699.1	0.03	0.25	0.21	0.002	0.011	0.002
AD-52679.1	0.03	0.51	0.24		0.345	0.008
AD-52689.1	0.03	0.44	0.42	0.000	0.039	0.002
AD-52700.1	0.03	0.56	0.57	0.005	0.044	0.020
AD-52637.1	0.04	0.27	0.23	0.001	0.003	0.005
AD-52730.1	0.04	0.61	0.59	0.005	0.053	0.014
AD-52725.1	0.04	0.62	0.61	0.002	0.027	0.012
AD-52688.1	0.04	0.23	0.20	0.006	0.012	0.011
AD-52661.1	0.04	0.61	0.25	0.001	0.449	0.009
AD-52667.1	0.04	0.28	0.22	0.004	0.018	0.013
AD-52665.1	0.04	0.43	0.48	0.007	0.019	0.009
AD-52638.1	0.04	0.28	0.25	0.000	0.016	0.027
AD-52724.1	0.05	0.86	0.76	0.001	0.055	0.011
AD-52705.1	0.05	0.74	0.65	0.004	0.022	0.016
AD-52708.1	0.05	0.53	0.52	0.001	0.034	0.013
AD-52659.1	0.05	0.56	0.48	0.000	0.000	0.033
AD-52678.1	0.05	0.53	0.53	0.002	0.034	0.000
AD-52670.1	0.05	0.35	0.33	0.002	0.009	0.003
AD-52695.1	0.05	0.63	0.67	0.001	0.012	0.013
AD-52704.1	0.05	0.55	0.53	0.002	0.005	0.034
AD-52683.1	0.05	0.36	0.28	0.002	0.021	0.011
AD-52673.1	0.05	0.22	0.19	0.023	0.010	0.002
AD-52721.1	0.05	0.60	0.53	0.003	0.006	0.029
AD-52710.1	0.05	0.56	0.40	0.007	0.073	0.000

AD-52714.1	0.05	0.40	0.51	0.000	0.016	0.003
AD-52686.1	0.05	0.57	0.60	0.003	0.014	0.000
AD-52645.1	0.05	0.62	0.59	0.004	0.030	0.003
AD-52662.1	0.05	0.55	0.52	0.002	0.030	0.008
AD-52720.1	0.05	0.50	0.46	0.003	0.007	0.011
AD-52654.1	0.05	0.29	0.36	0.008	0.037	0.014
AD-52680.1	0.06	0.48	0.41	0.001	0.019	0.026
AD-52723.1	0.06	0.84	0.76	0.001	0.041	0.004
AD-52726.1	0.06	0.72	0.66	0.003	0.028	0.016
AD-52701.1	0.06	0.67	0.39	0.001	0.003	0.002
AD-52694.1	0.06	0.68	0.59	0.004	0.040	0.012
AD-52685.1	0.06	0.30	0.25	0.002	0.013	0.016
AD-52728.1	0.06	0.80	0.79	0.005	0.043	0.015
AD-52676.1	0.06	0.68	0.67	0.002	0.023	0.029
AD-52639.1	0.06	0.47	0.45	0.000	0.005	0.007
AD-52722.1	0.06	0.81	0.93	0.005	0.004	0.027
AD-52682.1	0.06	0.87	0.73	0.009	0.038	0.014
AD-52660.1	0.07	0.69	0.68	0.002	0.014	0.017
AD-52709.1	0.07	0.89	0.82	0.001	0.013	0.020
AD-52643.1	0.07	0.27	0.24	0.006	0.016	0.012
AD-52696.1	0.07	0.53	0.46	0.003	0.026	0.007
AD-52657.1	0.08	0.60	0.58	0.008	0.030	0.006
AD-52706.1	0.08	0.84	0.78	0.001	0.021	0.019
AD-52653.1	0.08	0.41	0.45	0.057	0.004	0.029
AD-52656.1	0.08	0.65	0.50	0.004	0.022	0.012
AD-52693.1	0.09	0.61	0.62	0.007	0.021	0.018
AD-52692.1	0.09	0.54	0.52	0.023	0.018	0.033
AD-52674.1	0.10	0.79	0.64	0.001	0.008	0.028
AD-52648.1	0.10	0.67	0.53	0.002	0.013	0.028
AD-52651.1	0.10	0.84	0.73	0.000	0.000	0.007
AD-52641.1	0.10	0.62	0.50	0.004	0.172	0.002
AD-52707.1	0.10	0.92	0.81	0.001	0.018	0.032
AD-52671.1	0.11	0.87	0.84	0.005	0.034	0.025

AD-52650.1	0.12	0.88	0.94	0.007	0.013	0.041
AD-52642.1	0.12	0.90	0.76	0.015	0.022	0.004
AD-52675.1	0.13	0.94	0.89	0.001	0.018	0.044
AD-52647.1	0.13	0.80	0.79	0.031	0.008	0.023
AD-52716.1	0.14	0.61	0.69	0.010	0.060	0.013
AD-52649.1	0.14	0.31	0.29	0.136	0.020	0.006
AD-52677.1	0.16	1.01	0.72	0.059	0.040	0.007
AD-52697.1	0.16	0.86	0.77	0.012	0.021	0.015
AD-52715.1	0.17	0.90	0.89	0.005	0.009	0.022
AD-52691.1	0.18	0.93	0.88	0.004	0.036	0.017
AD-52698.1	0.20	0.97	0.87	0.010	0.028	0.000
AD-52672.1	0.20	0.70	0.66	0.170	0.014	0.019
AD-52712.1	0.29	0.92	0.90	0.007	0.036	0.004
AD-52690.1	0.30	0.95	0.85	0.115	0.032	0.004
AD-52640.1	0.30	1.04	0.91	0.018	0.046	0.013
AD-52684.1	0.31	0.90	0.94	0.014	0.018	0.014
AD-52666.1	0.32	1.04	0.91	0.013	0.005	0.004
AD-52703.1	0.32	1.02	0.96	0.016	0.015	0.005
AD-52729.1	0.33	1.02	0.87	0.032	0.020	0.008
AD-52668.1	0.35	0.94	0.90	0.029	0.046	0.026
AD-52681.1	0.57	1.00	0.99	0.003	0.034	0.039
AD-52702.1	0.72	1.02	0.92	0.658	0.060	0.014
AD-52727.1	0.73	1.03	0.91	0.004	0.065	0.027
AD-52663.1	0.78	1.05	0.96	0.027	0.010	0.005
AD-52669.1	0.91	0.91	0.94	0.004	0.049	0.032
AD-1955	0.95	0.84	0.95	0.005	0.021	0.019
AD-1955	0.97	1.07	1.03	0.000	0.021	0.015
AD-1955	1.01	1.08	1.01	0.035	0.011	0.005
mock	1.02	0.96	0.97	0.030	0.037	0.005
AD-1955	1.08	1.03	1.02	0.032	0.051	0.005
AD-52652.1	1.13	1.11	1.02	0.028	0.043	0.020
AD-52658.1	1.33	1.10	0.93	0.091	0.043	0.018
AD-52664.1	1.49	0.95	0.88	0.438	0.019	0.009

AD-52752.1	0.03	0.43	0.69	0.002	0.015	0.017
AD-52741.1	0.03	0.56	0.86	0.001	0.044	0.021
AD-52804.1	0.03	0.49	0.89	0.001	0.002	0.017
AD-52764.1	0.03	0.54	0.79	0.005	0.016	0.078
AD-52770.1	0.03	0.58	0.78	0.000	0.006	0.027
AD-52735.1	0.03	0.31	0.46	0.003	0.031	0.009
AD-52810.1	0.03	0.67	0.86	0.001	0.013	0.025
AD-52759.1	0.03	0.54	0.79	0.000	0.018	0.023
AD-52736.1	0.03	0.51	0.60	0.004	0.012	0.023
AD-52775.1	0.03	0.54	0.73	0.005	0.024	0.022
AD-52758.1	0.03	0.57	0.78	0.001	0.014	0.050
AD-52743.1	0.03	0.45	0.67	0.002	0.018	0.033
AD-52747.1	0.04	0.57	0.84	0.002	0.061	0.058
AD-52819.1	0.04	0.26	0.45	0.005	0.001	0.022
AD-52765.1	0.04	0.68	0.83	0.000	0.013	0.053
AD-52754.1	0.04	0.76	1.00	0.000	0.007	0.015
AD-52787.1	0.05	0.55	0.68	0.001	0.043	0.060
AD-52791.1	0.05	0.70	0.91	0.001	0.014	0.084
AD-52811.1	0.05	0.73	0.84	0.002	0.014	0.058
AD-52817.1	0.05	0.77	0.92	0.003	0.011	0.031
AD-52745.1	0.06	0.62	0.77	0.007	0.021	0.000
AD-52749.1	0.06	0.63	0.88	0.005	0.037	0.043
AD-52740.1	0.06	0.83	0.94	0.007	0.012	0.051
AD-52796.1	0.06	0.72	0.92	0.003	0.021	0.054
AD-52820.1	0.06	0.90	0.87	0.001	0.026	0.064
AD-52809.1	0.06	0.76	0.90	0.001	0.037	0.027
AD-52760.1	0.06	0.81	0.97	0.001	0.056	0.047
AD-52767.1	0.07	0.55	0.55	0.001	0.016	0.013
AD-52734.1	0.07	0.61	0.64	0.004	0.003	0.003
AD-52794.1	0.07	0.94	0.87	0.007	0.014	0.051
AD-52797.1	0.07	0.69	0.87	0.004	0.000	0.038
AD-52737.1	0.08	0.70	0.84	0.004	0.031	0.012
AD-52812.1	0.08	0.75	0.88	0.004	0.000	0.056

AD-52748.1	0.08	0.70	0.89	0.001	0.010	0.009
AD-52782.1	0.08	0.68	0.78	0.004	0.023	0.011
AD-52816.1	0.08	0.71	0.88	0.003	0.042	0.060
AD-52763.1	0.08	0.68	0.77	0.002	0.013	0.026
AD-52788.1	0.08	0.89	1.00	0.004	0.017	0.034
AD-52762.1	0.08	0.78	0.91	0.007	0.046	0.009
AD-52785.1	0.08	0.88	0.95	0.002	0.004	0.019
AD-52800.1	0.09	0.82	0.94	0.001	0.040	0.005
AD-52792.1	0.09	0.93	0.94	0.002	0.018	0.037
AD-52784.1	0.10	0.84	0.92	0.000	0.066	0.032
AD-52746.1	0.10	0.82	0.93	0.002	0.060	0.059
AD-52814.1	0.10	0.85	0.88	0.002	0.042	0.013
AD-52751.1	0.10	0.88	0.98	0.005	0.030	0.067
AD-52786.1	0.10	0.81	0.81	0.006	0.028	0.048
AD-52755.1	0.10	0.93	0.99	0.003	0.032	0.048
AD-52808.1	0.11	0.98	0.92	0.000	0.038	0.032
AD-52815.1	0.11	0.96	0.96	0.002	0.009	0.000
AD-52805.1	0.11	0.79	0.86	0.003	0.050	0.008
AD-52777.1	0.11	0.88	0.94	0.001	0.065	0.000
AD-52756.1	0.11	0.92	0.91	0.003	0.032	0.004
AD-52733.1	0.12	0.66	0.65	0.005	0.071	0.022
AD-52739.1	0.13	0.83	0.95	0.002	0.008	0.061
AD-52780.1	0.13	0.70	0.67	0.012	0.021	0.059
AD-52798.1	0.13	0.64	0.97	0.001	0.006	0.038
AD-52776.1	0.14	0.97	0.94	0.011	0.029	0.023
AD-52753.1	0.15	0.88	1.09	0.001	0.048	0.005
AD-52778.1	0.16	0.76	0.69	0.003	0.067	0.003
AD-52744.1	0.16	0.90	0.91	0.002	0.000	0.049
AD-52750.1	0.16	0.87	1.01	0.000	0.060	0.055
AD-52774.1	0.17	0.71	0.89	0.002	0.010	0.017
AD-52803.1	0.18	0.87	0.92	0.015	0.026	0.040
AD-52821.1	0.18	0.86	0.87	0.005	0.046	0.055
AD-52781.1	0.18	0.78	0.66	0.008	0.000	0.023

AD-52779.1	0.20	0.83	0.66	0.002	0.024	0.016
AD-52793.1	0.20	0.74	0.88	0.010	0.025	0.069
AD-52799.1	0.20	0.75	1.01	0.005	0.018	0.010
AD-52761.1	0.22	0.83	0.92	0.000	0.024	0.023
AD-52768.1	0.22	0.96	0.97	0.001	ND	0.028
AD-52757.1	0.23	1.02	0.95	0.018	0.040	0.042
AD-52806.1	0.24	0.96	0.87	0.011	0.084	0.055
AD-52771.1	0.25	0.92	0.98	0.010	0.018	0.048
AD-52802.1	0.30	0.95	1.00	0.010	0.019	0.005
AD-52731.1	0.30	0.85	0.75	0.001	0.067	0.022
AD-52813.1	0.30	1.07	0.98	0.001	0.109	0.014
AD-52742.1	0.31	0.95	1.03	0.005	0.028	0.056
AD-52766.1	0.35	0.97	1.00	0.010	0.024	0.044
AD-52732.1	0.41	0.79	0.73	0.004	0.016	0.039
AD-52773.1	0.43	0.99	0.92	0.004	0.029	0.022
AD-52772.1	0.43	1.00	1.02	0.006	0.000	0.065
AD-52822.1	0.44	0.68	0.81	0.004	0.010	0.016
AD-52783.1	0.45	0.66	0.76	0.009	0.036	0.019
AD-52789.1	0.50	0.68	0.78	0.010	0.053	0.004
AD-52795.1	0.50	0.82	0.69	0.000	0.080	0.054
AD-52801.1	0.54	0.70	0.79	0.018	0.038	0.035
AD-52807.1	0.57	0.76	0.93	0.006	0.011	0.032
AD-52769.1	0.76	0.97	0.92	0.015	0.085	0.045
AD-1955	0.90	0.96	1.04	0.018	0.165	0.010
AD-52818.1	0.92	1.03	0.92	0.009	0.010	0.063
AD-1955	1.01	0.90	0.96	0.005	0.031	0.019
AD-1955	1.05	1.09	1.00	0.046	0.085	0.005
AD-1955	1.05	1.07	1.00	0.010	0.031	0.039
mock	1.20	0.98	0.92	0.000	0.014	0.005
mock	1.25	0.99	1.00	0.006	0.005	0.034

Table 14. Results of a dose response screen using a subset of sequences from Table 13.

A subset of active ANGPTL3 siRNAs from Table 10 were tested by transfection in Hep3B cells in dose response screens.

Duplex	IC50 (nM)
AD-52819.1	0.0036
AD-52667.1	0.0037
AD-52638.1	0.0048
AD-52673.1	0.0049
AD-52711.1	0.0050
AD-52661.1	0.0054
AD-52654.1	0.0058
AD-52637.1	0.0058
AD-52643.1	0.0060
AD-52685.1	0.0062
AD-52670.1	0.0064
AD-52679.1	0.0064
AD-52649.1	0.0066
AD-52683.1	0.0069
AD-52688.1	0.0071
AD-52717.1	0.0072
AD-52699.1	0.0073
AD-52714.1	0.0086
AD-52718.1	0.0088
AD-52735.1	0.0093
AD-52653.1	0.0102
AD-52687.1	0.0109
AD-52680.1	0.0120
AD-52713.1	0.0133
AD-52720.1	0.0143
AD-52639.1	0.0161
AD-52696.1	0.0163

AD-52662.1	0.0179
AD-52659.1	0.0180
AD-52710.1	0.0195
AD-52689.1	0.0216
AD-52787.1	0.0242
AD-52765.1	0.0318

Table 15. IDs of duplex pairs for which both an unconjugated and a GalNac-conjugated version were synthesized and tested

These duplexes have the same sequence and modification pattern.

Unconjugated duplex ID	GalNac conjugated duplex ID
AD-52637.1	AD-52953.1
AD-52638.1	AD-52954.1
AD-52639.1	AD-52955.1
AD-52640.1	AD-52956.1
AD-52641.1	AD-52957.1
AD-52642.1	AD-52958.1
AD-52643.1	None
None	AD-52960.1
None	AD-52961.1
AD-52645.1	AD-52962.1
AD-52647.1	AD-52963.1
AD-52648.1	AD-52964.1
AD-52649.1	AD-52965.1
AD-52650.1	AD-52966.1
AD-52651.1	AD-52967.1
AD-52652.1	AD-52968.1
AD-52653.1	AD-52969.1
AD-52654.1	AD-52970.1
None	AD-52971.1
AD-52656.1	AD-52972.1
AD-52657.1	AD-52973.1
AD-52658.1	AD-52974.1
AD-52659.1	AD-52975.1
AD-52660.1	AD-52976.1
AD-52661.1	AD-52977.1
AD-52662.1	AD-52978.1
AD-52663.1	AD-52979.1

AD-52664.1	AD-52980.1
AD-52665.1	AD-52981.1
AD-52666.1	AD-52982.1
AD-52667.1	AD-52983.1
AD-52668.1	AD-52984.1
AD-52669.1	AD-52985.1
AD-52670.1	AD-52986.1
AD-52671.1	AD-52987.1
AD-52672.1	AD-52988.1
AD-52673.1	AD-52989.1
AD-52674.1	AD-52990.1
AD-52675.1	AD-52991.1
AD-52676.1	AD-52992.1
AD-52677.1	AD-52993.1
AD-52678.1	AD-52994.1
AD-52679.1	AD-52995.1
AD-52680.1	AD-52996.1
AD-52681.1	AD-52997.1
AD-52682.1	AD-52998.1
AD-52683.1	AD-52999.1
AD-52684.1	AD-53000.1
AD-52685.1	AD-53001.1
AD-52686.1	AD-53002.1
AD-52687.1	AD-53003.1
AD-52688.1	AD-53004.1
AD-52689.1	AD-53005.1
AD-52690.1	AD-53006.1
AD-52691.1	AD-53007.1
AD-52692.1	AD-53008.1
AD-52693.1	AD-53009.1
AD-52694.1	AD-53010.1
AD-52695.1	AD-53011.1
AD-52696.1	AD-53012.1
AD-52697.1	AD-53013.1

AD-52698.1	AD-53014.1
AD-52699.1	AD-53015.1
AD-52700.1	AD-53016.1
AD-52701.1	AD-53017.1
AD-52702.1	AD-53018.1
AD-52703.1	AD-53019.1
AD-52704.1	AD-53020.1
AD-52705.1	AD-53021.1
AD-52706.1	AD-53022.1
AD-52707.1	AD-53023.1
AD-52708.1	AD-53024.1
AD-52709.1	AD-53025.1
AD-52710.1	AD-53026.1
AD-52711.1	AD-53027.1
AD-52712.1	AD-53028.1
AD-52713.1	AD-53029.1
AD-52714.1	AD-53030.1
AD-52715.1	AD-53031.1
AD-52716.1	AD-53032.1
AD-52717.1	AD-53033.1
AD-52718.1	AD-53034.1
AD-52719.1	AD-53035.1
AD-52720.1	AD-53036.1
AD-52721.1	AD-53037.1
AD-52722.1	AD-53038.1
AD-52723.1	AD-53039.1
AD-52724.1	AD-53040.1
AD-52725.1	AD-53041.1
AD-52726.1	AD-53042.1
AD-52727.1	AD-53043.1
AD-52728.1	AD-53044.1
AD-52729.1	AD-53045.1
AD-52730.1	AD-53046.1
AD-52731.1	AD-53059.1

AD-52732.1	AD-53060.1
AD-52733.1	AD-53061.1
AD-52734.1	AD-53062.1
AD-52735.1	AD-53063.1
AD-52736.1	AD-53064.1
AD-52737.1	AD-53065.1
None	AD-53066.1
AD-52739.1	AD-53067.1
AD-52740.1	AD-53068.1
AD-52741.1	AD-53069.1
AD-52742.1	AD-53070.1
AD-52743.1	AD-53071.1
AD-52744.1	AD-53072.1
AD-52745.1	AD-53073.1
AD-52746.1	AD-53074.1
AD-52747.1	AD-53075.1
AD-52748.1	AD-53076.1
AD-52749.1	AD-53077.1
AD-52750.1	AD-53078.1
AD-52751.1	AD-53079.1
AD-52752.1	AD-53080.1
AD-52753.1	AD-53081.1
AD-52754.1	AD-53082.1
AD-52755.1	AD-53083.1
AD-52756.1	AD-53084.1
AD-52757.1	AD-53085.1
AD-52758.1	AD-53086.1
AD-52759.1	AD-53087.1
AD-52760.1	AD-53088.1
AD-52761.1	AD-53089.1
AD-52762.1	AD-53090.1
AD-52763.1	AD-53091.1
AD-52764.1	AD-53092.1
AD-52765.1	AD-53093.1

AD-52766.1	AD-53094.1
AD-52767.1	AD-53095.1
AD-52768.1	AD-53096.1
AD-52769.1	AD-53097.1
AD-52770.1	AD-53098.1
AD-52771.1	AD-53099.1
AD-52772.1	AD-53100.1
AD-52773.1	AD-53101.1
AD-52774.1	AD-53102.1
AD-52775.1	AD-53103.1
AD-52776.1	AD-53104.1
AD-52777.1	AD-53105.1
AD-52778.1	AD-53106.1
AD-52779.1	AD-53107.1
AD-52780.1	AD-53108.1
AD-52781.1	AD-53109.1
AD-52782.1	AD-53110.1
AD-52783.1	AD-53111.1
AD-52784.1	AD-53112.1
AD-52785.1	AD-53113.1
AD-52786.1	AD-53114.1
AD-52787.1	AD-53115.1
AD-52788.1	AD-53116.1
AD-52789.1	AD-53117.1
None	AD-53118.1
AD-52791.1	AD-53119.1
AD-52792.1	AD-53120.1
AD-52793.1	AD-53121.1
AD-52794.1	AD-53122.1
AD-52795.1	AD-53123.1
AD-52796.1	AD-53124.1
AD-52797.1	AD-53125.1
AD-52798.1	AD-53126.1
AD-52799.1	AD-53127.1

AD-52800.1	AD-53128.1
AD-52801.1	AD-53129.1
AD-52802.1	AD-53130.1
AD-52803.1	AD-53131.1
AD-52804.1	AD-53132.1
AD-52805.1	AD-53133.1
AD-52806.1	AD-53134.1
AD-52807.1	AD-53135.1
AD-52808.1	AD-53136.1
AD-52809.1	AD-53137.1
AD-52810.1	AD-53138.1
AD-52811.1	AD-53139.1
AD-52812.1	AD-53140.1
AD-52813.1	AD-53141.1
AD-52814.1	AD-53142.1
AD-52815.1	AD-53143.1
AD-52816.1	AD-53144.1
AD-52817.1	AD-53145.1
AD-52818.1	AD-53146.1
AD-52819.1	AD-53147.1
AD-52820.1	AD-53148.1
AD-52821.1	AD-53149.1
AD-52822.1	AD-53150.1

In Vivo Tests

Example 3.

Test articles

- 5 *In vivo* experiments were conducted using dsRNA sequences of the invention. The dsRNA sequence used in the experiments was GalNac-conjugated AD-52981 (“ANG”, sense sequence: AfcAfuAfuUfuGfAfUfcAfgUfcUfuUfuUfL96; antisense sequence: aAfaAfaGfaCfuGfaucAfaAfuAfuGfusUfsg). The dsRNA sequence used as a

negative control was luciferase-conjugated AD-48399B1 ("Luc", sense sequence: CfaCfuUfaCfGcfuGfaGfuAfcUfuCfGAIL96, antisense sequence: uCfGafaGfuAfcUfcAfgCfGufaAfgUfGsAfsu). Also used as a negative control was GalNal-conjugated AD-1955 containing alternating 2'-methyl and 2' fluoro
5 modifications.

Experimental procedure

The dsRNA sequences were tested in C57BL/6 (WT) and ob/ob mice. WT mice received five daily doses of dsRNAs in PBS, Luc at 20 mg/kg, or ANG at 5 or 20 mg/kg; and ob/ob mice received five daily doses of NPLs formulated with Luc at 20
10 mg/kg or ANG at 20 mg/kg. All test articles were administered by subcutaneous injection according to the procedure shown in Figure 1. Specifically, five daily doses of the test articles were administered on five consecutive days (day 0, 1, 2, 3 and 4), and blood samples were collected 5, 3 or 1 day prior to administration, as well as on days 0, 1, 2, 3, 4, 7, 9, 11, 15, 18, 21, 25, 30, 37, 45 and 50 post-administration. The collected
15 blood samples were used to measure the expression of ANGPTL3 protein using an ELISA assay. Levels of serum triglycerides (TGs), low density lipoprotein cholesterol (LDLc), high density lipoprotein cholesterol (HDLc) and total cholesterol (TC) were also measured using an Olympus Analyzer.

Results

Shown in Figure 2, Panel A, are levels of murine ANGPTL3 (mANGPTL3, protein measured in WT mice after administration of control or ANG at 5 or 20 mg/kg. Also shown in Figure 2, Panel B are levels of mANGPTL3 protein measured in ob/ob mice after administration of control or ANG at 20 mg/kg. The data indicates that, for both WT and ob/ob mice, administration of ANG results in decreased levels of
20 mANGPTL3 protein, as compared to controls.
25

Shown in Figure 3, Panel A, are levels of LDL-c measured in WT mice after administration of control or ANG at 20 mg/kg. Shown in Figure 3, Panel B are levels of LDL-c measured in ob/ob mice after administration of control or ANG at 20 mg/kg. The data indicates that administration of ANG causes decreased levels of LDL-c,
30 particularly in ob/ob mice, as compared to controls.

Shown in Figure 4, Panel A, are levels of triglycerides measured in WT mice after administration of control or ANG at 20 mg/kg. Shown in Figure 4, Panel B are levels of triglycerides measured in ob/ob mice after administration of control or ANG at 20 mg/kg. The data indicates that administration of ANG causes decreased levels of tryglycerides, particularly, in ob/ob mice, as compared to controls.

Shown in Figure 5, Panel A and B are levels of total cholesterol (TC) measured in WT and ob/ob mice, respectively, after administration of control or ANG at 20 mg/kg. The data indicates that administration of ANG causes a moderate decrease in TC levels in ob/ob mice, but not in WT mice. Similarly, administration of ANG causes a moderate decrease in HDL-c levels in ob/ob mice, but not in WT mice, as is shown in the graphs in Figure 6.

Example 4.

Test article

The effect of a single injection of dsRNA sequence of the invention on the level of ANGPTL3 protein was tested. The dsRNA sequence used in the experiments was GalNac-conjugated AD-52981 ("ANG", sense sequence: AfcAfuAfuUfuGfAfUfcAfgUfcUfuUfuUfL96; antisense sequence: aAfaAfaGfaCfuGfaucAfaAfuAfuGfusUfsg). PBS was used as a negative control.

Experimental procedure

The dsRNA sequences were tested in Human PCS Transgenic mouse characterized by liver-specific expression of full-length human PCSK9 gene. Human PCS transgenic mice were dosed with the AD-52981 or PBS using a single subcutaneous injection. The mice were divided into four groups, each group consisting of two males and two females. Each group received an injection of PBS or a 5 mg/kg, 20 mg/kg or 60 mg/kg dose of AD-52981. Blood samples were collected at day 1 and day 0 prior to dosing, and at 72 hours post dosing. ANGPTL3 protein levels were measured by ELISA and compared to levels at day1 and day 0 prior to dosing.

Results

Shown in Figure 7, are levels of murine ANGPTL3 protein (mANGPTL3) measured in Human PCS transgenic mice. The data shown is expressed relative to PBS control and represents an average for 2 males and 2 females in each group. Error bars represent standard deviation. The data indicates that administration of a single injection of AD-52981 reduces the levels of ANGPTL3 protein in the mice in a dose-dependent manner, with the dose of 60 mg/kg decreasing the levels of ANGPTL3 protein more than five-fold (see Figure 7).

SEQUENCES

SEQ ID NO:1

>gi|41327750|ref|NM_014495.2| Homo sapiens angiopoietin-like 3
(ANGPTL3), mRNA

```
5  TTCCAGAAGAAAACAGTTCCACGTTGCTTGAAATTGAAAATCAAGATAAAAAATGTTTACAATTAAGCTCCT
   TCTTTTATTGTTCTCTAGTTATTTTCTCCAGAATTGATCAAGACAATTCATCATTTTGATTCTCTATCTC
   CAGAGCCAAAATCAAGATTTGCTATGTTAGACGATGTAAAAATTTTAGCCAATGGCCTCCTTCAGTTGGGA
   CATGGTCTTAAAGACTTTGTCCATAAGACGAAGGGCCAAATTAATGACATATTTCAAAAACCAACATATT
   TGATCAGTCTTTTATGATCTATCGCTGCAAACCAAGTGAAATCAAAGAAGAAGAAAAGGAAGTGAAGAA
10  CTACATATAAACTACAAGTCAAAAAATGAAGAGGTAAAGAATATGTCACCTGAACTCAACTCAAACTTGAA
   AGCCTCCTAGAGAAAAAATTCTACTTCAACAAAAAGTGAAATATTTAGAAGAGCAACTAACTAACTTAAT
   TCAAAATCAACCTGAACTCCAGAACACCCAGAAGTAACTTCACTTAAACTTTTGTAGAAAAACAAGATA
   ATAGCATCAAAGACCTTCTCCAGACCGTGGAAGACCAATATAAACAATTAAACCAACAGCATAGTCAAATA
   AAAGAAATAGAAAAACAGCTCAGAAGGACTAGTATTCAAGAACCCACAGAAATTTCTCTATCTTCCAAGCC
15  AAGAGCACCAAGAAGTACTCCCTTTCTTCAGTTGAATGAAATAAGAAATGTAAAACATGATGGCATTCTTG
   CTGAATGTACCACCATTTATAACAGAGGTGAACATACAAGTGGCATGTATGCCATCAGACCCAGCAACTCT
   CAAGTTTTTTCATGTCTACTGTGATGTTATATCAGGTAGTCCATGGACATTAAATCAACATCGAATAGATGG
   ATCACAACAACTTCAATGAAACGTGGGAGAACTACAAATATGGTTTTGGGAGGCTTGATGGAGAAATTTGGT
   TGGGCTTAGAGAAGATATACTCCATAGTGAAGCAATCTAATTATGTTTTACGAATTGAGTTGGAAGACTGG
20  AAAGACAACAAACATTATATTGAATATCTTTTTTACTTGGGAAATCACGAAACCAACTATACGCTACATCT
   AGTTGCGATTACTGGCAATGTCCCAATGCAATCCCGGAAAAACAAAGATTTGGTGTTTTCTACTTGGGATC
   ACAAAGCAAAGGACACTTCAACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCATGATGAGTGTGGA
   GAAAAACAACCTAAATGGTAAATATAACAAACCAAGAGCAAAATCTAAGCCAGAGAGGAGAAGAGGATTATC
   TTGGAAGTCTCAAAATGGAAGGTTTACTCTATAAAATCAACCAAAATGTTGATCCATCCACAGATTTCAG
25  AAAGCTTTGAATGAAGTGAAGGCAAAATTTAAAGGCAATAATTTAAACATTAACTCATTCCAAGTTAATGT
   GGTCTAATAATCTGGTATTAAATCCTTAAGAGAAAGCTTGAGAAATAGATTTTTTTTATCTTAAAGTCACT
   GTCATTTTAAGATTAAACATACAATCACATAACCTTAAAGAATACCGTTTACATTTCTCAATCAAAATCTT
   TATAATACTATTTGTTTTAAATTTTGTGATGTGGGAATCAATTTTAGATGGTCACAATCTAGATTATAATC
   AATAGGTGAACCTATTAAATAACTTTTCTAAATAAAAAATTTAGAGACTTTTATTTTAAAGGCATCATAT
30  GAGCTAATATCACAACTTTCCAGTTTAAAAAAGCTAGTACTCTTGTTAAACTCTAACTTGACTAAATAC
   AGAGGACTGGTAAATGTACAGTTCTTAAATGTTGTAGTATTAATTTCAAACTAAAAATCGTCAGCACAGA
   GTATGTGTAAAAATCTGTAATACAAATTTTAAACTGATGCTTCATTTTGCTACAAAATAATTTGGAGTAA
   ATGTTTGATATGATTTATTTATGAACCTAATGAAGCAGAAATTAATACTGTATTAAAATAAGTTCGCTGT
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35

SEQ ID NO:2

>gi|297278846|ref|XM_001086114.2| PREDICTED: Macaca mulatta
angiopoietin-like 3 (ANGPTL3), mRNA

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   AGACAATTCATCATTTGATTCTGTATCTCCAGAGCCAAAATCAAGATTTGCTATGTTAGACGATGTAAAAA
   TTTTAGCCAATGGCCTCCTTCAGTTGGGACATGGTCTTAAAGACTTTGTCCATAAGACTAAGGGCCAAAT
   AATGACATATTTCAAAAACCAACATATTTGATCAGTCTTTTATGATCTATCACTGCAAACCAAGTGAAAT
   CAAAGAAGAAGAAAAGGAAGTGAAGAAGTACATATAAACTACAAGTCAAAAATGAAGAGGTAAAGAATA
45  TGTCACTTGAACCTCAACTCAAACTTGAAGCCTCCTAGAAAGAAAAAATTTACTTCAACAAAAAGTGAAA
   TATTTAGAAGAGCAACTAACTAACTTAATTCAAAATCAACCTGAAACTCCAGAACATCCAGAAGTAACTTC
   ACTTAAAGTTTTGTAGAAAAACAAGATAATAGCATCAAAGACCTTCTCCAGACTGTGGAAGAACAAATATA
   AGCAATTAACCAACAGCACAGTCAAATAAAAGAAATAGAAAATCAGCTCAGAATGACTAATATTCAAGAA
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 TGTCTAAGGAGAATAGATGGATCACAAAACCTTCAATGAAACGTGGGAGAACTACAAATATGGTTTCGGGAG
 5 GCTTGATGGAGAATTCTGGTTGGGCCTAGAGAAGATATACTCCATAGTGAAGCAATCTAATTACGTTTTAC
 GAATTGAGTTGGAAGACTGGAAAGACAACAAACATTATATTGAATATTCTTTTTACTTTGGGAAATCACGAA
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 GGTGTTTTCTACTTTGGGATCACAAAGCAAAGGACACTTCAGCTGTCCAGAGAGTTATTTCAGGAGGCTGGT
 10 GGTGGCATGATGAGTGTGGAGAAAACAACCTAAATGGTAAATATAACAAACCAAGAACAATACTAAGCCA
 GAGCGAGAAGAGGAATTATCCTGGGAAGTCTCAAAATGGGAAGGTTATACTCTATAAAATCAACCAAAATGTT
 GATCCATCCAACAGATTTCAGAAAGCTTTGAATGAAC TGAGGCAAAATTTAAAAGGCAATAAATTAAACATTA
 AACTCATTCCAAGTTAATGTGGTTTAATAATCTGGTATTAAATCCTTAAGAGAAGGCTTGAGAAATAGATT
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 ATTTCTCAATCAAAATTCCTACAACACTATTTGTTTTATATTTGTGATGTGGGAATCAATTTTAGATGGT
 15 CGCAATCTAAATTATAATCAACAGGTGAACCTTACTAAATAACTTTTCTAAATAAAAACTTAGAGACTTTA
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SEQ ID NO:3

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 25 (Angptl3), mRNA

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SEQ ID NO:4

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SEQ ID NO:5 Reverse Complement of SEQ ID NO:1

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SEQ ID NO:9

40 *Macaca fascicularis* angiopoietin-like 3 (Angptl3), mRNA

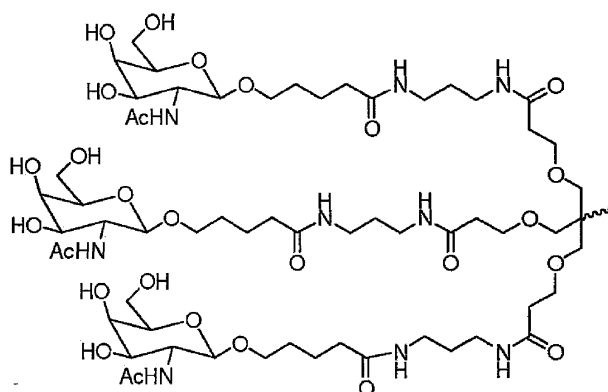
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 20

We claim:

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ANGPTL3, wherein said dsRNA comprises a sense strand and an antisense strand, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:5.
2. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ANGPTL3, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences listed in Tables 2, 3, 7, 8, 9 and 10.
3. The dsRNA of claim 2, wherein the sense and antisense strands comprise sequences selected from the group consisting of AD-52981.1, AD-53063.1, AD-53001.1, AD-53015.1, AD-52986.1, AD-52953.1, AD-53024.1, AD-53033.1, AD-53030.1, AD-53080.1, AD-53073.1, AD-53132.1, AD-52983.1, AD-52954.1, AD-52961.1, AD-52994.1, AD-52970.1, AD-53075.1, AD-53147.1, AD-53077.1 of Tables 7 and 8.
4. The dsRNA of claim 1 or 2, wherein said dsRNA comprises at least one modified nucleotide.
5. The dsRNA of claim 4, wherein at least one of said modified nucleotides is selected from the group consisting of a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group.
6. The dsRNA of claim 4, wherein said modified nucleotide is selected from the group consisting of a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

7. The dsRNA of claim 2, wherein the region of complementarity is at least 17 nucleotides in length.
8. The dsRNA of claim 2, wherein the region of complementarity is between 19 and 21 nucleotides in length.
- 5 9. The dsRNA of claim 8, wherein the region of complementarity is 19 nucleotides in length.
10. The dsRNA of claim 1 or 2, wherein each strand is no more than 30 nucleotides in length.
11. The dsRNA of claim 1 or 2, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.
- 10 12. The dsRNA of claim 1 or 2, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.
13. The dsRNA of claim 1 or 2, further comprising a ligand.
- 14 The dsRNA of claim 13, wherein the ligand is conjugated to the 3' end of the
- 15 sense strand of the dsRNA.
15. The dsRNA of claim 13, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.
16. The dsRNA of claim 15, wherein the ligand is



17. The dsRNA of claim 2, wherein the region of complementarity consists of one of the antisense sequences of Tables 2, 3, 7, 8, 9 and 10.
18. The dsRNA of claim 1 or 2, wherein the dsRNA comprises a sense strand consisting of a sense strand sequence selected from the sequence of Tables 2, 3, 9 and 10, and an antisense strand consisting of an antisense sequence selected from the sequences of Tables 2, 3, 7, 8, 9 and 10.
19. A cell containing the dsRNA of claim 1 or 2.
20. A vector encoding at least one strand of a dsRNA, wherein said dsRNA comprises a region of complementarity to at least a part of an mRNA encoding ANGPTL3, wherein said dsRNA is 30 base pairs or less in length, and wherein said dsRNA targets said mRNA for cleavage.
21. The vector of claim 20, wherein the region of complementarity is at least 15 nucleotides in length.
22. The vector of claim 20, wherein the region of complementarity is 19 to 21 nucleotides in length.
23. A cell comprising the vector of claim 20.
24. A pharmaceutical composition for inhibiting expression of an ANGPTL3 gene comprising the dsRNA of claim 1 or 2 or the vector of claim 20.
25. The pharmaceutical composition of claim 24, further comprising a lipid formulation.
26. The pharmaceutical composition of claim 24, wherein the lipid formulation comprises a SNALP, or XTC.
27. The pharmaceutical composition of claim 24, wherein the lipid formulation comprises a MC3.

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28. A method of inhibiting ANGPTL3 expression in a cell, the method comprising:
- (a) contacting the cell with the dsRNA of claim 1 or 2 or the vector of claim 20; and
 - (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of an ANGPTL3 gene, thereby inhibiting expression of the ANGPTL3 gene in the cell.
29. The method of claim 28, wherein said cell is within a subject.
30. The method of claim 29, wherein the subject is a human.
31. The method of claim 30, wherein the human subject suffers from a disorder of lipid metabolism.
32. The method of claim 31, wherein the disorder of lipid metabolism is hyperlipidemia or hypertriglyceridemia.
33. The method of any one of claims 28-32, wherein the ANGPTL3 expression is inhibited by at least about 30%.
34. A method of treating a subject having a disorder that would benefit from reduction in ANGPTL3 expression, comprising administering to the subject a therapeutically effective amount of the dsRNA of claim 1 or 2 or the vector of claim 20, thereby treating said subject.
35. The method of claim 34, wherein the disorder is a disorder of lipid metabolism.
36. The method of claim 35, wherein the disorder of lipid metabolism is hyperlipidemia or hypertriglyceridemia.
37. The method of claim 34, wherein the administration of the dsRNA to the subject causes a decrease in one or more serum lipid and/or a decrease in ANGPTL3 protein accumulation.
38. The method of claim 34, wherein the dsRNA is administered at a dose of about 0.01 mg/kg to about 10 mg/kg or about 5 mg/kg to about 50 mg/kg.

39. A method of inhibiting the expression of ANGPTL3 in a subject, the method comprising

administering to said subject a therapeutically effective amount of the dsRNA of claim 1 or 2 or the vector of claim 20, thereby inhibiting the expression of ANGPTL3 in
5 said subject.

40. The method of claim 39, wherein the dsRNA is administered to said subject at a dose of about 0.01 mg/kg to about 10 mg/kg or about 5 mg/kg to about 50 mg/kg.