

COMPOSITIONS AND METHODS FOR INHIBITING ANGPTL3 EXPRESSION

RELATED APPLICATIONS

[001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 62/991,335, filed March 18, 2020, the contents of which are herein incorporated by reference in their entireties.

TECHNICAL FIELD

[002] The disclosure relates to oligonucleotides that inhibit angiopoietin-like protein 3 (ANGPTL3) expression and uses thereof, particularly uses relating to treating diseases, disorders and/or conditions associated with ANGPTL3 expression.

REFERENCE TO THE SEQUENCE LISTING

[003] The disclosure is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 400930_182359_SL.txt, created on March 18, 2021, having a file size of 371 KB. The information in electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND

[004] Lipid metabolism disorders can result in elevated levels of serum lipids, such as triglycerides and/or cholesterol. Elevated serum lipids are strongly associated with high blood pressure, cardiovascular disease, diabetes and other pathological conditions. Despite treatment advances, there remains a very high, unmet, medical need for therapies to treat cardiovascular and metabolic diseases.

[005] Hypertriglyceridemia is a lipid metabolism disorder characterized by an abnormally elevated concentration of triglyceride in the blood (*e.g.*, >150 mg/dL). Hypertriglyceridemia has been associated with the development of cardiovascular diseases (*e.g.*, arteriosclerosis). Severe hypertriglyceridemia (*e.g.*, >500 mg/dL) may cause pancreatitis, eruptive xanthomas or *lipemia retinalis*. In some cases, extremely high levels of chylomicrons can cause chylomicronemia syndrome, which is characterized by recurrent abdominal pain, nausea, vomiting and pancreatitis (Pejic & Lee (2006) *J. Am. Board. Fam. Med.* 19:310-316). Hyperlipidemia is another lipid metabolism disorder that is characterized by elevated levels of any one or all lipids and/or lipoproteins in the blood.

[006] ANGPTL3 is a member of the angiopoietin-like family of secreted proteins that regulates lipid metabolism and that is primarily expressed in the liver (Koishi *et al.* (2002) *Nat. Genet.* 30:151-157). ANGPTL3 inhibits lipoprotein lipase (LPL), which catalyzes the hydrolysis of triglycerides, and inhibits endothelial lipase (EL), which hydrolyzes high density lipoprotein (HDL) phospholipids.

BRIEF SUMMARY

[007] Aspects of the disclosure relate to compositions and methods for treating a disease, disorder and/or condition related to ANGPTL3 expression. The disclosure is based, in part, on the discovery and development of oligonucleotides that selectively inhibit and/or reduce ANGPTL3 expression.

[008] In some embodiments, the disclosure provides an oligonucleotide for reducing ANGPTL3 expression, where the oligonucleotide comprises an antisense strand comprising a sequence as set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, and 116.

[009] In some embodiments, the disclosure provides an oligonucleotide for reducing ANGPTL3 expression, where the oligonucleotide comprises a sense strand comprising a sequence as set forth in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, and 115.

[0010] In some embodiments, the oligonucleotide for reducing ANGPTL3 expression comprises an antisense strand of 15 to 30 nucleotides in length and a sense strand of 15 to 40 nucleotides in length, where the antisense strand has a region of complementarity to a target sequence of ANGPTL3 as set forth in any one of SEQ ID NOs: 125, 126, 127, 118, 119, 120, 121, 122, 123, 124, and 117, and where the region of complementarity is at least 15 contiguous nucleotides in length.

[0011] In some embodiments, the antisense strand is 19 to 27 nucleotides in length or 21 to 27 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length.

[0012] In some embodiments, the sense strand is 19 to 40 nucleotides in length. In some embodiments, the sense strand is 36 nucleotides in length.

[0013] In some embodiments, the oligonucleotide for reducing ANGPTL3 expression has a duplex region of at least 19 nucleotides in length or at least 21 nucleotides in length. In some embodiments, the duplex region is 20 nucleotides in length.

[0014] In some embodiments, the region of complementarity to ANGPTL3 is at least 19 contiguous nucleotides in length or at least 21 contiguous nucleotides in length.

[0015] In some embodiments, the oligonucleotide for reducing ANGPTL3 expression comprises on the sense strand at its 3' end a stem-loop set forth as: S1-L-S2, wherein S1 is complementary to S2, and where L forms a loop between S1 and S2 of 3 to 5 nucleotides in length.

[0016] In some embodiments, an oligonucleotide for reducing ANGPTL3 expression comprises an antisense strand and a sense strand, where the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to ANGPTL3, where the sense strand comprises at its 3' end a stem-loop set forth as: S1-L-S2, where S1 is complementary to S2, where L forms a loop between S1 and S2 of 3 to 5 nucleotides in length, and where the antisense strand and the sense strand form a duplex structure of at least 19 nucleotides in length but are not covalently linked.

[0017] In some embodiments, the loop L is a tetraloop. In some embodiments, L is 4 nucleotides in length. In some embodiments, L comprises a sequence GAAA.

[0018] In some embodiments, the oligonucleotide for reducing ANGPTL3 expression comprises an antisense strand that is 27 nucleotides in length and a sense strand that is 25 nucleotides in length. In some embodiments, the oligonucleotide comprises an antisense strand that is 22 nucleotides in length and a sense strand that is 36 nucleotides in length.

[0019] In some embodiments, an oligonucleotide with a duplex region comprises a 3'-overhang sequence on the antisense strand. In some embodiments, the 3'-overhang sequence on the antisense strand is 2 nucleotides in length.

[0020] In some embodiments, the oligonucleotide for reducing ANGPTL3 expression comprises an antisense strand and a sense strand that are each in a range of 21 to 23 nucleotides in length. In some embodiments, the oligonucleotide comprises a duplex structure in a range of 19 to 21 nucleotides in length. In some such embodiments, the oligonucleotide comprises a 3'-overhang sequence of one or more nucleotides in length, where the 3'-overhang sequence is present on the antisense strand, the sense strand, or the antisense strand and sense strand. In some embodiments, the 3'-overhang sequence of 2 nucleotides in length, where the 3'-overhang sequence is on the antisense strand, and where the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length, such that the sense strand and antisense strand form a duplex of 21 nucleotides in length.

[0021] In some embodiments, the oligonucleotide for reducing ANGPTL3 expression comprises at least one modified nucleotide. In some embodiments, the modified nucleotide

comprises a 2'-modification. In some embodiments, all of the nucleotides of the oligonucleotide are modified, for example with a 2'-modification.

[0022] In some embodiments, the oligonucleotide for reducing ANGPTL3 expression comprises at least one modified internucleotide linkage, preferably a phosphorothioate linkage.

[0023] In some embodiments, the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand comprises a phosphate analog, for example, an oxymethylphosphonate, vinylphosphonate or malonylphosphonate.

[0024] In some embodiments, at least one nucleotide of the oligonucleotide is conjugated to one or more targeting ligands, such as a carbohydrate, amino sugar, cholesterol, polypeptide or lipid. In some embodiments, the targeting ligand comprises a N-acetylgalactosamine (GalNAc) moiety. In some embodiments, the GalNAc moiety comprises a monovalent GalNAc moiety, a bivalent GalNAc moiety, a trivalent GalNAc moiety, or a tetravalent GalNAc moiety.

[0025] In some embodiments, the targeting ligand is conjugated to one or more nucleotides of L of the stem-loop. In some embodiments, up to 4 nucleotides of L of the stem-loop are each conjugated to a monovalent GalNAc moiety.

[0026] In some embodiments, the oligonucleotide for reducing ANGPTL3 expression is an RNAi oligonucleotide.

[0027] In another aspect, the disclosure provides a method of reducing ANGPTL3 expression in a cell, a population of cells or a subject by administering an oligonucleotide herein. In some embodiments, a method of for reducing ANGPTL3 expression in a cell, a population of cells or a subject comprises a step of contacting the cell or the population of cells or administering to the subject an effective amount of an oligonucleotide herein, or a pharmaceutical composition thereof. In some embodiments, the method for reducing ANGPTL3 expression comprises reducing an amount or a level of ANGPTL3 mRNA, an amount or a level of ANGPTL3 protein, or both.

[0028] In some embodiments, the disclosure provides a method for reducing an amount or level of triglyceride (TG) in a subject by administering to the subject an effective amount of an oligonucleotide herein, or a pharmaceutical composition thereof.

[0029] In some embodiments, the disclosure provides a method for reducing an amount or level of cholesterol in a subject by administering to the subject an effective amount of the oligonucleotide herein, or a pharmaceutical composition thereof.

[0030] In some embodiments, a subject for treatment with an oligonucleotide herein has a disease, disorder or condition associated with ANGPTL3 expression. In some embodiments,

a method for treating a subject having a disease, disorder or condition associated with ANGPTL3 expression comprises administering to the subject in need thereof a therapeutically effective amount of an oligonucleotide herein, or a pharmaceutical composition thereof, thereby treating the subject.

[0031] In some embodiments, an oligonucleotide herein for administration comprises a sense strand of 15 to 50 nucleotides in length and an antisense strand of 15 to 30 nucleotides in length, where the sense strand forms a duplex region with the antisense strand, where the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, and 115, and where the antisense strand comprises a complementary sequence selected from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, and 116, or pharmaceutical composition thereof, thereby treating the subject.

[0032] In some embodiments, a method for treating a subject having a disease, disorder or condition associated with ANGPTL3 expression comprises administering to the subject in need thereof a therapeutically effective amount of an oligonucleotide comprising a pair of sense and antisense strands selected from a row of the table set forth in Table 5, or pharmaceutical composition thereof, thereby treating the subject.

[0033] In some embodiments, the disease, disorder or condition associated with ANGPTL3 expression is selected from the group consisting of hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, type II diabetes mellitus, cardiovascular disease, coronary artery disease, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), homozygous and heterozygous familial hypercholesterolemia, and statin-resistant hypercholesterolemia.

[0034] In some embodiments, the disease, disorder or condition associated with ANGPTL3 expression is cardiovascular disease, type II diabetes mellitus, hypertriglyceridemia, NASH, obesity, or a combination thereof.

[0035] In some embodiments, the oligonucleotide, or pharmaceutical composition thereof, is administered in combination with a second therapeutic agent or composition thereof.

[0036] In a further aspect, the present disclosure provides use of any of the oligonucleotides of the present disclosure, or the pharmaceutical composition thereof, in the manufacture of a

medicament for the treatment of a disease, disorder or condition associated with ANGPTL3 expression.

[0037] In some embodiments, the oligonucleotide of the disclosure, or the pharmaceutical composition of the disclosure, is for use, or adaptable for use, in the treatment of a disease, disorder or condition associated with ANGPTL3 expression.

[0038] In a further aspect, the oligonucleotide of the present disclosure is provided in the form of a kit for treating a disease, disorder or condition associated with ANGPTL3 expression. In some embodiments, the kit comprises an oligonucleotide herein, and a pharmaceutically acceptable carrier. In some embodiments, the kit further includes a package insert comprising instructions for administration to a subject having a disease, disorder or condition associated with ANGPTL3 expression.

[0039] In some embodiments of the use or kits, the disease, disorder or condition associated with ANGPTL3 expression is selected from the group consisting of hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, type II diabetes mellitus, cardiovascular disease, coronary artery disease, NASH, NAFLD, homozygous and heterozygous familial hypercholesterolemia, and statin-resistant hypercholesterolemia.

[0040] In some embodiments of the use or kits, the disease, disorder or condition associated with ANGPTL3 expression is cardiovascular disease, type II diabetes mellitus, hypertriglyceridemia, NASH, obesity, or a combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] FIG. 1 provides a graph depicting the percent (%) of ANGPTL3 mRNA in HuH-7 cells transfected with the indicated DsiRNAs relative to the % of ANGPTL3 mRNA control mock-treated cells.

[0042] FIG. 2 provides a graph depicting the percent (%) of ANGPTL3 mRNA in HuH-7 cells transfected with the indicated DsiRNAs relative to the % of ANGPTL3 mRNA control mock-treated cells.

[0043] FIG. 3 provides a schematic depicting the structure and chemical modification patterns of generic GalNAc-conjugated ANGPTL3 oligonucleotides.

[0044] FIG. 4 provides a graph depicting the percent (%) of ANGPTL3 mRNA in liver samples from mice treated with the indicated GalNAc-conjugated ANGPTL3 oligonucleotides relative to mice treated with phosphate buffered saline (PBS).

[0045] FIG. 5A-5C provides graphs depicting the percent (%) of ANGPTL3 mRNA in liver samples from non-human primates (NHPs) treated with the indicated GalNAc-conjugated ANGPTL3 oligonucleotides relative to NHPs treated with PBS on day 28 (FIG. 5A), day 56 (FIG. 5B) and day 84 (FIG. 5C) following treatment.

[0046] FIG. 6 provides a graph depicting the mean percent (%) of ANGPTL3 mRNA in liver samples from NHPs treated with the indicated GalNAc-conjugated ANGPTL3 oligonucleotides relative to NHPs treated with PBS over time.

[0047] FIG. 7 provides a graph depicting the mean percent (%) of ANGPTL3 protein in serum from NHPs treated with the indicated GalNAc-conjugated ANGPTL3 oligonucleotides relative to NHPs treated with PBS over time.

DETAILED DESCRIPTION

[0048] I. Definitions

[0049] As used herein, “about,” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0050] As used herein, “administer,” “administering,” “administration” and the like refer to providing a substance (*e.g.*, an oligonucleotide) to a subject in a manner that is pharmacologically useful (*e.g.*, to treat a condition in the subject).

[0051] As used herein, “ANGPTL3” refers to angiotensin-like protein 3, which is a member of the angiotensin-like family of secreted polypeptides. ANGPTL3 is expressed predominantly in the liver of mammals, and the ANGPTL3 protein has the characteristic structure of angiotensins, including a signal peptide, an N-terminal coiled-coil domain, and a C-terminal fibrinogen (FBN)-like domain. For the purposes of the disclosure, “ANGPTL3” refers to the ANGPTL3 from any vertebrate or mammal including, but not limited to, human, mouse, primate, monkey, bovine, chicken, rodent, rat, porcine, ovine and guinea pig. ANGPTL3 also refers to fragments and variants of native ANGPTL3 that maintain at least one *in vivo* or *in vitro* activity of a native ANGPTL3. ANGPTL3 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 FBN-like domain. An exemplary sequence of a human ANGPTL3 mRNA transcript is

publicly available (GenBank Accession No. GI: 41327750 (NM_014495.2)) and disclosed herein (SEQ ID NO:128). An exemplary sequence of cynomolgus monkey ANGPTL3 mRNA is publicly available (GenBank Accession No. GI: 102136264 (XM_005543185.2)) and disclosed herein (SEQ ID NO:129). An exemplary sequence of mouse ANGPTL3 mRNA is publicly available (GenBank Accession No. GI: 142388354 (NM_013913.3)) and disclosed herein (SEQ ID NO:130). An exemplary sequence of rat ANGPTL3 is publicly available (GenBank Accession No. GI: 68163568 (NM_001025065.1) and disclosed herein (SEQ ID NO:131).

[0052] As used herein, “asialoglycoprotein receptor” or “ASGPR” refers to a bipartite C-type lectin formed by a major 48 kDa subunit (ASGPR-1) and minor 40 kDa subunit (ASGPR-2). ASGPR is primarily expressed on the sinusoidal surface of hepatocyte cells and has a major role in binding, internalizing and subsequent clearing of circulating glycoproteins that contain terminal galactose or GalNAc residues (asialoglycoproteins).

[0053] As used herein, “attenuate,” “attenuating,” “attenuation” and the like refer to reducing or effectively halting. As a non-limiting example, one or more of the treatments herein may reduce or effectively halt the onset or progression of dyslipidemia/hypertriglyceridemia/hyperlipidemia in a subject. This attenuation may be exemplified by, for example, a decrease in one or more aspects (*e.g.*, symptoms, tissue characteristics, and cellular, inflammatory or immunological activity, *etc.*) of dyslipidemia/hypertriglyceridemia/hyperlipidemia, no detectable progression (worsening) of one or more aspects of dyslipidemia/hypertriglyceridemia/hyperlipidemia, or no detectable aspects of dyslipidemia/hypertriglyceridemia/hyperlipidemia in a subject when they might otherwise be expected.

[0054] As used herein, “complementary” refers to a structural relationship between two nucleotides (*e.g.*, on two opposing nucleic acids or on opposing regions of a single nucleic acid strand) that permits the two nucleotides to form base pairs with one another. For example, a purine nucleotide of one nucleic acid that is complementary to a pyrimidine nucleotide of an opposing nucleic acid may base pair together by forming hydrogen bonds with one another. In some embodiments, complementary nucleotides can base pair in the Watson-Crick manner or in any other manner that allows for the formation of stable duplexes. In some embodiments, two nucleic acids may have regions of multiple nucleotides that are complementary with each other to form regions of complementarity, as described herein.

[0055] As used herein, “deoxyribonucleotide” refers to a nucleotide having a hydrogen in place of a hydroxyl at the 2' position of its pentose sugar when compared with a ribonucleotide.

A modified deoxyribonucleotide is a deoxyribonucleotide having one or more modifications or substitutions of atoms other than at the 2' position, including modifications or substitutions in or of the sugar, phosphate group or base.

[0056] As used herein, “double-stranded oligonucleotide” or “ds oligonucleotide” refers to an oligonucleotide that is substantially in a duplex form. In some embodiments, the complementary base-pairing of duplex region(s) of a ds oligonucleotide is formed between antiparallel sequences of nucleotides of covalently separate nucleic acid strands. In some embodiments, complementary base-pairing of duplex region(s) of a ds oligonucleotide is formed between antiparallel sequences of nucleotides of nucleic acid strands that are covalently linked. In some embodiments, complementary base-pairing of duplex region(s) of a ds oligonucleotide is formed from single nucleic acid strand that is folded (*e.g.*, via a hairpin) to provide complementary antiparallel sequences of nucleotides that base pair together. In some embodiments, a ds oligonucleotide comprises two covalently separate nucleic acid strands that are fully duplexed with one another. However, in some embodiments, a ds oligonucleotide comprises two covalently separate nucleic acid strands that are partially duplexed (*e.g.*, having overhangs at one or both ends). In some embodiments, a ds oligonucleotide comprises antiparallel sequence of nucleotides that are partially complementary, and thus, may have one or more mismatches, which may include internal mismatches or end mismatches.

[0057] As used herein, “duplex,” in reference to nucleic acids (*e.g.*, oligonucleotides), refers to a structure formed through complementary base pairing of two antiparallel sequences of nucleotides.

[0058] As used herein, “excipient” refers to a non-therapeutic agent that may be included in a composition, for example, to provide or contribute to a desired consistency or stabilizing effect.

[0059] As used herein, “hepatocyte” or “hepatocytes” refers to cells of the parenchymal tissues of the liver. These cells make up about 70%-85% of the liver's mass and manufacture serum albumin, FBN and the prothrombin group of clotting factors (except for Factors 3 and 4). Markers for hepatocyte lineage cells include, but are not limited to, transthyretin (Ttr), glutamine synthetase (Glul), hepatocyte nuclear factor 1a (Hnf1a) and hepatocyte nuclear factor 4a (Hnf4a). Markers for mature hepatocytes may include, but are not limited to, cytochrome P450 (Cyp3a11), fumarylacetoacetate hydrolase (Fah), glucose 6-phosphate (G6p), albumin (Alb) and OC2-2F8. *See, e.g.*, Huch *et al.* (2013) *Nature* 494:247-250.

[0060] As used herein, a “hepatotoxic agent” refers to a chemical compound, virus or other substance that is itself toxic to the liver or can be processed to form a metabolite that is toxic

to the liver. Hepatotoxic agents may include, but are not limited to, carbon tetrachloride (CCl₄), acetaminophen (paracetamol), vinyl chloride, arsenic, chloroform, nonsteroidal anti-inflammatory drugs (such as aspirin and phenylbutazone).

[0061] As used herein, “labile linker” refers to a linker that can be cleaved (*e.g.*, by acidic pH). A “fairly stable linker” refers to a linker that cannot be cleaved.

[0062] As used herein, “liver inflammation” or “hepatitis” refers to a physical condition in which the liver becomes swollen, dysfunctional and/or painful, especially as a result of injury or infection, as may be caused by exposure to a hepatotoxic agent. Symptoms may include jaundice (yellowing of the skin or eyes), fatigue, weakness, nausea, vomiting, appetite reduction and weight loss. Liver inflammation, if left untreated, may progress to fibrosis, cirrhosis, liver failure or liver cancer.

[0063] As used herein, “liver fibrosis” or “fibrosis of the liver” refers to an excessive accumulation in the liver of extracellular matrix proteins, which could include collagens (I, III, and IV), FBN, undulin, elastin, laminin, hyaluronan and proteoglycans resulting from inflammation and liver cell death. Liver fibrosis, if left untreated, may progress to cirrhosis, liver failure or liver cancer.

[0064] As used herein, “loop” refers to an unpaired region of a nucleic acid (*e.g.*, oligonucleotide) that is flanked by two antiparallel regions of the nucleic acid that are sufficiently complementary to one another, such that under appropriate hybridization conditions (*e.g.*, in a phosphate buffer, in a cells), the two antiparallel regions, which flank the unpaired region, hybridize to form a duplex (referred to as a “stem”).

[0065] As used herein, “modified internucleotide linkage” refers to an internucleotide linkage having one or more chemical modifications when compared with a reference internucleotide linkage comprising a phosphodiester bond. In some embodiments, a modified nucleotide is a non-naturally occurring linkage. Typically, a modified internucleotide linkage confers one or more desirable properties to a nucleic acid in which the modified internucleotide linkage is present. For example, a modified nucleotide may improve thermal stability, resistance to degradation, nuclease resistance, solubility, bioavailability, bioactivity, reduced immunogenicity, *etc.*

[0066] As used herein, “modified nucleotide” refers to a nucleotide having one or more chemical modifications when compared with a corresponding reference nucleotide selected from: adenine ribonucleotide, guanine ribonucleotide, cytosine ribonucleotide, uracil ribonucleotide, adenine deoxyribonucleotide, guanine deoxyribonucleotide, cytosine deoxyribonucleotide and thymidine deoxyribonucleotide. In some embodiments, a modified

nucleotide is a non-naturally occurring nucleotide. In some embodiments, a modified nucleotide has one or more chemical modification in its sugar, nucleobase and/or phosphate group. In some embodiments, a modified nucleotide has one or more chemical moieties conjugated to a corresponding reference nucleotide. Typically, a modified nucleotide confers one or more desirable properties to a nucleic acid in which the modified nucleotide is present. For example, a modified nucleotide may improve thermal stability, resistance to degradation, nuclease resistance, solubility, bioavailability, bioactivity, reduced immunogenicity, *etc.*

[0067] As used herein, “nicked tetraloop structure” refers to a structure of a RNAi oligonucleotide that is characterized by separate sense (passenger) and antisense (guide) strands, in which the sense strand has a region of complementarity with the antisense strand, and in which at least one of the strands, generally the sense strand, has a tetraloop configured to stabilize an adjacent stem region formed within the at least one strand.

[0068] As used herein, “oligonucleotide” refers to a short nucleic acid (*e.g.*, less than about 100 nucleotides in length). An oligonucleotide may be single-stranded (ss) or ds. An oligonucleotide may or may not have duplex regions. As a set of non-limiting examples, an oligonucleotide may be, but is not limited to, a small interfering RNA (siRNA), microRNA (miRNA), short hairpin RNA (shRNA), dicer substrate interfering RNA (dsiRNA), antisense oligonucleotide, short siRNA or ss siRNA. In some embodiments, a ds oligonucleotide is an RNAi oligonucleotide.

[0069] As used herein, “overhang” refers to terminal non-base pairing nucleotide(s) resulting from one strand or region extending beyond the terminus of a complementary strand with which the one strand or region forms a duplex. In some embodiments, an overhang comprises one or more unpaired nucleotides extending from a duplex region at the 5' terminus or 3' terminus of a ds oligonucleotide. In certain embodiments, the overhang is a 3' or 5' overhang on the antisense strand or sense strand of a ds oligonucleotides.

[0070] As used herein, “phosphate analog” refers to a chemical moiety that mimics the electrostatic and/or steric properties of a phosphate group. In some embodiments, a phosphate analog is positioned at the 5' terminal nucleotide of an oligonucleotide in place of a 5'-phosphate, which is often susceptible to enzymatic removal. In some embodiments, a 5' phosphate analog contains a phosphatase-resistant linkage. Examples of phosphate analogs include, but are not limited to, 5' phosphonates, such as 5' methylenephosphonate (5'-MP) and 5'-(E)-vinylphosphonate (5'-VP). In some embodiments, an oligonucleotide has a phosphate analog at a 4'-carbon position of the sugar (referred to as a “4'-phosphate analog”) at a 5'-terminal nucleotide. An example of a 4'-phosphate analog is oxymethylphosphonate, in which

the oxygen atom of the oxymethyl group is bound to the sugar moiety (*e.g.*, at its 4'-carbon) or analog thereof. *See, e.g.*, US Provisional Patent Application Nos. 62/383,207 (filed on 2 September 2016) and 62/393,401 (filed on 12 September 2016). Other modifications have been developed for the 5' end of oligonucleotides (*see, e.g.*, Intl. Patent Application No. WO 2011/133871; US Patent No. 8,927,513; and Prakash *et al.* (2015) *Nucleic Acids Res.* 43:2993-3011).

[0071] As used herein, “reduced expression” of a gene (*e.g.*, ANGPTL3) refers to a decrease in the amount or level of RNA transcript (*e.g.*, ANGPTL3 mRNA) or protein encoded by the gene and/or a decrease in the amount or level of activity of the gene in a cell, a population of cells, a sample or a subject, when compared to an appropriate reference (*e.g.*, a reference cell, population of cells, sample or subject). For example, the act of contacting a cell with an oligonucleotide herein (*e.g.*, an oligonucleotide comprising an antisense strand having a nucleotide sequence that is complementary to a nucleotide sequence comprising ANGPTL3 mRNA) may result in a decrease in the amount or level of ANGPTL3 mRNA, protein and/or activity (*e.g.*, via degradation of ANGPTL3 mRNA by the RNAi pathway) when compared to a cell that is not treated with the ds oligonucleotide. Similarly, and as used herein, “reducing expression” refers to an act that results in reduced expression of a gene (*e.g.*, ANGPTL3). As used herein, “reduction of ANGPTL3 expression” refers to a decrease in the amount or level of ANGPTL3 mRNA, ANGPTL3 protein and/or ANGPTL3 activity in a cell, a population of cells, a sample or a subject when compared to an appropriate reference (*e.g.*, a reference cell, population of cells, sample, or subject).

[0072] As used herein, “region of complementarity” refers to a sequence of nucleotides of a nucleic acid (*e.g.*, a ds oligonucleotide) that is sufficiently complementary to an antiparallel sequence of nucleotides to permit hybridization between the two sequences of nucleotides under appropriate hybridization conditions (*e.g.*, in a phosphate buffer, in a cell, *etc.*). In some embodiments, an oligonucleotide herein comprises a targeting sequence having a region of complementarity to a mRNA target sequence.

[0073] As used herein, “ribonucleotide” refers to a nucleotide having a ribose as its pentose sugar, which contains a hydroxyl group at its 2' position. A modified ribonucleotide is a ribonucleotide having one or more modifications or substitutions of atoms other than at the 2' position, including modifications or substitutions in or of the ribose, phosphate group or base.

[0074] As used herein, “RNAi oligonucleotide” refers to either (a) a ds oligonucleotide having a sense strand (passenger) and antisense strand (guide), in which the antisense strand or part of the antisense strand is used by the Argonaute 2 (Ago2) endonuclease in the cleavage of

a target mRNA or (b) a ss oligonucleotide having a single antisense strand, where that antisense strand (or part of that antisense strand) is used by the Ago2 endonuclease in the cleavage of a target mRNA.

[0075] As used herein, “strand” refers to a single, contiguous sequence of nucleotides linked together through internucleotide linkages (*e.g.*, phosphodiester linkages or phosphorothioate linkages). In some embodiments, a strand has two free ends (*e.g.*, a 5' end and a 3' end).

[0076] As used herein, “subject” means any mammal, including mice, rabbits and humans. In one embodiment, the subject is a human or NHP. Moreover, “individual” or “patient” may be used interchangeably with “subject.”

[0077] As used herein, “synthetic” refers to a nucleic acid or other molecule that is artificially synthesized (*e.g.*, using a machine (*e.g.*, a solid-state nucleic acid synthesizer)) or that is otherwise not derived from a natural source (*e.g.*, a cell or organism) that normally produces the molecule.

[0078] As used herein, “targeting ligand” refers to a molecule (*e.g.*, a carbohydrate, amino sugar, cholesterol, polypeptide or lipid) that selectively binds to a cognate molecule (*e.g.*, a receptor) of a tissue or cell of interest and that is conjugatable to another substance for purposes of targeting the other substance to the tissue or cell of interest. For example, in some embodiments, a targeting ligand may be conjugated to an oligonucleotide for purposes of targeting the oligonucleotide to a specific tissue or cell of interest. In some embodiments, a targeting ligand selectively binds to a cell surface receptor. Accordingly, in some embodiments, a targeting ligand when conjugated to an oligonucleotide facilitates delivery of the oligonucleotide into a particular cell through selective binding to a receptor expressed on the surface of the cell and endosomal internalization by the cell of the complex comprising the oligonucleotide, targeting ligand and receptor. In some embodiments, a targeting ligand is conjugated to an oligonucleotide via a linker that is cleaved following or during cellular internalization such that the oligonucleotide is released from the targeting ligand in the cell.

[0079] As used herein, “tetraloop” refers to a loop that increases stability of an adjacent duplex formed by hybridization of flanking sequences of nucleotides. The increase in stability is detectable as an increase in melting temperature (T_m) of an adjacent stem duplex that is higher than the T_m of the adjacent stem duplex expected, on average, from a set of loops of comparable length consisting of randomly selected sequences of nucleotides. For example, a tetraloop can confer a T_m of at least about 50°C, at least about 55°C, at least about 56°C, at least about 58°C, at least about 60°C, at least about 65°C, or at least about 75°C in 10 mM NaHPO₄ to a hairpin comprising a duplex of at least 2 base pairs (bp) in length. In some

embodiments, a tetraloop may stabilize a bp in an adjacent stem duplex by stacking interactions. In addition, interactions among the nucleotides in a tetraloop include, but are not limited to, non-Watson-Crick base pairing, stacking interactions, hydrogen bonding and contact interactions (Cheong *et al.* (1990) *Nature* 346:680-682; Heus & Pardi (1991) *Science* 253:191-194). In some embodiments, a tetraloop comprises or consists of 3 to 6 nucleotides and is typically 4 to 5 nucleotides. In certain embodiments, a tetraloop comprises or consists of 3, 4, 5 or 6 nucleotides, which may or may not be modified (*e.g.*, which may or may not be conjugated to a targeting moiety). In one embodiment, a tetraloop consists of 4 nucleotides. Any nucleotide may be used in the tetraloop and standard IUPAC-IUB symbols for such nucleotides may be used as described in Cornish-Bowden (1985) *Nucleic Acids Res.* 13:3021-3030. For example, the letter “N” may be used to mean that any base may be in that position, the letter “R” may be used to show that A (adenine) or G (guanine) may be in that position, and “B” may be used to show that C (cytosine), G (guanine), or T (thymine) may be in that position. Examples of tetraloops include the UCG family of tetraloops (*e.g.*, UUCG), the GNRA family of tetraloops (*e.g.*, GAAA), and the CUUG tetraloop (Woese *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:8467-8471; Antao *et al.* (1991) *Nucleic Acids Res.* 19:5901-5905). Examples of DNA tetraloops include the d(GNNA) family of tetraloops (*e.g.*, d(GTTA)), the d(GNRA) family of tetraloops, the d(GNAB) family of tetraloops, the d(CNNG) family of tetraloops, and the d(TNCG) family of tetraloops (*e.g.*, d(TTCG)). *See, e.g.*, Nakano *et al.* (2002) *Biochem.* 41:4281-4292; Shinji *et al.* (2000) *Nippon Kagakkai Koen Yokoshu* 78:731. In some embodiments, the tetraloop is contained within a nicked tetraloop structure.

[0080] As used herein, “treat” or “treating” refers to the act of providing care to a subject in need thereof, for example, by administering a therapeutic agent (*e.g.*, an oligonucleotide herein) to the subject, for purposes of improving the health and/or well-being of the subject with respect to an existing condition (*e.g.*, a disease, disorder) or to prevent or decrease the likelihood of the occurrence of a condition. In some embodiments, treatment involves reducing the frequency or severity of at least one sign, symptom or contributing factor of a condition (*e.g.*, disease, disorder) experienced by a subject.

[0081] II. Oligonucleotide Inhibitors of ANGPTL3 Expression

[0082] The disclosure provides, *inter alia*, oligonucleotides that inhibit ANGPTL3 expression. In some embodiments, an oligonucleotide that inhibits ANGPTL3 expression herein is targeted to an ANGPTL3 mRNA.

[0083] i. ANGPTL3 Target Sequences

[0084] In some embodiments, the oligonucleotide is targeted to a target sequence comprising an ANGPTL3 mRNA. In some embodiments, the oligonucleotide, or a portion, fragment or strand thereof (*e.g.*, an antisense strand or a guide strand of a ds oligonucleotide) binds or anneals to a target sequence comprising an ANGPTL3 mRNA, thereby inhibiting ANGPTL3 expression. In some embodiments, the oligonucleotide is targeted to an ANGPTL3 target sequence for the purpose of inhibiting ANGPTL3 expression *in vivo*. In some embodiments, the amount or extent of inhibition of ANGPTL3 expression by an oligonucleotide targeted to an ANGPTL3 target sequence correlates with the potency of the oligonucleotide. In some embodiments, the amount or extent of inhibition of ANGPTL3 expression by an oligonucleotide targeted to an ANGPTL3 target sequence correlates with the amount or extent of therapeutic benefit in a subject or patient having a disease, disorder or condition associated with the expression of ANGPTL3 treated with the oligonucleotide.

[0085] Through examination of the nucleotide sequence of mRNAs encoding ANGPTL3, including mRNAs of multiple different species (*e.g.*, human, cynomolgus monkey, mouse, and rat; *see, e.g.*, Example 1) and as a result of *in vitro* and *in vivo* testing (*see, e.g.*, Example 2 and Example 3), it has been discovered that certain nucleotide sequences of ANGPTL3 mRNA are more amenable than others to oligonucleotide-based inhibition and are thus useful as target sequences for the oligonucleotides herein. In some embodiments, a sense strand of an oligonucleotide (*e.g.*, a ds oligonucleotide) described herein (*e.g.*, in **Table 5**) comprises an ANGPTL3 target sequence. In some embodiments, a portion or region of the sense strand of a ds oligonucleotide described herein (*e.g.*, in **Table 5**) comprises an ANGPTL3 target sequence. In some embodiments, an ANGPTL3 target sequence comprises, or consists of, a sequence of any one of SEQ ID NOs: 117, 118, 119, 120, 121, 122, 123, 124, 125, 126 and 127.

[0086] ii. ANGPTL3-Targeting Sequences

[0087] In some embodiments, the oligonucleotides herein have regions of complementarity to ANGPTL3 mRNA (*e.g.*, within a target sequence of ANGPTL3 mRNA) for purposes of targeting the mRNA in cells and inhibiting its expression. In some embodiments, the oligonucleotides herein comprise an ANGPTL3 targeting sequence (*e.g.*, an antisense strand or a guide strand of a ds oligonucleotide) having a region of complementarity that binds or anneals to an ANGPTL3 target sequence by complementary (Watson-Crick) base pairing. The targeting sequence or region of complementarity is generally of a suitable length and base content to enable binding or annealing of the oligonucleotide (or a strand thereof) to an ANGPTL3 mRNA for purposes of inhibiting its expression. In some embodiments, the

targeting sequence or region of complementarity is at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 26, at least about 27, at least about 28, at least about 29, or at least about 30 nucleotides in length. In some embodiments, the targeting sequence or region of complementarity is about 12 to about 30 (*e.g.*, 12 to 30, 12 to 22, 15 to 25, 17 to 21, 18 to 27, 19 to 27, or 15 to 30) nucleotides in length. In some embodiments, the targeting sequence or region of complementarity is about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In some embodiments, the targeting sequence or region of complementarity is 18 nucleotides in length. In some embodiments, the targeting sequence or region of complementarity is 19 nucleotides in length. In some embodiments, the targeting sequence or region of complementarity is 20 nucleotides in length. In some embodiments, the targeting sequence or region of complementarity is 21 nucleotides in length. In some embodiments, the targeting sequence or region of complementarity is 22 nucleotides in length. In some embodiments, the targeting sequence or region of complementarity is 23 nucleotides in length. In some embodiments, the targeting sequence or region of complementarity is 24 nucleotides in length.

[0088] In some embodiments, an oligonucleotide herein comprises a targeting sequence or a region of complementarity (*e.g.*, an antisense strand or a guide strand of a double-stranded oligonucleotide) that is fully complementary to an ANGPTL3 target sequence. In some embodiments, the targeting sequence or region of complementarity is partially complementary to an ANGPTL3 target sequence. In some embodiments, the oligonucleotide comprises a targeting sequence or region of complementarity that is fully complementary to a sequence of any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, and 115. In some embodiments, the oligonucleotide comprises a targeting sequence or region of complementarity that is partially complementary to a sequence of any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, and 115.

[0089] In some embodiments, the oligonucleotide herein comprises a targeting sequence or region of complementarity that is complementary to a contiguous sequence of nucleotides comprising an ANGPTL3 mRNA, where the contiguous sequence of nucleotides is about 12

to about 30 nucleotides in length (*e.g.*, 12 to 30, 12 to 28, 12 to 26, 12 to 24, 12 to 20, 12 to 18, 12 to 16, 14 to 22, 16 to 20, 18 to 20, or 18 to 19 nucleotides in length). In some embodiments, the oligonucleotide comprises a targeting sequence or region of complementarity that is complementary to a contiguous sequence of nucleotides comprising an ANGPTL3 mRNA, wherein the contiguous sequence of nucleotides is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length. In some embodiments, the oligonucleotide comprises a targeting sequence or region of complementarity that is complementary to a contiguous sequence of nucleotides comprising an ANGPTL3 mRNA, where the contiguous sequence of nucleotides is 19 nucleotides in length. In some embodiments, the oligonucleotide comprises a targeting sequence or a region of complementarity that is complementary to a contiguous sequence of nucleotides of any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, and 115, optionally where the contiguous sequence of nucleotides is 19 nucleotides in length.

[0090] In some embodiments, a targeting sequence or region of complementarity of an oligonucleotide that is complementary to contiguous nucleotides of a sequence as set forth in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, and 115 spans the entire length of an antisense strand. In some embodiments, a region of complementarity of an oligonucleotide that is complementary to contiguous nucleotides of a sequence as set forth in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, and 115 spans a portion of the entire length of an antisense strand. In some embodiments, an oligonucleotide herein comprises a region of complementarity (*e.g.*, on an antisense strand of a ds oligonucleotide) that is at least partially (*e.g.*, fully) complementary to a contiguous stretch of nucleotides spanning nucleotides 1-20 of a sequence as set forth in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, and 115.

[0091] In some embodiments, an oligonucleotide herein comprises a targeting sequence or region of complementarity having one or more bp mismatches with the corresponding ANGPTL3 target sequence. In some embodiments, the targeting sequence or region of complementarity may have up to about 1, up to about 2, up to about 3, up to about 4, up to

about 5, *etc.* mismatches with the corresponding ANGPTL3 target sequence provided that the ability of the targeting sequence or region of complementarity to bind or anneal to the ANGPTL3 mRNA under appropriate hybridization conditions and/or the ability of the oligonucleotide to inhibit ANGPTL3 expression is maintained. Alternatively, the targeting sequence or region of complementarity may have no more than 1, no more than 2, no more than 3, no more than 4, or no more than 5 mismatches with the corresponding ANGPTL3 target sequence provided that the ability of the targeting sequence or region of complementarity to bind or anneal to the ANGPTL3 mRNA under appropriate hybridization conditions and/or the ability of the oligonucleotide to inhibit ANGPTL3 expression is maintained. In some embodiments, the oligonucleotide comprises a targeting sequence or region of complementarity having 1 mismatch with the corresponding target sequence. In some embodiments, the oligonucleotide comprises a targeting sequence or region of complementarity having 2 mismatches with the corresponding target sequence. In some embodiments, the oligonucleotide comprises a targeting sequence or region of complementarity having 3 mismatches with the corresponding target sequence. In some embodiments, the oligonucleotide comprises a targeting sequence or region of complementarity having 4 mismatches with the corresponding target sequence. In some embodiments, the oligonucleotide comprises a targeting sequence or region of complementarity having 5 mismatches with the corresponding target sequence. In some embodiments, the oligonucleotide comprises a targeting sequence or region of complementarity more than one mismatch (*e.g.*, 2, 3, 4, 5 or more mismatches) with the corresponding target sequence, wherein at least 2 (*e.g.*, all) of the mismatches are positioned consecutively (*e.g.*, 2, 3, 4, 5 or more mismatches in a row), or where in the mismatches are interspersed throughout the targeting sequence or region of complementarity.

[0092] iii. Types of Oligonucleotides

[0093] A variety of oligonucleotide types and/or structures are useful for targeting ANGPTL3 in the methods herein including, but not limited to, RNAi oligonucleotides, antisense oligonucleotides, miRNAs, *etc.* Any of the oligonucleotide types described herein or elsewhere are contemplated for use as a framework to incorporate an ANGPTL3 targeting sequence herein.

[0094] In some embodiments, the oligonucleotides herein inhibit ANGPTL3 expression by engaging with RNA interference (RNAi) pathways upstream or downstream of Dicer involvement. For example, RNAi oligonucleotides have been developed with each strand having sizes of about 19-25 nucleotides with at least one 3' overhang of 1 to 5 nucleotides (*see*,

e.g., US Patent No. 8,372,968). Longer oligonucleotides also have been developed that are processed by Dicer to generate active RNAi products (*see, e.g.*, US Patent No. 8,883,996). Further work produced extended ds oligonucleotides where at least one end of at least one strand is extended beyond a duplex targeting region, including structures where one of the strands includes a thermodynamically-stabilizing tetraloop structure (*see, e.g.*, US Patent Nos. 8,513,207 and 8,927,705, as well as Intl. Patent Application Publication No. WO 2010/033225). Such structures may include ss extensions (on one or both sides of the molecule) as well as ds extensions.

[0095] In some embodiments, the oligonucleotides herein engage with the RNAi pathway downstream of the involvement of Dicer (*e.g.*, Dicer cleavage). In some embodiments, the oligonucleotide has an overhang (*e.g.*, of 1, 2, or 3 nucleotides in length) in the 3' end of the sense strand. In some embodiments, the oligonucleotide (*e.g.*, siRNA) comprises a 21-nucleotide guide strand that is antisense to a target RNA and a complementary passenger strand, in which both strands anneal to form a 19-bp duplex and 2 nucleotide overhangs at either or both 3' ends. Longer oligonucleotide designs also are available including oligonucleotides having a guide strand of 23 nucleotides and a passenger strand of 21 nucleotides, where there is a blunt end on the right side of the molecule (3' end of passenger strand/5' end of guide strand) and a two nucleotide 3'-guide strand overhang on the left side of the molecule (5' end of the passenger strand/3' end of the guide strand). In such molecules, there is a 21 bp duplex region. *See, e.g.*, US Patent Nos. 9,012,138; 9,012,621 and 9,193,753.

[0096] In some embodiments, the oligonucleotides herein comprise sense and antisense strands that are both in the range of about 17 to 26 (*e.g.*, 17 to 26, 20 to 25 or 21-23) nucleotides in length. In some embodiments, an oligonucleotide herein comprises a sense and antisense strand that are both in the range of about 19-22 nucleotides in length. In some embodiments, the sense and antisense strands are of equal length. In some embodiments, an oligonucleotide comprises sense and antisense strands, such that there is a 3'-overhang on either the sense strand or the antisense strand, or both the sense and antisense strand. In some embodiments, for oligonucleotides that have sense and antisense strands that are both in the range of about 21-23 nucleotides in length, a 3' overhang on the sense, antisense, or both sense and antisense strands is 1 or 2 nucleotides in length. In some embodiments, the oligonucleotide has a guide strand of 22 nucleotides and a passenger strand of 20 nucleotides, where there is a blunt end on the right side of the molecule (3' end of passenger strand/5' end of guide strand) and a 2 nucleotide 3'-guide strand overhang on the left side of the molecule (5' end of the passenger strand/3' end of the guide strand). In such molecules, there is a 20 bp duplex region.

[0097] Other oligonucleotide designs for use with the compositions and methods herein include: 16-mer siRNAs (*see, e.g.*, NUCLEIC ACIDS IN CHEMISTRY AND BIOLOGY. Blackburn (ed.), Royal Society of Chemistry, 2006), shRNAs (*e.g.*, having 19 bp or shorter stems; *see, e.g.*, Moore *et al.* (2010) *Methods Mol. Biol.* 629:141-158), blunt siRNAs (*e.g.*, of 19 bps in length; *see, e.g.*, Kraynack & Baker (2006) *RNA* 12:163-176), asymmetrical siRNAs (aiRNA; *see, e.g.*, Sun *et al.* (2008) *Nat. Biotechnol.* 26:1379-1382), asymmetric shorter-duplex siRNA (*see, e.g.*, Chang *et al.* (2009) *Mol. Ther.* 17:725-732), fork siRNAs (*see, e.g.*, Hohjoh (2004) *FEBS Lett.* 557:193-198), ss siRNAs (Elsner (2012) *Nat. Biotechnol.* 30:1063), dumbbell-shaped circular siRNAs (*see, e.g.*, Abe *et al.* (2007) *J. Am. Chem. Soc.* 129:15108-15109), and small internally segmented interfering RNA (siRNA; *see, e.g.*, Bramsen *et al.* (2007) *Nucleic Acids Res.* 35:5886-5897). Further non-limiting examples of an oligonucleotide structures that may be used in some embodiments to reduce or inhibit the expression of ANGPTL3 are microRNA (miRNA), short hairpin RNA (shRNA) and short siRNA (*see, e.g.*, Hamilton *et al.* (2002) *EMBO J.* 21:4671-4679; *see also*, US Patent Application Publication No. 2009/0099115).

[0098] Still, in some embodiments, an oligonucleotide for reducing or inhibiting ANGPTL3 expression herein is ss. Such structures may include but are not limited to ss RNAi molecules. Recent efforts have demonstrated the activity of ss RNAi molecules (*see, e.g.*, Matsui *et al.* (2016) *Mol. Ther.* 24:946-955). However, in some embodiments, oligonucleotides herein are antisense oligonucleotides (ASOs). An antisense oligonucleotide is a ss oligonucleotide that has a nucleobase sequence which, when written in the 5' to 3' direction, comprises the reverse complement of a targeted segment of a particular nucleic acid and is suitably modified (*e.g.*, as a gapmer) so as to induce RNaseH-mediated cleavage of its target RNA in cells or (*e.g.*, as a mixmer) so as to inhibit translation of the target mRNA in cells. ASOs for use herein may be modified in any suitable manner known in the art including, for example, as shown in US Patent No. 9,567,587 (including, *e.g.*, length, sugar moieties of the nucleobase (pyrimidine, purine), and alterations of the heterocyclic portion of the nucleobase). Further, ASOs have been used for decades to reduce expression of specific target genes (*see, e.g.*, Bennett *et al.* (2017) *Annu. Rev. Pharmacol.* 57:81-105).

[0099] iv. Double-Stranded Oligonucleotides

[00100] The disclosure provides ds oligonucleotides for targeting ANGPTL3 mRNA and inhibiting ANGPTL3 expression (*e.g.*, via the RNAi pathway) comprising a sense strand (also referred to herein as a passenger strand) and an antisense strand (also referred to herein as a guide strand). In some embodiments, the sense strand and antisense strand are separate strands

and are not covalently linked. In some embodiments, the sense strand and antisense strand are covalently linked.

[00101] In some embodiments, the sense strand has a first region (R1) and a second region (R2), wherein R2 comprises a first subregion (S1), a tetraloop (L) or triloop (triL), and a second subregion (S2), wherein L or triL is located between S1 and S2, and wherein S1 and S2 form a second duplex (D2). D2 may have various length. In some embodiments, D2 is about 1-6 bp in length. In some embodiments, D2 is 2-6, 3-6, 4-6, 5-6, 1-5, 2-5, 3-5 or 4-5 bp in length. In some embodiments, D2 is 1, 2, 3, 4, 5 or 6 bp in length. In some embodiments, D2 is 6 bp in length.

[00102] In some embodiments, R1 of the sense strand and the antisense strand form a first duplex (D1). In some embodiments, D1 is at least about 15 (*e.g.*, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21) nucleotides in length. In some embodiments, D1 is in the range of about 12 to 30 nucleotides in length (*e.g.*, 12 to 30, 12 to 27, 15 to 22, 18 to 22, 18 to 25, 18 to 27, 18 to 30, or 21 to 30 nucleotides in length). In some embodiments, D1 is at least 12 nucleotides in length (*e.g.*, at least 12, at least 15, at least 20, at least 25, or at least 30 nucleotides in length). In some embodiments, D1 is 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides in length. In some embodiments, D1 is 20 nucleotides in length. In some embodiments, D1 comprising sense strand and antisense strand does not span the entire length of the sense strand and/or antisense strand. In some embodiments, D1 comprising the sense strand and antisense strand spans the entire length of either the sense strand or antisense strand or both. In certain embodiments, D1 comprising the sense strand and antisense strand spans the entire length of both the sense strand and the antisense strand.

[00103] In some embodiments, a ds oligonucleotide herein comprises a sense strand having a sequence of any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, and 115 and an antisense strand comprising a complementary sequence selected from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, and 116, as is arranged **Table 3**. In some embodiments, the sense strand comprises the sequence of SEQ ID NO: 99 and the antisense strand comprises the sequence of SEQ ID NO: 100.

[00104] In some embodiments, a ds oligonucleotide herein comprises a sense strand comprising a sequence of any one of SEQ ID NOs: 19, 25, 49, 71, 73, 75, 79, 99, 101, 103, and 113 and an antisense strand comprising a complementary sequence selected from SEQ ID NOs: 20, 26, 50, 72, 74, 76, 80, 100, 102, 104, and 114, as is arranged **Table 4**. In some embodiments, the sense strand comprises the sequence of SEQ ID NO: 99 and the antisense strand comprises the sequence of SEQ ID NO: 100.

[00105] It should be appreciated that, in some embodiments, sequences presented in the Sequence Listing may be referred to in describing the structure of an oligonucleotide or other nucleic acid. In such embodiments, the actual oligonucleotide or other nucleic acid may have one or more alternative nucleotides (*e.g.*, an RNA counterpart of a DNA nucleotide or a DNA counterpart of an RNA nucleotide) and/or one or more modified nucleotides and/or one or more modified internucleotide linkages and/or one or more other modification when compared with the specified sequence while retaining essentially same or similar complementary properties as the specified sequence.

[00106] In some embodiments, a ds oligonucleotide herein comprises a 25-nucleotide sense strand and a 27-nucleotide antisense strand that when acted upon by a Dicer enzyme results in an antisense strand that is incorporated into the mature RISC. In some embodiments, the sense strand of the ds oligonucleotide is longer than 27 nucleotides (*e.g.*, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 nucleotides). In some embodiments, the sense strand of the ds oligonucleotide is longer than 25 nucleotides (*e.g.*, 26, 27, 28, 29 or 30 nucleotides).

[00107] In some embodiments, oligonucleotides herein have one 5' end that is thermodynamically less stable when compared to the other 5' end. In some embodiments, an asymmetry oligonucleotide is provided that includes a blunt end at the 3' end of a sense strand and a 3'-overhang at the 3' end of an antisense strand. In some embodiments, the 3'-overhang on the antisense strand is about 1-8 nucleotides in length (*e.g.*, 1, 2, 3, 4, 5, 6, 7 or 8 nucleotides in length). Typically, an oligonucleotide for RNAi has a two-nucleotide overhang on the 3' end of the antisense (guide) strand. However, other overhangs are possible. In some embodiments, an overhang is a 3'-overhang comprising a length of between 1 and 6 nucleotides, optionally 1 to 5, 1 to 4, 1 to 3, 1 to 2, 2 to 6, 2 to 5, 2 to 4, 2 to 3, 3 to 6, 3 to 5, 3 to 4, 4 to 6, 4 to 5, 5 to 6 nucleotides, or 1, 2, 3, 4, 5 or 6 nucleotides. However, in some embodiments, the overhang is a 5'-overhang comprising a length of between 1 and 6 nucleotides, optionally 1 to 5, 1 to 4, 1 to 3, 1 to 2, 2 to 6, 2 to 5, 2 to 4, 2 to 3, 3 to 6, 3 to 5, 3 to 4, 4 to 6, 4 to 5, 5 to 6 nucleotides, or 1, 2, 3, 4, 5 or 6 nucleotides.

[00108] In some embodiments, two terminal nucleotides on the 3' end of an antisense strand are modified. In some embodiments, the two terminal nucleotides on the 3' end of the antisense strand are complementary with the target. In some embodiments, the two terminal nucleotides on the 3' end of the antisense strand are not complementary with the target. In some embodiments, two terminal nucleotides on each 3' end of an oligonucleotide in the nicked tetraloop structure are GG. Typically, one or both of the two terminal GG nucleotides on each 3' end of an oligonucleotide is not complementary with the target.

[00109] In some embodiments, there is one or more (*e.g.*, 1, 2, 3, 4 or 5) mismatch between a sense and antisense strand. If there is more than one mismatch between a sense and antisense strand, they may be positioned consecutively (*e.g.*, 2, 3 or more in a row), or interspersed throughout the region of complementarity. In some embodiments, the 3' end of the sense strand contains one or more mismatches. In one embodiment, two mismatches are incorporated at the 3' end of the sense strand. In some embodiments, base mismatches or destabilization of segments at the 3' end of the sense strand of the oligonucleotide improved the potency of synthetic duplexes in RNAi, possibly through facilitating processing by Dicer.

[00110] a. Antisense Strands

[00111] In some embodiments, an oligonucleotide disclosed herein for targeting ANGPTL3 comprises an antisense strand comprising or consisting of a sequence as set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, and 116. In some embodiments, an oligonucleotide comprises an antisense strand comprising or consisting of at least about 12 (*e.g.*, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, or at least 23) contiguous nucleotides of a sequence as set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, and 116.

[00112] In some embodiments, a ds oligonucleotide comprises an antisense strand of up to about 40 nucleotides in length (*e.g.*, up to 40, up to 35, up to 30, up to 27, up to 25, up to 21, up to 19, up to 17, or up to 12 nucleotides in length). In some embodiments, an oligonucleotide may have an antisense strand of at least about 12 nucleotides in length (*e.g.*, at least 12, at least 15, at least 19, at least 21, at least 22, at least 25, at least 27, at least 30, at least 35, or at least 38 nucleotides in length). In some embodiments, an oligonucleotide may have an antisense strand in a range of about 12 to about 40 (*e.g.*, 12 to 40, 12 to 36, 12 to 32, 12 to 28, 15 to 40,

15 to 36, 15 to 32, 15 to 28, 17 to 22, 17 to 25, 19 to 27, 19 to 30, 20 to 40, 22 to 40, 25 to 40, or 32 to 40) nucleotides in length. In some embodiments, an oligonucleotide may have an antisense strand of 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 or 40 nucleotides in length.

[00113] In some embodiments, an antisense strand of an oligonucleotide may be referred to as a “guide strand.” For example, if an antisense strand can engage with RNA-induced silencing complex (RISC) and bind to an Argonaute protein such as Ago2, or engage with or bind to one or more similar factors, and direct silencing of a target gene, it may be referred to as a guide strand. In some embodiments, a sense strand complementary to a guide strand may be referred to as a “passenger strand.”

[00114] b. Sense Strands

[00115] In some embodiments, an oligonucleotide herein for targeting ANGPTL3 comprises or consists of a sense strand sequence as set forth in in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, and 115. In some embodiments, an oligonucleotide has a sense strand that comprises or consists of at least about 12 (*e.g.*, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, or at least 23) contiguous nucleotides of a sequence as set forth in in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, and 115.

[00116] In some embodiments, an oligonucleotide comprises a sense strand (or passenger strand) of up to about 40 nucleotides in length (*e.g.*, up to 40, up to 36, up to 30, up to 27, up to 25, up to 21, up to 19, up to 17, or up to 12 nucleotides in length). In some embodiments, an oligonucleotide may have a sense strand of at least about 12 nucleotides in length (*e.g.*, at least 12, at least 15, at least 19, at least 21, at least 25, at least 27, at least 30, at least 36, or at least 38 nucleotides in length). In some embodiments, an oligonucleotide may have a sense strand in a range of about 12 to about 40 (*e.g.*, 12 to 40, 12 to 36, 12 to 32, 12 to 28, 15 to 40, 15 to 36, 15 to 32, 15 to 28, 17 to 21, 17 to 25, 19 to 27, 19 to 30, 20 to 40, 22 to 40, 25 to 40, or 32 to 40) nucleotides in length. In some embodiments, an oligonucleotide may have a sense strand of 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 or 40 nucleotides in length.

[00117] In some embodiments, a sense strand comprises a stem-loop structure at its 3' end. In some embodiments, a sense strand comprises a stem-loop structure at its 5' end. In some embodiments, a stem is a duplex of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 bp in length. In some embodiments, a stem-loop provides the molecule protection against degradation (*e.g.*, enzymatic degradation) and facilitates targeting characteristics for delivery to a target cell. For example, in some embodiments, a loop provides added nucleotides on which modification can be made without substantially affecting the gene expression inhibition activity of an oligonucleotide. In certain embodiments, an oligonucleotide is herein in which the sense strand comprises (*e.g.*, at its 3' end) a stem-loop set forth as: S1-L-S2, in which S1 is complementary to S2, and in which L forms a loop between S1 and S2 of up to about 10 nucleotides in length (*e.g.*, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides in length). FIG. 3 depicts a non-limiting example of such an oligonucleotide.

[00118] In some embodiments, a loop (F) of a stem-loop is a tetraloop (*e.g.*, within a nicked tetraloop structure). A tetraloop may contain ribonucleotides, deoxyribonucleotides, modified nucleotides and combinations thereof. Typically, a tetraloop has 4 to 5 nucleotides.

[00119] v. Oligonucleotide Modifications

[00120] a. Sugar Modifications

[00121] In some embodiments, a modified sugar (also referred herein to a sugar analog) includes a modified deoxyribose or ribose moiety in which, for example, one or more modifications occur at the 2', 3', 4' and/or 5' carbon position of the sugar. In some embodiments, a modified sugar may also include non-natural alternative carbon structures such as those present in locked nucleic acids ("LNA"; *see, e.g.*, Koshkin *et al.* (1998) *Tetrahedron* 54:3607-3630), unlocked nucleic acids ("UNA"; *see, e.g.*, Snead *et al.* (2013) *Mol. Ther-Nucl. Acids* 2:e103), and bridged nucleic acids ("BNA"; *see, e.g.*, Imanishi & Obika (2002) *Chem Commun. (Camb)* 21:1653-1659).

[00122] In some embodiments, a nucleotide modification in a sugar comprises a 2'-modification. In some embodiments, a 2'-modification may be 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-fluoro (2'-F), 2'-aminoethyl (EA), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), 2'-O-[2-(methylamino)-2-oxoethyl] (2'-O-NMA), or 2'-deoxy-2'-fluoro-β-d-arabinonucleic acid (2'-FANA). In some embodiments, the modification is 2'-F, 2'-OMe or 2'-MOE. In some embodiments, a modification in a sugar comprises a modification of the sugar ring, which may comprise modification of one or more carbons of the sugar ring. For example, a modification of a sugar of a nucleotide may comprise a 2'-oxygen of a sugar is linked to a 1'-carbon or 4'-carbon of the sugar, or a 2'-oxygen is linked to the 1'-carbon or 4'-

carbon *via* an ethylene or methylene bridge. In some embodiments, a modified nucleotide has an acyclic sugar that lacks a 2'-carbon to 3'-carbon bond. In some embodiments, a modified nucleotide has a thiol group, *e.g.*, in the 4' position of the sugar.

[00123] In some embodiments, the oligonucleotide described herein comprises at least about 1 modified nucleotide (*e.g.*, at least 1, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, or more). In some embodiments, the sense strand of the oligonucleotide comprises at least about 1 modified nucleotide (*e.g.*, at least 1, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, or more). In some embodiments, the antisense strand of the oligonucleotide comprises at least about 1 modified nucleotide (*e.g.*, at least 1, at least 5, at least 10, at least 15, at least 20, or more).

[00124] In some embodiments, all the nucleotides of the sense strand of the oligonucleotide are modified. In some embodiments, all the nucleotides of the antisense strand of the oligonucleotide are modified. In some embodiments, all the nucleotides of the oligonucleotide (*i.e.*, both the sense strand and the antisense strand) are modified. In some embodiments, the modified nucleotide comprises a 2'-modification (*e.g.*, a 2'-F or 2'-OMe, 2'-MOE, and 2'-deoxy-2'-fluoro- β -D-arabinonucleic acid). In some embodiments, the modified nucleotide comprises a 2'-modification (*e.g.*, a 2'-F or 2'-OMe).

[00125] The disclosure provides oligonucleotides having different modification patterns. In some embodiments, the modified oligonucleotides comprise a sense strand sequence having a modification pattern as set forth in any one of **Tables 3** and **4** (as well as FIG. 3) and an antisense strand having a modification pattern as set forth in any one of **Tables 3** and **4** (as well as FIG. 3). In some embodiments, for these oligonucleotides, one or more of positions 8, 9, 10 or 11 of the sense strand is modified with a 2'-F group. In other embodiments, for these oligonucleotides, the sugar moiety at each of nucleotides at positions 1-7 and 12-20 in the sense strand is modified with a 2'-OMe.

[00126] In some embodiments, the present invention provide an oligonucleotide, which is, or comprises, a modified or unmodified sense strand selected from those listed in Table A. In some embodiments, the present invention provide an oligonucleotide, which is, or comprises, a modified or unmodified antisense strand selected from those listed in Table A. In some embodiments, the present invention provide a modified or unmodified double-stranded oligonucleotide selected from those listed in Table A. In some embodiments, the present invention provide a sense strand modification pattern selected from those listed in Table A. In

some embodiments, the present invention provide an antisense strand modification pattern selected from those listed in Table A.

[00127] In some embodiments, the antisense strand has 3 nucleotides that are modified at the 2'-position of the sugar moiety with a 2'-F. In some embodiments, the sugar moiety at positions 2, 5 and 14 and optionally up to 3 of the nucleotides at positions 1, 3, 7 and 10 of the antisense strand are modified with a 2'-F. In other embodiments, the sugar moiety at each of the positions at positions 2, 5 and 14 of the antisense strand is modified with the 2'-F. In other embodiments, the sugar moiety at each of the positions at positions 1, 2, 5 and 14 of the antisense strand is modified with the 2'-F. In still other embodiments, the sugar moiety at each of the positions at positions 1, 2, 3, 5, 7 and 14 of the antisense strand is modified with the 2'-F. In yet another embodiment, the sugar moiety at each of the positions at positions 1, 2, 3, 5, 10 and 14 of the antisense strand is modified with the 2'-F. In another embodiment, the sugar moiety at each of the positions at positions 2, 3, 5, 7, 10 and 14 of the antisense strand is modified with the 2'-F.

[00128] b. 5' Terminal Phosphates

[00129] In some embodiments, 5'-terminal phosphate groups of oligonucleotides enhance the interaction with Ago2. However, oligonucleotides comprising a 5'-phosphate group may be susceptible to degradation *via* phosphatases or other enzymes, which can limit their bioavailability *in vivo*. In some embodiments, oligonucleotides include analogs of 5' phosphates that are resistant to such degradation. In some embodiments, a phosphate analog may be oxymethylphosphonate, vinylphosphonate or malonylphosphonate. In certain embodiments, the 1' end of an oligonucleotide strand is attached to chemical moiety that mimics the electrostatic and steric properties of a natural 5'-phosphate group ("phosphate mimic").

[00130] In some embodiments, an oligonucleotide has a phosphate analog at a 4'-carbon position of the sugar (referred to as a "4'-phosphate analog"). *See, e.g.*, Intl. Patent Application Publication No. WO 2018/045317. In some embodiments, an oligonucleotide herein comprises a 4'-phosphate analog at a 5'-terminal nucleotide. In some embodiments, a phosphate analog is an oxymethylphosphonate, in which the oxygen atom of the oxymethyl group is bound to the sugar moiety (*e.g.*, at its 4'-carbon) or analog thereof. In other embodiments, a 4'-phosphate analog is a thiomethylphosphonate or an aminomethylphosphonate, in which the sulfur atom of the thiomethyl group or the nitrogen atom of the amino methyl group is bound to the 4'-carbon of the sugar moiety or analog thereof. In certain embodiments, a 4'-phosphate analog is an oxymethylphosphonate. In some embodiments, an oxymethylphosphonate is represented by the formula $-O-CH_2-PO(OH)_2$ or $-O-CH_2-PO(OR)_2$, in which R is independently selected

from H, CH₃, an alkyl group, CH₂CH₂CN, CH₂OCOC(CH₃)₃, CH₂OCH₂CH₂Si(CH₃)₃ or a protecting group. In certain embodiments, the alkyl group is CH₂CH₃. More typically, R is independently selected from H, CH₃ or CH₂CH₃.

[00131] c. Modified Intranucleoside Linkages

[00132] In some embodiments, an oligonucleotide may comprise a modified internucleoside linkage. In some embodiments, phosphate modifications or substitutions may result in an oligonucleotide that comprises at least about 1 (*e.g.*, at least 1, at least 2, at least 3 or at least 5) modified internucleotide linkage. In some embodiments, any one of the oligonucleotides disclosed herein comprises about 1 to about 10 (*e.g.*, 1 to 10, 2 to 8, 4 to 6, 3 to 10, 5 to 10, 1 to 5, 1 to 3, or 1 to 2) modified internucleotide linkages. In some embodiments, any one of the oligonucleotides disclosed herein comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 modified internucleotide linkages.

[00133] A modified internucleotide linkage may be a phosphorodithioate linkage, a phosphorothioate linkage, a phosphotriester linkage, a thionoalkylphosphonate linkage, a thionalkylphosphotriester linkage, a phosphoramidite linkage, a phosphonate linkage or a boranophosphate linkage. In some embodiments, at least one modified internucleotide linkage of any one of the oligonucleotides as disclosed herein is a phosphorothioate linkage.

[00134] In some embodiments, the oligonucleotide described herein has a phosphorothioate linkage between one or more of positions 1 and 2 of the sense strand, positions 1 and 2 of the antisense strand, positions 2 and 3 of the antisense strand, positions 3 and 4 of the antisense strand, positions 20 and 21 of the antisense strand, and positions 21 and 22 of the antisense strand. In some embodiments, the oligonucleotide described herein has a phosphorothioate linkage between each of positions 1 and 2 of the sense strand, positions 1 and 2 of the antisense strand, positions 2 and 3 of the antisense strand, positions 20 and 21 of the antisense strand, and positions 21 and 22 of the antisense strand.

[00135] d. Base Modifications

[00136] In some embodiments, oligonucleotides herein have one or more modified nucleobases. In some embodiments, modified nucleobases (also referred to herein as base analogs) are linked at the 1' position of a nucleotide sugar moiety. In certain embodiments, a modified nucleobase is a nitrogenous base. In certain embodiments, a modified nucleobase does not contain nitrogen atom. *See, e.g.*, US Patent Application Publication No. 2008/0274462. In some embodiments, a modified nucleotide comprises a universal base. However, in certain embodiments, a modified nucleotide does not contain a nucleobase (abasic).

[00137] In some embodiments, a universal base is a heterocyclic moiety located at the 1' position of a nucleotide sugar moiety in a modified nucleotide, or the equivalent position in a nucleotide sugar moiety substitution, that, when present in a duplex, can be positioned opposite more than one type of base without substantially altering structure of the duplex. In some embodiments, compared to a reference single-stranded nucleic acid (*e.g.*, oligonucleotide) that is fully complementary to a target nucleic acid, a single-stranded nucleic acid containing a universal base forms a duplex with the target nucleic acid that has a lower T_m than a duplex formed with the complementary nucleic acid. However, in some embodiments, when compared to a reference single-stranded nucleic acid in which the universal base has been replaced with a base to generate a single mismatch, the single-stranded nucleic acid containing the universal base forms a duplex with the target nucleic acid that has a higher T_m than a duplex formed with the nucleic acid comprising the mismatched base.

[00138] Non-limiting examples of universal-binding nucleotides include, but are not limited to, inosine, 1- β -D-ribofuranosyl-5-nitroindole and/or 1- β -D-ribofuranosyl-3-nitropyrrole (*see*, US Patent Application Publication No. 2007/0254362; Van Aerschot *et al.* (1995) *Nucleic Acids Res.* 23:4363-4370; Loakes *et al.* (1995) *Nucleic Acids Res.* 23:2361-2366; and Loakes & Brown (1994) *Nucleic Acids Res.* 22:4039-4043).

[00139] e. Reversible Modifications

[00140] While certain modifications to protect an oligonucleotide from the *in vivo* environment before reaching target cells can be made, they can reduce the potency or activity of the oligonucleotide once it reaches the cytosol of the target cell. Reversible modifications can be made such that the molecule retains desirable properties outside of the cell, which are then removed upon entering the cytosolic environment of the cell. Reversible modification can be removed, for example, by the action of an intracellular enzyme or by the chemical conditions inside of a cell (*e.g.*, through reduction by intracellular glutathione).

[00141] In some embodiments, a reversibly modified nucleotide comprises a glutathione-sensitive moiety. Typically, nucleic acid molecules have been chemically modified with cyclic disulfide moieties to mask the negative charge created by the internucleotide diphosphate linkages and improve cellular uptake and nuclease resistance. *See* US Patent Application Publication No. 2011/0294869, Intl. Patent Application Publication Nos. WO 2014/088920 and WO 2015/188197, and Meade *et al.* (2014) *Nat. Biotechnol.* 32:1256-1263. This reversible modification of the internucleotide diphosphate linkages is designed to be cleaved intracellularly by the reducing environment of the cytosol (*e.g.*, glutathione). Earlier examples

include neutralizing phosphotriester modifications that were reported to be cleavable inside cells (*see, Dellinger et al. (2003) J. Am. Chem. Soc. 125:940-950*).

[00142] In some embodiments, such a reversible modification allows protection during *in vivo* administration (*e.g.*, transit through the blood and/or lysosomal/endosomal compartments of a cell) where the oligonucleotide will be exposed to nucleases and other harsh environmental conditions (*e.g.*, pH). When released into the cytosol of a cell where the levels of glutathione are higher compared to extracellular space, the modification is reversed, and the result is a cleaved oligonucleotide. Using reversible, glutathione-sensitive moieties, it is possible to introduce sterically larger chemical groups into the oligonucleotide of interest when compared to the options available using irreversible chemical modifications. This is because these larger chemical groups will be removed in the cytosol and, therefore, should not interfere with the biological activity of the oligonucleotides inside the cytosol of a cell. As a result, these larger chemical groups can be engineered to confer various advantages to the nucleotide or oligonucleotide, such as nuclease resistance, lipophilicity, charge, thermal stability, specificity and reduced immunogenicity. In some embodiments, the structure of the glutathione-sensitive moiety can be engineered to modify the kinetics of its release.

[00143] In some embodiments, a glutathione-sensitive moiety is attached to the sugar of the nucleotide. In some embodiments, a glutathione-sensitive moiety is attached to the 2'-carbon of the sugar of a modified nucleotide. In some embodiments, the glutathione-sensitive moiety is located at the 5'-carbon of a sugar, particularly when the modified nucleotide is the 5'-terminal nucleotide of the oligonucleotide. In some embodiments, the glutathione-sensitive moiety is located at the 3'-carbon of sugar, particularly when the modified nucleotide is the 3'-terminal nucleotide of the oligonucleotide. In some embodiments, the glutathione-sensitive moiety comprises a sulfonyl group. *See, e.g.*, US Provisional Patent Application No. 62/378,635, entitled *Compositions Comprising Reversibly Modified Oligonucleotides and Uses Thereof*, which was filed on August 23, 2016.

[00144] vi. Targeting Ligands

[00145] In some embodiments, it is desirable to target the oligonucleotides of the disclosure to one or more cells or one or more organs. Such a strategy can help to avoid undesirable effects in other organs or avoid undue loss of the oligonucleotide to cells, tissue or organs that would not benefit from the oligonucleotide. Accordingly, in some embodiments, oligonucleotides disclosed herein are modified to facilitate targeting and/or delivery of a particular tissue, cell or organ (*e.g.*, to facilitate delivery of the oligonucleotide to the liver). In certain embodiments, oligonucleotides disclosed herein are modified to facilitate delivery of

the oligonucleotide to the hepatocytes of the liver. In some embodiments, an oligonucleotide comprises at least one nucleotide (*e.g.*, 1, 2, 3, 4, 5, 6 or more nucleotides) conjugated to one or more targeting ligand(s).

[00146] In some embodiments, the targeting ligand comprises a carbohydrate, amino sugar, cholesterol, peptide, polypeptide, protein or part of a protein (*e.g.*, an antibody or antibody fragment), or lipid. In some embodiments, the targeting ligand is an aptamer. For example, a targeting ligand may be an RGD peptide that is used to target tumor vasculature or glioma cells, CREKA peptide to target tumor vasculature or stoma, transferring, lactoferrin, or an aptamer to target transferrin receptors expressed on CNS vasculature, or an anti-EGFR antibody to target EGFR on glioma cells. In certain embodiments, the targeting ligand is one or more GalNAc moieties.

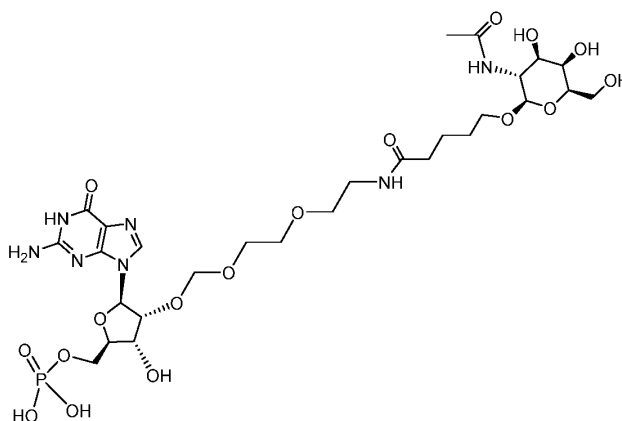
[00147] In some embodiments, 1 or more (*e.g.*, 1, 2, 3, 4, 5 or 6) nucleotides of an oligonucleotide are each conjugated to a separate targeting ligand. In some embodiments, 2 to 4 nucleotides of an oligonucleotide are each conjugated to a separate targeting ligand. In some embodiments, targeting ligands are conjugated to 2 to 4 nucleotides at either ends of the sense or antisense strand (*e.g.*, targeting ligands are conjugated to a 2 to 4 nucleotide overhang or extension on the 5' or 3' end of the sense or antisense strand) such that the targeting ligands resemble bristles of a toothbrush and the oligonucleotide resembles a toothbrush. For example, an oligonucleotide may comprise a stem-loop at either the 5' or 3' end of the sense strand and 1, 2, 3 or 4 nucleotides of the loop of the stem may be individually conjugated to a targeting ligand. In some embodiments, an oligonucleotide (*e.g.*, a ds oligonucleotide) provided by the disclosure comprises a stem-loop at the 3' end of the sense strand, wherein the loop of the stem-loop comprises a triloop or a tetraloop, and wherein the 3 or 4 nucleotides comprising the triloop or tetraloop, respectfully, are individually conjugated to a targeting ligand.

[00148] GalNAc is a high affinity ligand for the ASGPR, which is primarily expressed on the sinusoidal surface of hepatocyte cells and has a major role in binding, internalizing and subsequent clearing circulating glycoproteins that contain terminal galactose or GalNAc residues (asialoglycoproteins). Conjugation (either indirect or direct) of GalNAc moieties to oligonucleotides of the instant disclosure can be used to target these oligonucleotides to the ASGPR expressed on cells. In some embodiments, an oligonucleotide of the instant disclosure is conjugated to at least one or more GalNAc moieties, wherein the GalNAc moieties target the oligonucleotide to an ASGPR expressed on human liver cells (*e.g.*, human hepatocytes). In some embodiments, the GalNAc moiety target the oligonucleotide to the liver.

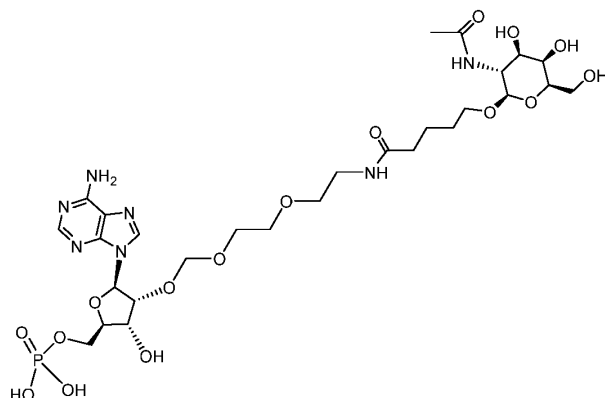
[00149] In some embodiments, an oligonucleotide of the instant disclosure is conjugated directly or indirectly to a monovalent GalNAc. In some embodiments, the oligonucleotide is conjugated directly or indirectly to more than one monovalent GalNAc (*i.e.*, is conjugated to 2, 3 or 4 monovalent GalNAc moieties, and is typically conjugated to 3 or 4 monovalent GalNAc moieties). In some embodiments, an oligonucleotide is conjugated to one or more bivalent GalNAc, trivalent GalNAc or tetravalent GalNAc moieties.

[00150] In some embodiments, 1 or more (*e.g.*, 1, 2, 3, 4, 5 or 6) nucleotides of an oligonucleotide are each conjugated to a GalNAc moiety. In some embodiments, 2 to 4 nucleotides of a tetraloop are each conjugated to a separate GalNAc. In some embodiments, 1 to 3 nucleotides of a triloop are each conjugated to a separate GalNAc. In some embodiments, targeting ligands are conjugated to 2 to 4 nucleotides at either ends of the sense or antisense strand (*e.g.*, ligands are conjugated to a 2 to 4 nucleotide overhang or extension on the 5' or 3' end of the sense or antisense strand) such that the GalNAc moieties resemble bristles of a toothbrush and the oligonucleotide resembles a toothbrush. In some embodiments, GalNAc moieties are conjugated to a nucleotide of the sense strand. For example, 4 GalNAc moieties can be conjugated to nucleotides in the tetraloop of the sense strand where each GalNAc moiety is conjugated to 1 nucleotide.

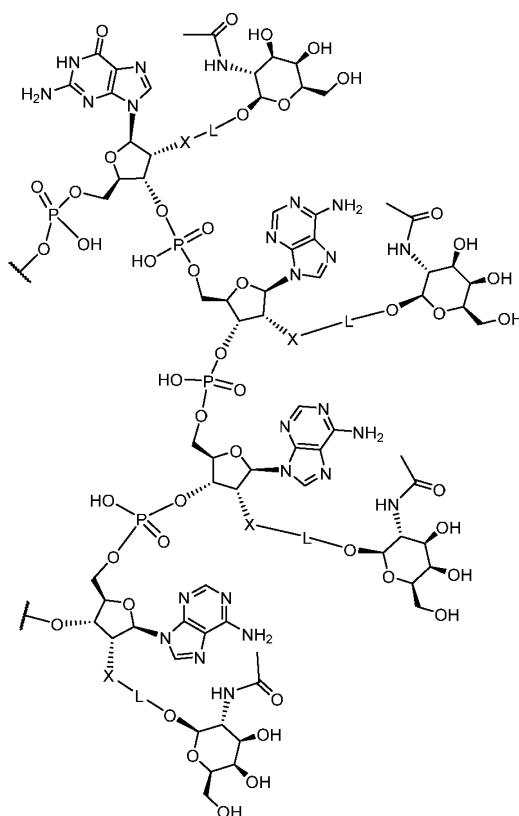
[00151] In some embodiments, an oligonucleotide herein comprises a monovalent GalNAc attached to a guanine nucleotide referred to as [ademG-GalNAc] or 2'-aminodiethoxymethanol-Guanine-GalNAc, as depicted below:



[00152] In some embodiments, an oligonucleotide herein comprises a monovalent GalNAc attached to an adenine nucleotide, referred to as [ademA-GalNAc] or 2'-aminodiethoxymethanol-Adenine-GalNAc, as depicted below:

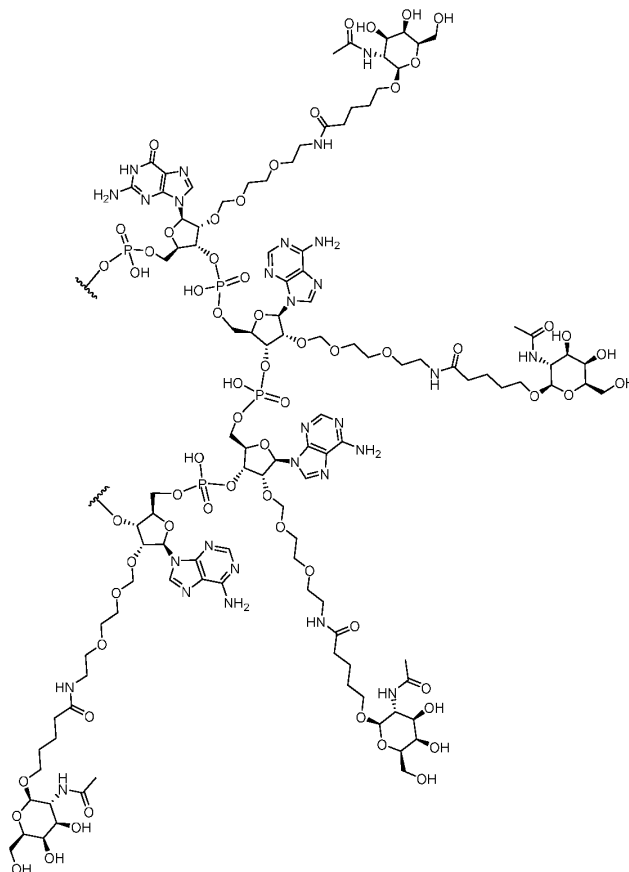


[00153] An example of such conjugation is shown below for a loop comprising from 5' to 3' the nucleotide sequence GAAA (L = linker, X = heteroatom) stem attachment points are shown. Such a loop may be present, for example, at positions 27-30 of the sense strand listed in **Table 5** and as shown in **FIG. 3**. In the chemical formula, $\frac{3}{2}$ is used to describe an attachment point to the oligonucleotide strand.



[00154] Appropriate methods or chemistry (*e.g.*, click chemistry) can be used to link a targeting ligand to a nucleotide. In some embodiments, a targeting ligand is conjugated to a nucleotide using a click linker. In some embodiments, an acetal-based linker is used to conjugate a targeting ligand to a nucleotide of any one of the oligonucleotides described herein. Acetal-based linkers are disclosed, for example, in Intl. Patent Application Publication No. WO 2016/100401. In some embodiments, the linker is a labile linker. However, in other embodiments, the linker is stable. An example is shown below for a loop comprising from 5' to 3' the nucleotides GAAA, in which GalNAc moieties are attached to nucleotides of the loop using an acetal linker. Such a loop may be present, for example, at positions 27-30 of the any one of the sense strand listed in **Table 5** and as shown in **FIG. 3**. In the chemical formula,

 is an attachment point to the oligonucleotide strand.



[00155] As mentioned, various appropriate methods or chemistry synthetic techniques (*e.g.*,

click chemistry) can be used to link a targeting ligand to a nucleotide. In some embodiments, a targeting ligand is conjugated to a nucleotide using a click linker. In some embodiments, an acetal-based linker is used to conjugate a targeting ligand to a nucleotide of any one of the oligonucleotides described herein. Acetal-based linkers are disclosed, for example, in Intl. Patent Application Publication No. WO 2016/100401. In some embodiments, the linker is a labile linker. However, in other embodiments, the linker is a stable linker.

[00156] In some embodiments, a duplex extension (*e.g.*, of up to 3, 4, 5 or 6 bp in length) is provided between a targeting ligand (*e.g.*, a GalNAc moiety) and a ds oligonucleotide. In some embodiments, the oligonucleotides herein do not have a GalNAc conjugated thereto.

[00157] III. Formulations

[00158] Various formulations have been developed to facilitate oligonucleotide use. For example, oligonucleotides can be delivered to a subject or a cellular environment using a formulation that minimizes degradation, facilitates delivery and/or uptake, or provides another beneficial property to the oligonucleotides in the formulation. In some embodiments, an oligonucleotide is formulated in buffer solutions such as phosphate buffered saline solutions, liposomes, micellar structures and capsids.

[00159] Formulations of oligonucleotides with cationic lipids can be used to facilitate transfection of the oligonucleotides into cells. For example, cationic lipids, such as lipofectin, cationic glycerol derivatives, and polycationic molecules (*e.g.*, polylysine, can be used. Suitable lipids include Oligofectamine, Lipofectamine (Life Technologies), NC388 (Ribozyme Pharmaceuticals, Inc., Boulder, Colo.), or FuGene 6 (Roche) all of which can be used according to the manufacturer's instructions.

[00160] Accordingly, in some embodiments, a formulation comprises a lipid nanoparticle. In some embodiments, an excipient comprises a liposome, a lipid, a lipid complex, a microsphere, a microparticle, a nanosphere or a nanoparticle, or may be otherwise formulated for administration to the cells, tissues, organs, or body of a subject in need thereof (*see, e.g.*, Remington: THE SCIENCE AND PRACTICE OF PHARMACY, 22nd edition, Pharmaceutical Press, 2013).

[00161] In some embodiments, the formulations herein comprise an excipient. In some embodiments, an excipient confers to a composition improved stability, improved absorption, improved solubility and/or therapeutic enhancement of the active ingredient. In some embodiments, an excipient is a buffering agent (*e.g.*, sodium citrate, sodium phosphate, a tris base, or sodium hydroxide) or a vehicle (*e.g.*, a buffered solution, petrolatum, dimethyl

sulfoxide or mineral oil). In some embodiments, an oligonucleotide is lyophilized for extending its shelf-life and then made into a solution before use (*e.g.*, administration to a subject). Accordingly, an excipient in a composition comprising any one of the oligonucleotides described herein may be a lyoprotectant (*e.g.*, mannitol, lactose, polyethylene glycol or polyvinylpyrrolidone) or a collapse temperature modifier (*e.g.*, dextran, Ficoll™ or gelatin).

[00162] In some embodiments, a pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral (*e.g.*, intravenous, intramuscular, intraperitoneal, intradermal, subcutaneous), oral (*e.g.*, inhalation), transdermal (*e.g.*, topical), transmucosal and rectal administration.

[00163] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.), or phosphate buffered saline (PBS). The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Sterile injectable solutions can be prepared by incorporating the oligonucleotides in a required amount in a selected solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

[00164] In some embodiments, a composition may contain at least about 0.1% of the therapeutic agent or more, although the percentage of the active ingredient(s) may be between about 1% to about 80% or more of the weight or volume of the total composition. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

[00165] Even though several embodiments are directed to liver-targeted delivery of any of the oligonucleotides herein, targeting of other tissues is also contemplated.

[00166] IV. Methods of Use

[00167] i. Reducing ANGPTL3 Expression in Cells

[00168] The disclosure provides methods for contacting or delivering to a cell or population of cells an effective amount any one of oligonucleotides herein for purposes of reducing ANGPTL3 expression. The methods can include the steps described herein, and these may be, but not necessarily, carried out in the sequence as described. Other sequences, however, also are conceivable. Moreover, individual or multiple steps may be carried out either in parallel and/or overlapping in time and/or individually or in multiply repeated steps. Furthermore, the methods may include additional, unspecified steps.

[00169] Methods herein are useful in any appropriate cell type. In some embodiments, a cell is any cell that expresses mRNA (*e.g.*, hepatocytes, macrophages, monocyte-derived cells, prostate cancer cells, cells of the brain, endocrine tissue, bone marrow, lymph nodes, lung, gall bladder, liver, duodenum, small intestine, pancreas, kidney, gastrointestinal tract, bladder, adipose, and soft tissue and skin). In some embodiments, the cell is a primary cell obtained from a subject. In some embodiments, the primary cell has undergone a limited number of passages such that the cell substantially maintains its natural phenotypic properties. In some embodiments, a cell to which the oligonucleotide is delivered is *ex vivo* or *in vitro* (*i.e.*, can be delivered to a cell in culture or to an organism in which the cell resides).

[00170] In some embodiments, the oligonucleotides herein are delivered using appropriate nucleic acid delivery methods including, but not limited to, injection of a solution containing the oligonucleotides, bombardment by particles covered by the oligonucleotides, exposing the cell or population of cells to a solution containing the oligonucleotides, or electroporation of cell membranes in the presence of the oligonucleotides. Other appropriate methods for delivering oligonucleotides to cells may be used, such as lipid-mediated carrier transport, chemical-mediated transport, and cationic liposome transfection such as calcium phosphate, and others.

[00171] In some embodiments, reduction of ANGPTL3 expression can be determined by an appropriate assay or technique to evaluate one or more properties or characteristics of a cell or population of cells associated with ANGPTL3 expression (*e.g.*, using an ANGPTL3 expression biomarker) or by an assay or technique that evaluates molecules that are directly indicative of ANGPTL3 expression (*e.g.*, ANGPTL3 mRNA or ANGPTL3 protein). In some embodiments, the extent to which an oligonucleotide herein reduces ANGPTL3 expression is evaluated by comparing ANGPTL3 expression in a cell or population of cells contacted with the oligonucleotide to an appropriate control (*e.g.*, an appropriate cell or population of cells not contacted with the oligonucleotide or contacted with a control oligonucleotide). In some embodiments, an appropriate control level of mRNA expression into protein, after delivery of

a RNAi molecule may be a predetermined level or value, such that a control level need not be measured every time. The predetermined level or value can take a variety of forms. In some embodiments, a predetermined level or value can be single cut-off value, such as a median or mean.

[00172] In some embodiments, administration of an oligonucleotide herein results in a reduction in ANGPTL3 expression in a cell or population of cells. In some embodiments, the reduction in ANGPTL3 expression is about 1% or lower, about 5% or lower, about 10% or lower, about 15% or lower, about 20% or lower, about 25% or lower, about 30% or lower, about 35% or lower, about 40% or lower, about 45% or lower, about 50% or lower, about 55% or lower, about 60% or lower, about 70% or lower, about 80% or lower, or about 90% or lower when compared with an appropriate control level of mRNA. The appropriate control level may be a level of mRNA expression and/or protein translation in a cell or population of cells that has not been contacted with an oligonucleotide herein. In some embodiments, the effect of delivery of an oligonucleotide to a cell according to a method herein is assessed after a finite period. For example, levels of mRNA may be analyzed in a cell at least about 8 hours, about 12 hours, about 18 hours, or about 24 hours; or at least about 1, 2, 3, 4, 5, 6, 7 or even up to 14 days after introduction of the oligonucleotide into the cell.

[00173] In some embodiments, an oligonucleotide is delivered in the form of a transgene that is engineered to express in a cell the oligonucleotide or strands comprising the oligonucleotide (*e.g.*, its sense and antisense strands). In some embodiments, an oligonucleotide is delivered using a transgene engineered to express any oligonucleotide disclosed herein. Transgenes may be delivered using viral vectors (*e.g.*, adenovirus, retrovirus, vaccinia virus, poxvirus, adeno-associated virus or herpes simplex virus) or non-viral vectors (*e.g.*, plasmids or synthetic mRNAs). In some embodiments, transgenes can be injected directly to a subject.

[00174] ii. Medical Use

[00175] The disclosure also provides oligonucleotides for use, or adaptable for use, to treat a subject (*e.g.*, a human having a disease, disorder or condition associated with ANGPTL3 expression) that would benefit from reducing ANGPTL3 expression. In some respects, the disclosure provides oligonucleotides for use, or adapted for use, to treat a subject having a disease, disorder or condition associated with expression of ANGPTL3. The disclosure also provides oligonucleotides for use, or adaptable for use, in the manufacture of a medicament or pharmaceutical composition for treating a disease, disorder or condition associated with ANGPTL3 expression. In some embodiments, the oligonucleotides for use, or adaptable for use, target ANGPTL3 mRNA and reduce ANGPTL3 expression (*e.g.*, via the RNAi pathway).

In some embodiments, the oligonucleotides for use, or adaptable for use, target ANGPTL3 mRNA and reduce the amount or level of ANGPTL3 mRNA, ANGPTL3 protein and/or ANGPTL3 activity.

[00176] In addition, the methods below can include selecting a subject having a disease, disorder or condition associated with ANGPTL3 expression or is predisposed to the same. In some instances, the methods can include selecting an individual having a marker for ANGPTL3 expression such as elevated TG or cholesterol (or even altered LPL and/or EL activity) or is predisposed to the same.

[00177] Likewise, and as detailed below, the methods also may include steps such as measuring or obtaining a baseline value for a marker of ANGPTL3 expression, and then comparing such obtained value to one or more other baseline values or values obtained after being administered the oligonucleotide to assess the effectiveness of treatment.

[00178] iii. Methods of Treatment

[00179] The disclosure also provides methods of treating a subject having, suspected of having, or at risk of developing a disease, disorder or condition with an oligonucleotide herein. In some aspects, the disclosure provides methods of treating or attenuating the onset or progression of a disease, disorder or condition associated with ANGPTL3 expression using the oligonucleotides herein. In other aspects, the disclosure provides methods to achieve one or more therapeutic benefits in a subject having a disease, disorder or condition associated with ANGPTL3 expression using the oligonucleotides herein. In some embodiments of the methods herein, the subject is treated by administering a therapeutically effective amount of any one or more of the oligonucleotides herein. In some embodiments, treatment comprises reducing ANGPTL3 expression. In some embodiments, the subject is treated therapeutically. In some embodiments, the subject is treated prophylactically.

[00180] In some embodiments of the methods herein, an oligonucleotide herein, or a pharmaceutical composition comprising the oligonucleotide, is administered to a subject having a disease, disorder or condition associated with ANGPTL3 expression such that ANGPTL3 expression is reduced in the subject, thereby treating the subject. In some embodiments, an amount or level of ANGPTL3 mRNA is reduced in the subject. In some embodiments, an amount or level of ANGPTL3 protein is reduced in the subject. In some embodiments, an amount or level of ANGPTL3 activity is reduced in the subject. In some embodiments, an amount or level of triglyceride (TG) (*e.g.*, one or more TG(s) or total TGs) is reduced in the subject. In some embodiments, an amount or level of cholesterol (*e.g.*, total cholesterol, LDL cholesterol, and/or HDL cholesterol) is reduced in the subject. In some

embodiments, an amount or level of low-density lipoprotein (LDL) cholesterol is reduced in the subject. In some embodiments, an amount or activity of LPL is altered in the subject. In some embodiments, an amount or activity of EL is altered in the subject. In some embodiments, any combination of the following is reduced or altered in the subject: ANGPTL3 expression, an amount or level of ANGPTL3 mRNA, an amount or level of ANGPTL3 protein, an amount or level of ANGPTL3 activity, an amount or level of TG, an amount or level of cholesterol, and/or an amount or activity of LPL and/or EL.

[00181] In some embodiments of the methods herein, an oligonucleotide herein, or a pharmaceutical composition comprising the oligonucleotide, is administered to a subject having a disease, disorder or condition associated with ANGPTL3 such that ANGPTL3 expression is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to ANGPTL3 expression prior to administration of the oligonucleotide or pharmaceutical composition. In some embodiments, ANGPTL3 expression is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to ANGPTL3 expression in a subject (*e.g.*, a reference or control subject) not receiving the oligonucleotide or pharmaceutical composition or receiving a control oligonucleotide, pharmaceutical composition or treatment.

[00182] In some embodiments of the methods herein, an oligonucleotide herein, or a pharmaceutical composition comprising the oligonucleotide, is administered to a subject having a disease, disorder or condition associated with ANGPTL3 expression such that an amount or level of ANGPTL3 mRNA is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to the amount or level of ANGPTL3 mRNA prior to administration of the oligonucleotide or pharmaceutical composition. In some embodiments, an amount or level of ANGPTL3 mRNA is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of ANGPTL3 mRNA in a subject (*e.g.*, a reference or control subject) not receiving the oligonucleotide or pharmaceutical composition or receiving a control oligonucleotide, pharmaceutical composition or treatment.

[00183] In some embodiments of the methods herein, an oligonucleotide herein, or a pharmaceutical composition comprising the oligonucleotide, is administered to a subject having a disease, disorder or condition associated with ANGPTL3 expression such that an amount or level of ANGPTL3 protein is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to the amount or level of ANGPTL3 protein prior to administration of the oligonucleotide or pharmaceutical composition. In some embodiments, an amount or level of ANGPTL3 protein is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of ANGPTL3 protein in a subject (*e.g.*, a reference or control subject) not receiving the oligonucleotide or pharmaceutical composition or receiving a control oligonucleotide, pharmaceutical composition or treatment.

[00184] In some embodiments of the methods herein, an oligonucleotide herein, or a pharmaceutical composition comprising the oligonucleotide, is administered to a subject having a disease, disorder or condition associated with ANGPTL3 such that an amount or level of ANGPTL3 activity/expression is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to the amount or level of ANGPTL3 activity prior to administration of the oligonucleotide or pharmaceutical composition. In some embodiments, an amount or level of ANGPTL3 activity is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of ANGPTL3 activity in a subject (*e.g.*, a reference or control subject) not receiving the oligonucleotide or pharmaceutical composition or receiving a control oligonucleotide, pharmaceutical composition or treatment.

[00185] In some embodiments of the methods herein, an oligonucleotide herein, or a pharmaceutical composition comprising the oligonucleotide, is administered to a subject having a disease, disorder or condition associated with ANGPTL3 expression such that an amount or level of TG (*e.g.*, one or more TGs or total TGs) is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater

than 99% when compared to the amount or level of TG prior to administration of the oligonucleotide or pharmaceutical composition. In some embodiments, an amount or level of TG is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of TG in a subject (*e.g.*, a reference or control subject) not receiving the oligonucleotide or pharmaceutical composition or receiving a control oligonucleotide, pharmaceutical composition or treatment.

[00186] Generally, a normal or desirable TG range for a human subject is <150 mg/dL of blood, with <100 mg/dL being considered ideal. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of TG of ≥ 150 mg/dL. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of TG in the range of 150 mg/dL to 199 mg/dL, which is considered borderline high TG levels. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of TG in the range of 200 to 499 mg/dL, which is considered high TG levels. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of TG in the range of 500 mg/dL or higher (*i.e.*, ≥ 500 mg/dL), which is considered very high TG levels. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of TG which is ≥ 150 mg/dL, ≥ 200 mg/dL or ≥ 500 mg/dL. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of TG of 200 mg/dL to 499 mg/dL, or 500 mg/dL or higher. In some embodiments, the patient selected for treatment or treated is identified or determined to have an amount or level of TG which is ≥ 200 mg/dL.

[00187] In some embodiments of the methods herein, an oligonucleotide herein, or a pharmaceutical composition comprising the oligonucleotide, is administered to a subject having a disease, disorder or condition associated with ANGPTL3 expression such that an amount or level of cholesterol (*e.g.*, total cholesterol, LDL cholesterol, and/or HDL cholesterol) is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to the amount or level of cholesterol prior to administration of the oligonucleotide or pharmaceutical composition. In some embodiments, an amount or level of cholesterol is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about

70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of cholesterol in a subject (*e.g.*, a reference or control subject) not receiving the oligonucleotide or pharmaceutical composition or receiving a control oligonucleotide, pharmaceutical composition or treatment.

[00188] Generally, a normal or desirable cholesterol range (total cholesterol) for an adult human patient is <200 mg/dL of blood. In some embodiments, the patient selected for treatment or treated is identified or determined to have an amount or level of cholesterol of ≥ 200 mg/dL. In some embodiments, the patient selected for treatment or treated is identified or determined to have an amount or level of cholesterol in the range of 200 mg/dL to 239 mg/dL, which is considered borderline high cholesterol levels. In some embodiments, the patient selected for treatment or treated is identified or determined to have an amount or level of cholesterol in the range of 240 mg/dL and higher (*i.e.*, ≥ 240 mg/dL), which is considered high cholesterol levels. In some embodiments, the patient selected from treatment or treated is identified or determined to have an amount or level of cholesterol of 200 mg/dL to 239 mg/dL, or 240 mg/dL or higher. In some embodiments, the patient selected for treatment or treated is identified or determined to have an amount or level of cholesterol which is ≥ 200 mg/dL or ≥ 240 mg/dL or higher.

[00189] In some embodiments of the methods herein, an oligonucleotide herein, or a pharmaceutical composition comprising the oligonucleotide, is administered to a subject having a disease, disorder, or condition associated with ANGPTL3 expression such that an amount or level of LDL cholesterol is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to the amount or level of LDL cholesterol prior to administration of the oligonucleotide or pharmaceutical composition. In some embodiments, an amount or level of LDL cholesterol is reduced in the subject by at least about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or greater than 99% when compared to an amount or level of LDL cholesterol in a subject (*e.g.*, a reference or control subject) not receiving the oligonucleotide or pharmaceutical composition or receiving a control oligonucleotide, pharmaceutical composition or treatment.

[00190] Generally, a normal or desirable LDL cholesterol range for an adult human subject is <100 mg/dL of blood. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of cholesterol of ≥ 100 mg/dL. In some

embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of LDL cholesterol in the range of 100 mg/dL to 129 mg/dL, which is considered above optimal. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of LDL cholesterol in the range of 130 mg/dL to 159 mg/dL, which is considered borderline high levels. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of LDL cholesterol in the range of 160 mg/dL to 189 mg/dL, which is considered high LDL cholesterol levels. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of LDL cholesterol in the range of 190 mg/dL and higher (*i.e.*, ≥ 190 mg/dL), which is considered very high LDL cholesterol levels. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of LDL cholesterol which is ≥ 100 mg/dL, ≥ 130 mg/dL, ≥ 160 mg/dL, or ≥ 190 mg/dL or higher, preferably ≥ 160 mg/dL, or ≥ 190 mg/dL or higher. In some embodiments, the subject selected for treatment or treated is identified or determined to have an amount or level of LDL cholesterol of 100 mg/dL to 129 mg/dL, 130 mg/dL to 159 mg/dL, 160 mg/dL to 189 mg/dL, or 190 mg/dL and higher.

[00191] Suitable methods for determining ANGPTL3 expression, the amount or level of ANGPTL3 mRNA, ANGPTL3 protein, ANGPTL3 activity, TG and/or LDL cholesterol, LPL and/or EL amount or activity in the subject, or in a sample from the subject, are known in the art. Further, the Examples set forth herein illustrate methods for determining ANGPTL3 expression.

[00192] In some embodiments, ANGPTL3 expression, the amount or level of ANGPTL3 mRNA, ANGPTL3 protein, ANGPTL3 activity, TG, LDL cholesterol, LPL protein, LPL activity, EL protein, EL activity or any combination thereof, is reduced in a cell (*e.g.*, a hepatocyte), a population or a group of cells (*e.g.*, an organoid), an organ (*e.g.*, liver), blood or a fraction thereof (*e.g.*, plasma), a tissue (*e.g.*, liver tissue), a sample (*e.g.*, a liver biopsy sample), or any other appropriate biological material obtained or isolated from the subject. In some embodiments, ANGPTL3 expression, the amount or level of ANGPTL3 mRNA, ANGPTL3 protein, ANGPTL3 activity, TG, LDL cholesterol, LPL protein, LPL activity, EL protein, EL activity or any combination thereof, is reduced in more than one type of cell (*e.g.*, a hepatocyte and one or more other type(s) of cell), more than one groups of cells, more than one organ (*e.g.*, liver and one or more other organ(s)), more than one fraction of blood (*e.g.*, plasma and one or more other blood fraction(s)), more than one type of tissue (*e.g.*, liver tissue and one or more other type(s) of tissue), more than one type of sample (*e.g.*, a liver biopsy

sample and one or more other type(s) of biopsy sample) isolated or other.

[00193] Examples of a disease, disorder or condition associated with ANGPTL3 expression include, but are not limited to, hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, type II diabetes mellitus (T2D), cardiovascular disease, chronic kidney disease, coronary artery disease, NASH, NAFLD, homozygous and heterozygous familial hypercholesterolemia, statin-resistant hypercholesterolemia and other ANGPTL3-associated metabolic-related disorders and diseases. Of particular interest herein are cardiovascular disease, T2D, hypertriglyceridemia, NASH, obesity or a combination thereof.

[00194] Because of their high specificity, the oligonucleotides herein specifically target mRNAs of target genes of diseased cells and tissues. In preventing disease, the target gene may be one that is required for initiation or maintenance of the disease or that has been identified as being associated with a higher risk of contracting the disease. In treating disease, the oligonucleotide can be brought into contact with the cells or tissue exhibiting the disease. For example, an oligonucleotide substantially identical to all or part of a wild-type (*i.e.*, native) or mutated gene associated with a disorder or condition associated with ANGPTL3 expression may be brought into contact with or introduced into a cell or tissue type of interest such as a hepatocyte or other liver cell.

[00195] In some embodiments, the target gene may be a target gene from any mammal, such as a human. Any gene may be silenced according to the method described herein.

[00196] Methods described herein are typically involve administering to a subject in an effective amount of an oligonucleotide, that is, an amount capable of producing a desirable therapeutic result. A therapeutically acceptable amount may be an amount that can therapeutically treat a disease or disorder. The appropriate dosage for any one subject will depend on certain factors, including the subject's size, body surface area, age, the particular composition to be administered, the active ingredient(s) in the composition, time and route of administration, general health, and other drugs being administered concurrently.

[00197] In some embodiments, a subject is administered any one of the compositions herein either enterally (*e.g.*, orally, by gastric feeding tube, by duodenal feeding tube, via gastrostomy or rectally), parenterally (*e.g.*, subcutaneous injection, intravenous injection or infusion, intra-arterial injection or infusion, intraosseous infusion, intramuscular injection, intracerebral injection, intracerebroventricular injection, intrathecal), topically (*e.g.*, epicutaneous, inhalational, *via* eye drops, or through a mucous membrane), or by direct injection into a target organ (*e.g.*, the liver of a subject). Typically, oligonucleotides herein are administered

intravenously or subcutaneously.

[00198] As a non-limiting set of examples, the oligonucleotides herein would typically be administered quarterly (once every three months), bi-monthly (once every two months), monthly or weekly. For example, the oligonucleotides may be administered every week or at intervals of two, or three weeks. Alternatively, the oligonucleotides may be administered daily. In some embodiments, a subject is administered one or more loading doses of the oligonucleotide followed by one or more maintenance doses of the oligonucleotide.

[00199] In some embodiments, the subject to be treated is a human or non-human primate or other mammalian subject. Other exemplary subjects include domesticated animals such as dogs and cats; livestock such as horses, cattle, pigs, sheep, goats, and chickens; and animals such as mice, rats, guinea pigs, and hamsters.

[00200] V. Kits

[00201] In some embodiments, the disclosure provides a kit comprising an oligonucleotide herein, and instructions for use. In some embodiments, the kit comprises an oligonucleotide herein, and a package insert containing instructions for use of the kit and/or any component thereof. In some embodiments, the kit comprises, in a suitable container, an oligonucleotide herein, one or more controls, and various buffers, reagents, enzymes, and other standard ingredients well known in the art. In some embodiments, the container comprises at least one vial, well, test tube, flask, bottle, syringe or other container means, into which the oligonucleotide is placed, and in some instances, suitably aliquoted. In some embodiments where an additional component is provided, the kit contains additional containers into which this component is placed. The kits can also include a means for containing the oligonucleotide and any other reagent in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained. Containers and/or kits can include labeling with instructions for use and/or warnings.

[00202] In some embodiments, a kit comprises an oligonucleotide herein, and a pharmaceutically acceptable carrier, or a pharmaceutical composition comprising the oligonucleotide and instructions for treating or delaying progression of a disease, disorder or condition associated with ANGPTL3 expression in a subject in need thereof.

EXAMPLES

[00203] While the disclosure has been described with reference to the specific embodiments set forth in the following Examples, it should be understood by those skilled in the art that

various changes may be made, and equivalents may be substituted without departing from the true spirit and scope of the disclosure. Further, the following Examples are offered by way of illustration and are not intended to limit the scope of the disclosure in any manner. In addition, modifications may be made to adapt to a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the disclosure. All such modifications are intended to be within the scope of the disclosure. Standard techniques well known in the art or the techniques specifically described below are utilized.

[00204] **Example 1: Preparation of Double-Stranded RNAi Oligonucleotides**

[00205] *Oligonucleotide Synthesis and Purification*

[00206] The ds RNAi oligonucleotides described in the foregoing Examples are chemically synthesized using methods described herein. Generally, ds RNAi oligonucleotides are synthesized using solid phase oligonucleotide synthesis methods as described for 19-23mer siRNAs (see, e.g., Scaringe *et al.* (1990) *Nucleic Acids Res.* 18:5433-5441 and Usman *et al.* (1987) *J. Am. Chem. Soc.* 109:7845-7845; see also, US Patent Nos. 5,804,683; 5,831,071; 5,998,203; 6,008,400; 6,111,086; 6,117,657; 6,353,098; 6,362,323; 6,437,117 and 6,469,158).

[00207] Individual RNA strands are synthesized and HPLC purified according to standard methods (Integrated DNA Technologies; Coralville, IA). For example, RNA oligonucleotides are synthesized using solid phase phosphoramidite chemistry, deprotected and desalted on NAP-5 columns (Amersham Pharmacia Biotech; Piscataway, NJ) using standard techniques (Damha & Olgivie (1993) *Methods Mol. Biol.* 20:81-114; Wincott *et al.* (1995) *Nucleic Acids Res.* 23:2677-2684). The oligomers are purified using ion-exchange high performance liquid chromatography (IE-HPLC) on an Amersham Source 15Q column (1.0 cm×25 cm; Amersham Pharmacia Biotech) using a 15 min step-linear gradient. The gradient varies from 90:10 Buffers A:B to 52:48 Buffers A:B, where Buffer A is 100 mM Tris pH 8.5 and Buffer B is 100 mM Tris pH 8.5, 1 M NaCl. Samples are monitored at 260 nm and peaks corresponding to the full-length oligonucleotide species are collected, pooled, desalted on NAP-5 columns, and lyophilized.

[00208] The purity of each oligomer is determined by capillary electrophoresis (CE) on a Beckman PACE 5000 (Beckman Coulter, Inc.; Fullerton, CA). The CE capillaries have a 100 µm inner diameter and contain ssDNA 100R Gel (Beckman-Coulter). Typically, about 0.6 nmole of oligonucleotide is injected into a capillary, is run in an electric field of 444 V/cm and is detected by UV absorbance at 260 nm. Denaturing Tris-Borate-7 M-urea running buffer is purchased from Beckman-Coulter. Oligoribonucleotides are obtained that are at least 90%

pure as assessed by CE for use in experiments described below. Compound identity is verified by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectroscopy on a Voyager DE™ Biospectrometry Work Station (Applied Biosystems; Foster City, CA) following the manufacturer's recommended protocol. Relative molecular masses of all oligomers are obtained, often within 0.2% of expected molecular mass.

[00209] *Preparation of Duplexes*

[00210] ssRNA oligomers are resuspended (*e.g.*, at 100 μ M concentration) in duplex buffer consisting of 100 mM potassium acetate, 30 mM HEPES, pH 7.5. Complementary sense and antisense strands are mixed in equal molar amounts to yield a final solution of, for example, 50 μ M duplex. Samples are heated to 100°C for 5' in RNA buffer (IDT) and are allowed to cool to room temperature before use. The ds RNA oligonucleotides are stored at -20° C. ss RNA oligomers are stored lyophilized or in nuclease-free water at -80° C.

[00211] **Example 2: RNAi Oligonucleotide Inhibition of ANGPTL3 Expression *In Vitro***

[00212] *ANGPTL3 Target Sequence Identification*

[00213] To identify RNAi oligonucleotide inhibitors of ANGPTL3 expression, a computer-based algorithm is used to computationally generate ANGPTL3 target sequences suitable for assaying inhibition of ANGPTL3 expression by the RNAi pathway. The algorithm provides RNAi oligonucleotide guide strand sequences that are complementary to suitable ANGPTL3 target sequences of human ANGPTL3 mRNA (*e.g.*, SEQ ID NO: 128; **Table 1**). Exemplary target sequences of human ANGPTL3 mRNA are provided in **Table 2**. Some of the guide strand sequences identified by the algorithm are also complementary to the corresponding ANGPTL3 target sequence of monkey and/or mouse ANGPTL3 mRNA (SEQ ID NO: 129 and 130, respectively; **Table 1**). 384 ds RNAi oligonucleotides (formatted as DsiRNA oligonucleotides) are generated, each with a unique guide strand having a region of complementarity to an ANGPTL3 target sequence identified by the algorithm.

[00214] **Table 1:** Sequences of Human, Monkey and Mouse ANGPTL3 mRNA

Species	GenBank Ref Seq #	SEQ ID NO
Human (Hs)	NM_014495.4	128
Cynomolgus monkey (Mf)	XM_005543185.2	129
Mouse (Mm)	NM_013913.4	130
Rat (Rn)	NM_001025065.1	131

[00215] **Table 2:** Exemplary Human ANGPTL3 mRNA Target Sequences

Target Sequence	SEQ ID NO
CUCAACAUAUUUGAUCAGU	117
AGAGCCAAAAUCAAGAUUU	118
CAAAAUCAAGAUUUGCUAU	119
GAGAAGAACUACAUUAAA	120
GUAGAAAAACAAGAUAAUA	121
UAGAAAAACAAGAUAAUAG	122
AGAAAAACAAGAUAAUAGC	123
AACAGCAUAGUCAAAUAAA	124
UCAAAAUUGGAAGGUUAUAC*	125
AAAUUGGAAGGUUAUACUCU	126
GAAGGUUAUACUCUAUAAA	127

[00216] *In Vitro Cell-Based Assays*

[00217] The ability of each of the 384 DsiRNAs above to inhibit ANGPTL3 expression is determined using *in vitro* cell-based assays. Briefly, HuH-7 human liver cells stably expressing ANGPTL3 are transfected with each of the DsiRNAs (0.5 nM) in separate wells of a multi-well cell-culture plate. Cells are maintained for 24 hr following transfection, and then levels of remaining ANGPTL3 mRNA from the transfected cells are determined using TAQMAN®-based qPCR assays. Two qPCR assays, a 3' assay and a 5' assay, are used to determine mRNA levels as measured by HEX and FAM probes, respectively.

[00218] The results of the HuH-7 cell-based assay with the 384 DsiRNAs are shown in **FIG. 1** and **FIG. 2**. **FIG. 1** shows the results of the HuH-7 cell-based assay with 109 DsiRNAs that have guide strands that are complementary to human, monkey and mouse ANGPTL3 mRNA ("triple common"). Transfection of a triple common DsiRNA that results in less than or equal to 35% ANGPTL3 mRNA remaining in the cells when compared to negative controls is considered a candidate ANGPTL3 expression inhibitor (referred to herein as a "hit"). **FIG. 2** shows the results of the HuH-7 cell-based assay with 275 DsiRNAs that have guide strands that are complementary to human and monkey ANGPTL3 mRNA ("human-monkey"). Human-monkey DsiRNAs resulting in less than or equal to 30% ANGPTL3 mRNA remaining when compared to negative controls are also considered hits. In **FIG. 1** and **FIG. 2**, the percent mRNA remaining is shown for each of the 3' assay (circle shapes) and the 5' assay (diamond shapes).

[00219] These results show that DsiRNAs designed to target human ANGPTL3 mRNA inhibit ANGPTL3 expression in cells (as determined by a reduced amount of ANGPTL3 mRNA in DsiRNA-transfected cells) and that the nucleotide sequences comprising the DsiRNA hits are

useful for generating RNAi oligonucleotides to inhibit ANGPTL3 expression. Further, these results demonstrate that multiple ANGPTL3 target sequences are suitable for the RNAi-mediated inhibition of ANGPTL3 expression.

[00220] **Example 3: RNAi Oligonucleotide Inhibition of ANGPTL3 Expression *In Vivo***

[00221] Of the 384 DsiRNAs screened in the HuH-7 cell-based assays described in **Example 2**, the nucleotide sequences of 55 DsiRNAs hits (**Table 3**) are selected for further evaluation *in vivo*. Briefly, the nucleotide sequences of the 55 selected DsiRNAs are used to generate 55 corresponding double-stranded RNAi oligonucleotides comprising a nicked tetraloop GalNAc-conjugated structure (referred to herein as “GalNAc-conjugated ANGPTL3 oligonucleotides”) having a 36-mer passenger strand and a 22-mer guide strand. Further, the nucleotide sequences comprising the passenger strand and guide strand of the GalNAc-conjugated ANGPTL3 oligonucleotides have a distinct pattern of modified nucleotides and phosphorothioate linkages (*see, e.g.*, **FIG. 3** for a schematic of the generic structure and chemical modification patterns of the GalNAc-conjugated ANGPTL3 oligonucleotides). The three adenosine nucleotides comprising the tetraloop are each conjugated to a GalNAc moiety (CAS#: 14131-60-3).

[00222] **Table 3:** GalNAc-Conjugated ANGPTL3 Oligonucleotides Evaluated in Mice

Oligonucleotide	DP#	SEQ ID NO (Sense)	SEQ ID NO (Antisense)
ANGPTL3-0099-M1	DP14993P:DP14992G	1	2
ANGPTL3-0108-M1	DP14995P:DP14994G	3	4
ANGPTL3-0111-M1	DP14997P:DP14996G	5	6
ANGPTL3-0112-M1	DP14999P:DP14998G	7	8
ANGPTL3-0143-M1	DP15001P:DP15000G	9	10
ANGPTL3-0165-M1	DP15003P:DP15002G	11	12
ANGPTL3-0167-M1	DP15005P:DP15004G	13	14
ANGPTL3-0170-M1	DP15007P:DP15006G	15	16
ANGPTL3-0196-M1	DP15009P:DP15008G	17	18
ANGPTL3-0197-M1	DP15011P:DP15010G	19	20
ANGPTL3-0198-M1	DP15013P:DP15012G	21	22
ANGPTL3-0201-M1	DP15015P:DP15014G	23	24
ANGPTL3-0202-M1	DP15017P:DP15016G	25	26
ANGPTL3-0203-M1	DP15019P:DP15018G	27	28
ANGPTL3-0212-M1	DP15021P:DP15020G	29	30
ANGPTL3-0303-M1	DP15023P:DP15022G	31	32

ANGPTL3-0310-M1	DP15025P:DP15024G	33	34
ANGPTL3-0330-M1	DP15027P:DP15026G	35	36
ANGPTL3-0332-M1	DP15029P:DP15028G	37	38
ANGPTL3-0333-M1	DP15031P:DP15030G	39	40
ANGPTL3-0337-M1	DP15033P:DP15032G	41	42
ANGPTL3-0394-M1	DP15035P:DP15034G	43	44
ANGPTL3-0396-M1	DP15037P:DP15036G	45	46
ANGPTL3-0400-M1	DP15039P:DP15038G	47	48
ANGPTL3-0401-M1	DP15041P:DP15040G	49	50
ANGPTL3-0437-M1	DP15043P:DP15042G	51	52
ANGPTL3-0447-M1	DP15045P:DP15044G	53	54
ANGPTL3-0517-M1	DP15047P:DP15046G	55	56
ANGPTL3-0518-M1	DP15049P:DP15048G	57	58
ANGPTL3-0532-M1	DP15051P:DP15050G	59	60
ANGPTL3-0541-M1	DP15053P:DP15052G	61	62
ANGPTL3-0582-M1	DP15055P:DP15054G	63	64
ANGPTL3-0602-M1	DP15057P:DP15056G	65	66
ANGPTL3-0603-M1	DP15059P:DP15058G	67	68
ANGPTL3-0604-M1	DP15061P:DP15060G	69	70
ANGPTL3-0606-M1	DP15063P:DP15062G	71	72
ANGPTL3-0607-M1	DP15065P:DP15064G	73	74
ANGPTL3-0608-M1	DP15067P:DP15066G	75	76
ANGPTL3-0610-M1	DP15069P:DP15068G	77	78
ANGPTL3-0676-M1	DP15071P:DP15070G	79	80
ANGPTL3-0738-M1	DP15073P:DP15072G	81	82
ANGPTL3-0796-M1	DP15075P:DP15074G	83	84
ANGPTL3-0893-M1	DP15077P:DP15076G	85	86
ANGPTL3-0894-M1	DP15079P:DP15078G	87	88
ANGPTL3-0895-M1	DP15081P:DP15080G	89	90
ANGPTL3-1059-M1	DP15083P:DP15082G	91	92
ANGPTL3-1062-M1	DP15085P:DP15084G	93	94
ANGPTL3-1065-M1	DP15087P:DP15086G	95	96
ANGPTL3-1071-M1	DP15089P:DP15088G	97	98
ANGPTL3-1412-M1	DP15091P:DP15090G	99	100
ANGPTL3-1415-M1	DP15093P:DP15092G	101	102
ANGPTL3-1420-M1	DP15095P:DP15094G	103	104

ANGPTL3-1421-M1	DP15097P:DP15096G	105	106
ANGPTL3-1422-M1	DP15099P:DP15098G	107	108
ANGPTL3-1468-M1	DP15101P:DP15100G	109	110
ANGPTL3-0204-M2	DP13439P:DP13438G	111	112
ANGPTL3-0327-M2	DP13443P:DP13442G	113	114
ANGPTL3-1327-M2	DP13465P:DP13464G	115	116

[00223] *Mouse Studies*

[00224] The GalNAc-conjugated ANGPTL3 oligonucleotides listed in **Table 3** are evaluated in mice engineered to transiently express human ANGPTL3 mRNA in hepatocytes. Three GalNAc-conjugated ANGPTL3 oligonucleotides (ANGPTL3-0204-M2, ANGPTL3-0327-M2 and ANGPTL3-1327-M2) are used as benchmark controls. Briefly, 6-8-week-old female CD-1 mice are treated subcutaneously with a GalNAc-conjugated ANGPTL3 oligonucleotide at a dose level of 1 mg/kg. Three days later (72 h), the mice are hydrodynamically injected with a DNA plasmid encoding the full human ANGPTL3 gene under control of a ubiquitous cytomegalovirus (CMV) promoter sequence. One day after introduction of the plasmid, liver samples are collected. Total RNA derived from these mice are subjected to qRT-PCR analysis for ANGPTL3 mRNA, relative to mice treated only with an identical volume of PBS. The values are normalized for transfection efficiency using the NeoR gene included on the plasmid.

[00225] As shown in **FIG. 4**, all the GalNAc-conjugated ANGPTL3 oligonucleotides tested inhibit ANGPTL3 expression, as determined by a reduced amount of ANGPTL3 mRNA in liver samples from oligonucleotide-treated mice relative to mice treated with PBS. The mean % of remaining ANGPTL3 mRNA in liver samples of mice treated with the benchmark GalNAc-conjugated ANGPTL3 oligonucleotide ANGPTL3-0327 relative to mice treated with PBS is shown as a solid bar. **FIG. 4** shows that 26 out of the 55 GalNAc-conjugated ANGPTL3 oligonucleotides tested inhibit ANGPTL3 expression to a greater extent than the benchmark GalNAc-conjugated ANGPTL3 oligonucleotide ANGPTL3-0327. Based on these results, 10 of the 55 GalNAc-conjugated ANGPTL3 oligonucleotides, indicated by arrows in **FIG. 4** and listed in **Table 4**, are selected for evaluation of their ability to inhibit ANGPTL3 expression in NHPs. The 10 GalNAc-conjugated ANGPTL3 oligonucleotides listed in **Table 4** comprise chemically modified nucleotides having either pattern M1 or M2 as described in **FIG. 3**.

[00226] **Table 4:** GalNAc-Conjugated ANGPTL3 Oligonucleotides Evaluated in NHPs

Oligonucleotide	DP#	SEQ ID NO (Sense)	SEQ ID NO (Antisense)
-----------------	-----	----------------------	--------------------------

ANGPTL3-0327-M2	DP13443P:DP13442G	113	114
ANGPTL3-0197-M1	DP15011P:DP15010G	19	20
ANGPTL3-0202-M1	DP15017P:DP15016G	25	26
ANGPTL3-0401-M1	DP15041P:DP15040G	49	50
ANGPTL3-0606-M1	DP15063P:DP15062G	71	72
ANGPTL3-0607-M1	DP15065P:DP15064G	73	74
ANGPTL3-0608-M1	DP15067P:DP15066G	75	76
ANGPTL3-0676-M1	DP15071P:DP15070G	79	80
ANGPTL3-1412 -M1	DP15091P:DP15090G	99	100
ANGPTL3-1415-M1	DP15093P:DP15092G	101	102
ANGPTL3-1420-M1	DP15095P:DP15094G	103	104

Table A: Sequence information for the oligonucleotides in Tables 3 and 4.

DP number passenger Guide	Modification Pattern	Sequence with Modifications	Corresponding unmodified sequence
DP1499 3P:DP1 4992G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mU][mA][mA][mA][mA][fU][fG][fU][fU][mC][mA][mC][mA][mA][mU][mU][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:132)	AUAAAAUGUUCA CAAUUAAGCAGCC GAAAGGCUGC (SEQ ID NO:1)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fAs][fA][fU][mU][fG][mU][mG][fA][mA][mC][mA][fU][mU][mU][mU][mU][mA][mUs][mGs][mG] (SEQ ID NO:133)	UUAAUUGUGAACA UUUUUAUGG (SEQ ID NO:2)
DP1499 5P:DP1 4994G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mUs][mU][mC][mA][mC][mA][mA][fU][fU][fA][fA][mG][mC][mU][mC][mC][mU][mU][mC][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:134)	UUCACAAUUAAGC UCCUUCAGCAGCC GAAAGGCUGC (SEQ ID NO:3)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fGs][fAs][fA][fG][mG][fA][mG][mC][fU][mU][mA][mA][fU][mU][mG][mU][mG][mA][mAs][mGs][mG] (SEQ ID NO:135)	UGAAGGAGCUUAA UUGUGAAGG (SEQ ID NO:4)

DP1499 7P:DP1 4996G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mC][mA][mU][mU][mA][fA][fG][fC][fU][mC][mC][mU][mU][mC][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:136)	ACAAUUAAGCUCC UUCUUUAGCAGCC GAAAGGCUGC (SEQ ID NO:5)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fA][fG][mA][fA][mG][mG][fA][mG][mC][mU][fU][mA][mA][mU][mU][mG][mUs][mGs][mG] (SEQ ID NO:137)	UAAAGAAGGAGCU UAAUUGUGG (SEQ ID NO:6)
DP1499 9P:DP1 4998G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mCs][mA][mA][mU][mU][mA][mA][fG][fC][fU][fC][mC][mU][mU][mC][mU][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:138)	CAAUUAAGCUCCU UCUUUAGCAGCC GAAAGGCUGC (SEQ ID NO:7)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fA][fA][mG][fA][mA][mG][fG][mA][m G][mC][fU][mU][mA][mA][mU][mU][mGs][mGs][m G] (SEQ ID NO:139)	UAAAAGAAGGAGC UUAUUGGG (SEQ ID NO:8)
DP1500 1P:DP1 5000G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mG][mU][mU][mA][mU][mU][fU][fC][fC][fU][mC][mC][mA][mG][mA][mA][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:140)	AGUUUUUCCUCC AGAAUAGCAGCC GAAAGGCUGC (SEQ ID NO:9)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fU][fU][mC][fU][mG][mG][fA][mG][mG][mA][fA][mA][mU][mA][mA][mC][mUs][mGs][mG] (SEQ ID NO:141)	UAAUUCUGGAGGA AAUAACUGG (SEQ ID NO:10)
DP1500 3P:DP1 5002G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mCs][mA][mA][mG][mA][mC][mA][fA][fU][fU][fC][mA][mU][mC][mA][mU][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:142)	CAAGACAAUUAU CAUUUGAGCAGCC GAAAGGCUGC (SEQ ID NO:11)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fCs][fAs][fA][fA][mU][fG][mA][mU][fG][mA][m G] (SEQ ID NO:12)	UCAAUGAUGAAU UGUCUUGGG (SEQ ID NO:12)

		A][mU][fU][mG][mU][mC][mU][mU][mGs][mGs][mG] (SEQ ID NO:143)	
DP1500 5P:DP1 5004G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mG][mA][mC][mA][mA][mU][fU][fC][fA][fU][mC][mA][mU][mU][mU][mG][mA][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:144)	AGACAAUUCAUCA UUUGAUAGCAGCC GAAAGGCUGC (SEQ ID NO:13)
	M{MS}{MS}MMMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fUs][fC][fA][mA][fA][mU][mG][fA][mU][m G][mA][fA][mU][mU][mG][mU][mC][mUs][mGs][m G] (SEQ ID NO:145)	UAUCAAUGAUGA AUUGUCUGG (SEQ ID NO:14)
DP1500 7P:DP1 5006G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mCs][mA][mA][mU][mU][mC][mA][fU][fC][fA][fU][mU][mU][mG][mA][mU][mU][mC][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:146)	CAAUUCAUAUUU GAUUCUAGCAGCC GAAAGGCUGC (SEQ ID NO:15)
	M{MS}{MS}MMMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fGs][fA][fA][mU][fC][mA][mA][fA][mU][m G][mA][fU][mG][mA][mA][mU][mU][mGs][mGs][m G] (SEQ ID NO:147)	UAGAAUCAAUGA UGAAUUGGG (SEQ ID NO:16)
DP1500 9P:DP1 5008G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mCs][mA][mG][mA][mG][mC][mC][fA][fA][fA][fA][mU][mC][mA][mA][mG][mA][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:148)	CAGAGCCAAAAUC AAGAUUAGCAGCC GAAAGGCUGC (SEQ ID NO:17)
	M{MS}{MS}MMMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fU][fC][mU][fU][mG][mA][fU][mU][mU][mU][fG][mG][mC][mU][mC][mU][mGs][mGs][mG] (SEQ ID NO:149)	UAAUCUUGAUUUU GGCUCUGGG (SEQ ID NO:18)
DP1501 1P:DP1 5010G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mG][mA][mG][mC][mC][mA][fA][fA][fA][fU][mC][mA][mA][mG][mA][mU][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:150)	AGAGCCAAAAUCA AGAUUUAGCAGCC GAAAGGCUGC (SEQ ID NO:19)

	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fA][fU][mC][fU][mU][mG][fA][mU][m U][mU][fU][mG][mG][mC][mU][mC][mUs][mGs][m G] (SEQ ID NO:151)	UAAAUUCUUGAUUU UGGCUCUGG (SEQ ID NO:20)
DP1501 3P:DP1 5012G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mGs][mA][mG][mC][mC][mA][mA][fA][fA][fU][fC][mA][mA][mG][mA][mU][mU][mU][mG][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:152)	GAGCCAAAAUCA GAUUUGAGCAGCC GAAAGGCUGC (SEQ ID NO:21)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fCs][fAs][fA][fA][mU][fC][mU][mU][fG][mA][m U][mU][fU][mU][mG][mG][mC][mU][mCs][mGs][m G] (SEQ ID NO:153)	UCAAUUCUUGAUU UUGGCUCGG (SEQ ID NO:22)
DP1501 5P:DP1 5014G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mCs][mC][mA][mA][mA][mA][mU][fC][fA][fA][fG][mA][mU][mU][mU][mG][mC][mU][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:154)	CCAAAAUCAAGAU UUGCUAAGCAGCC GAAAGGCUGC (SEQ ID NO:23)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fAs][fG][fC][mA][fA][mA][mU][fC][mU][m U][mG][fA][mU][mU][mU][mU][mG][mGs][mGs][m G] (SEQ ID NO:155)	UUAGCAAUUCUUG AUUUUGGGG (SEQ ID NO:24)
DP1501 7P:DP1 5016G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mCs][mA][mA][mA][mA][mU][mC][fA][fA][fG][fA][mU][mU][mU][mG][mC][mU][mA][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:156)	CAAAUCAAGAUU UGCUAUAGCAGCC GAAAGGCUGC (SEQ ID NO:25)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fUs][fA][fG][mC][fA][mA][mA][fU][mC][m U][mU][fG][mA][mU][mU][mU][mU][mG][mGs][mGs][m G] (SEQ ID NO:157)	UAUAGCAAUUCUU GAUUUUGGG (SEQ ID NO:26)
DP1501 9P:DP1 5018G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMM[adem- GalNAc][adem-	[mAs][mA][mA][mA][mU][mC][mA][fA][fG][fA][fU][mU][mU][mG][mC][mU][mA][mU][mG][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC]	AAAAUCAAGAUUU GCUAUGAGCAGCC GAAAGGCUGC (SEQ ID NO:27)

	GaINAc][adem-GaINAc]MMMMMM	(SEQ ID NO:158)	
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fCs][fAs][fU][fA][mG][fC][mA][mA][fA][mU][m C][mU][fU][mG][mA][mU][mU][mU][mUs][mGs][m G] (SEQ ID NO:159)	UCAUAGCAAAUCU UGAUUUUUGG (SEQ ID NO:28)
DP1502 1P:DP1 5020G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GaINAc][adem- GaINAc][adem- GaINAc]MMMMMM	[mAs][mU][mU][mU][mG][mC][mU][fA][fU][fG][fU][mU][mA][mG][mA][mC][mG][mA][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GaINAc][ademA-GaINAc][ademA- GaINAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:160)	AUUUGCUAUGUUA GACGAUAGCAGCC GAAAGGCUGC (SEQ ID NO:29)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fUs][fC][fG][mU][fC][mU][mA][fA][mC][mA][mU][fA][mG][mC][mA][mA][mA][mUs][mGs][mG] (SEQ ID NO:161)	UAUCGUCUAACAU AGCAAAUGG (SEQ ID NO:30)
DP1502 3P:DP1 5022G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GaINAc][adem- GaINAc][adem- GaINAc]MMMMMM	[mCs][mA][mA][mA][mU][mU][mA][fA][fU][fG][fA][mC][mA][mU][mA][mU][mU][mU][mC][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GaINAc][ademA-GaINAc][ademA- GaINAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:162)	CAAAUUAUGACA UAUUUCAGCAGCC GAAAGGCUGC (SEQ ID NO:31)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fGs][fAs][fA][fA][mU][fA][mU][mG][fU][mC][mA][mU][fU][mA][mA][mU][mU][mU][mGs][mGs][mG] (SEQ ID NO:163)	UGAAUAUGUCAU UAAUUUGGG (SEQ ID NO:32)
DP1502 5P:DP1 5024G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GaINAc][adem- GaINAc][adem- GaINAc]MMMMMM	[mAs][mU][mG][mA][mC][mA][mU][fA][fU][fU][fU][mC][mA][mA][mA][mA][mA][mC][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GaINAc][ademA-GaINAc][ademA- GaINAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:164)	AUGACAUUUUCA AAAACUAGCAGCC GAAAGGCUGC (SEQ ID NO:33)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fGs][fU][fU][mU][fU][mU][mG][fA][mA][mA][mU][fA][mU][mG][mU][mC][mA][mUs][mGs][mG] (SEQ ID NO:165)	UAGUUUUUGAAAU AUGUCAUGG (SEQ ID NO:34)
DP1502 7P:DP1 5026G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem-	[mAs][mA][mC][mA][mU][mA][mU][fU][fU][fG][fA][mU][mC][mA][mG][mU][mC][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA-	AACAUUUUGAUC AGUCUUAGCAGCC GAAAGGCUGC

	GalNAc][adem-GalNAc][adem-GalNAc]MMMMMM	GalNAc][ademA-GalNAc][ademA-GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:166)	(SEQ ID NO:35)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fG][fA][mC][fU][mG][mA][fU][mC][mA][mA][fA][mU][mA][mU][mG][mU][mUs][mGs][mG] (SEQ ID NO:167)	UAAGACUGAUCAA AUAUGUUGG (SEQ ID NO:36)
DP1502 9P:DP1 5028G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mCs][mA][mU][mA][mU][mU][mU][fG][fA][fU][fC][mA][mG][mU][mC][mU][mU][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:168)	CAUAAUUUGAUCAG UCUUUUAGCAGCC GAAAGGCUGC (SEQ ID NO:37)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fA][fA][mG][fA][mC][mU][fG][mA][m U][mC][fA][mA][mA][mU][mA][mU][mGs][mGs][m G] (SEQ ID NO:169)	UAAAAGACUGAUC AAAUAUGGG (SEQ ID NO:38)
DP1503 1P:DP1 5030G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mU][mA][mU][mU][mU][mG][fA][fU][fC][fA][mG][mU][mC][mU][mU][mU][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:170)	AUAUUUGAUCAGU CUUUUUAGCAGCC GAAAGGCUGC (SEQ ID NO:39)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fA][fA][mA][fG][mA][mC][fU][mG][m A][mU][fC][mA][mA][mA][mU][mA][mUs][mGs][m G] (SEQ ID NO:171)	UAAAAAGACUGAU CAAAUAUGG (SEQ ID NO:40)
DP1503 3P:DP1 5032G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mUs][mU][mG][mA][mU][mC][mA][fG][fU][fC][fU][mU][mU][mU][mU][mA][mU][mG][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:172)	UUGAUCAGUCUUU UUAUGAAGCAGCC GAAAGGCUGC (SEQ ID NO:41)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fCs][fA][fU][mA][fA][mA][mA][fA][mG][m A][mC][fU][mG][mA][mU][mC][mA][mAs][mGs][m G] (SEQ ID NO:173)	UUCAUAAAAAGAC UGAUCAGG (SEQ ID NO:42)

DP1503 5P:DP1 5034G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mG][mG][mA][mA][mC][mU][fG][fA][fG][fA][mA][mG][mA][mA][mC][mU][mA][mC][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:174)	AGGAACUGAGAAG AACUACAGCAGCC GAAAGGCUGC (SEQ ID NO:43)
	M{MS}{MS}MMMMM FMMMFMMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fGs][fUs][fA][fG][mU][fU][mC][mU][fU][mC][mU][mC][fA][mG][mU][mU][mC][mC][mUs][mGs][mG] (SEQ ID NO:175)	UGUAGUUCUUCUC AGUUCUGG (SEQ ID NO:44)
DP1503 7P:DP1 5036G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mGs][mA][mA][mC][mU][mG][mA][fG][fA][fG][fA][mA][mA][mC][mU][mA][mC][mA][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:176)	GAACUGAGAAGAA CUACAUAGCAGCC GAAAGGCUGC (SEQ ID NO:45)
	M{MS}{MS}MMMMM FMMMFMMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fUs][fG][fU][mA][fG][mU][mU][fC][mU][mU][mC][fU][mC][mA][mG][mU][mU][mCs][mGs][mG] (SEQ ID NO:177)	UAUGUAGUUCUUC UCAGUUCGG (SEQ ID NO:46)
DP1503 9P:DP1 5038G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mUs][mG][mA][mG][mA][mA][mG][fA][fA][fC][fU][mA][mC][mA][mU][mA][mU][mA][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:178)	UGAGAAGAACUAC AUUAUAAAGCAGCC GAAAGGCUGC (SEQ ID NO:47)
	M{MS}{MS}MMMMM FMMMFMMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fUs][fA][fU][mA][fU][mG][mU][fA][mG][mU][mU][fC][mU][mU][mC][mU][mC][mAs][mGs][mG] (SEQ ID NO:179)	UUUAUAUGUAGUU CUUCUCAGG (SEQ ID NO:48)
DP1504 1P:DP1 5040G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mGs][mA][mG][mA][mA][mG][mA][fA][fC][fU][fA][mC][mA][mU][mA][mU][mA][mA][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:180)	GAGAAGAACUACA UAUAAAAGCAGCC GAAAGGCUGC (SEQ ID NO:49)
	M{MS}{MS}MMMMM FMMMFMMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fUs][fU][fA][mU][fA][mU][mG][fU][mA][mG][mU][fU][mC][mU][mU][mC][mU][mCs][mGs][mG] (SEQ ID NO:181)	UUUAUAUGUAGU UCUUCUCGG (SEQ ID NO:50)

DP1504 3P:DP1 5042G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mG][mA][mG][mG][mU][mA][fA][fG][fA][mA][mU][mA][mU][mG][mU][mC][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:182)	AGAGGUAAAGAAU AUGUCAAGCAGCC GAAAGGCUGC (SEQ ID NO:51)
	M{MS}{MS}MMMMMM FMMFMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fGs][fA][fC][mA][fU][mA][mU][fU][mC][mU][mU][fU][mA][mC][mC][mU][mC][mUs][mGs][mG] (SEQ ID NO:183)	UUGACAUUUCUU UACCUCUGG (SEQ ID NO:52)
DP1504 5P:DP1 5044G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mA][mU][mA][mU][mG][mU][fC][fA][fC][fU][mU][mG][mA][mA][mC][mU][mC][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:184)	AAUAUGUCACUUG AACUCAAGCAGCC GAAAGGCUGC (SEQ ID NO:53)
	M{MS}{MS}MMMMMM FMMFMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fGs][fA][fG][mU][fU][mC][mA][fA][mG][mU][mG][fA][mC][mA][mU][mA][mU][mUs][mGs][mG] (SEQ ID NO:185)	UUGAGUUCAAGUG ACAUUUGG (SEQ ID NO:54)
DP1504 7P:DP1 5046G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mUs][mG][mA][mA][mA][mU][mA][fU][fU][fU][fA][mG][mA][mA][mG][mA][mG][mC][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:186)	UGAAUAUUUAGA AGAGCAAGCAGCC GAAAGGCUGC (SEQ ID NO:55)
	M{MS}{MS}MMMMMM FMMFMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fGs][fC][fU][mC][fU][mU][mC][fU][mA][mA][mA][fU][mA][mU][mU][mU][mC][mAs][mGs][mG] (SEQ ID NO:187)	UUGCUCUUCUAAA UAUUUCAGG (SEQ ID NO:56)
DP1504 9P:DP1 5048G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mGs][mA][mA][mA][mU][mA][mU][fU][fU][fA][fG][mA][mA][mG][mA][mG][mC][mA][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:188)	GAAUAUUUAGAA GAGCAAAGCAGCC GAAAGGCUGC (SEQ ID NO:57)
	M{MS}{MS}MMMMMM FMMFMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fUs][fG][fC][mU][fC][mU][mU][fC][mU][mU][fUs][fGs][fC][fU][mC][fU][mU][mC][fU][mA][mA][mA][fU][mA][mU][mU][mU][mC][mAs][mGs][mG] (SEQ ID NO:189)	UUUGCUCUUCUAA AUUUUCGG (SEQ ID NO:58)

		mA][mA][fA][mU][mA][mU][mU][mU][mCs][mGs][mG] (SEQ ID NO:189)	
DP1505 1P:DP1 5050G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mG][mC][mA][mA][mC][mU][fA][fA][fC][fU][mA][mA][mC][mU][mU][mA][mA][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:190)	AGCAACUAAACUAA CUUAAUAGCAGCC GAAAGGCUGC (SEQ ID NO:59)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fUs][fU][fA][mA][fG][mU][mU][fA][mG][mU][mU][fA][mG][mU][mU][mG][mC][mUs][mGs][mG] (SEQ ID NO:191)	UAUUAAGUUAGUU AGUUGCUGG (SEQ ID NO:60)
DP1505 3P:DP1 5052G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mCs][mU][mA][mA][mC][mU][mU][fA][fA][fU][fU][mC][mA][mA][mA][mA][mU][mC][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:192)	CUAACUUAAUUA AAAUCAAGCAGCC GAAAGGCUGC (SEQ ID NO:61)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fGs][fA][fU][mU][fU][mU][mG][fA][mA][mU][mU][fA][mA][mG][mU][mU][mA][mGs][mGs][mG] (SEQ ID NO:193)	UUGAUUUUGAAU AAGUUAGGG (SEQ ID NO:62)
DP1505 5P:DP1 5054G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mGs][mA][mA][mG][mU][mA][mA][fC][fU][fU][fC][mA][mC][mU][mU][mA][mA][mA][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:194)	GAAGUAAUCUAC UUAAAAAGCAGCC GAAAGGCUGC (SEQ ID NO:63)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fUs][fU][fU][mA][fA][mG][mU][fG][mA][mA][mG][fU][mU][mA][mC][mU][mU][mCs][mGs][mG] (SEQ ID NO:195)	UUUUUAAGUGAAG UUACUUCGG (SEQ ID NO:64)
DP1505 7P:DP1 5056G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mUs][mU][mU][mU][mG][mU][mA][fG][fA][fA][fA][mA][mA][mC][mA][mA][mG][mA][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:196)	UUUUGUAGAAAA CAAGAUAGCAGCC GAAAGGCUGC (SEQ ID NO:65)

	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fUs][fC][fU][mU][fG][mU][mU][fU][mU][mU][mC][fU][mA][mC][mA][mA][mA][mAs][mGs][mG] (SEQ ID NO:197)	UAUCUUGUUUUUC UACAAAAGG (SEQ ID NO:66)
DP1505 9P:DP1 5058G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mUs][mU][mU][mG][mU][mA][mG][fA][fA][fA][fA][mA][mC][mA][mA][mG][mA][mU][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:198)	UUUGUAGAAAAAC AAGAUAGCAGCC GAAAGGCUGC (SEQ ID NO:67)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fAs][fU][fC][mU][fU][mG][mU][fU][mU][mU][mU][fC][mU][mA][mC][mA][mA][mA][mAs][mGs][mG] (SEQ ID NO:199)	UUAUCUUGUUUUU CUACAAAGG (SEQ ID NO:68)
DP1506 1P:DP1 5060G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mUs][mU][mG][mU][mA][mG][mA][fA][fA][fA][fA][mC][mA][mA][mG][mA][mU][mA][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:200)	UUGUAGAAAAACA AGAUAGCAGCC GAAAGGCUGC (SEQ ID NO:69)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fUs][fA][fU][mC][fU][mU][mG][fU][mU][mU][mU][fU][mC][mU][mA][mC][mA][mA][mAs][mGs][mG] (SEQ ID NO:201)	UUUAUCUUGUUUU UCUACAAGG (SEQ ID NO:70)
DP1506 3P:DP1 5062G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mGs][mU][mA][mG][mA][mA][mA][fA][fA][fC][fA][mA][mG][mA][mU][mA][mA][mU][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:202)	GUAGAAAAACAAG AUAAUAGCAGCC GAAAGGCUGC (SEQ ID NO:71)
	M{MS}{MS}MMMMM FMMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fAs][fU][fU][mA][fU][mC][mU][fU][mG][mU][mU][fU][mU][mU][mC][mU][mA][mCs][mGs][mG] (SEQ ID NO:203)	UUUAUCUUGUU UUUCUACGG (SEQ ID NO:72)
DP1506 5P:DP1 5064G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMM[adem- GalNAc][adem-	[mUs][mA][mG][mA][mA][mA][mA][fA][fC][fA][fA][mG][mA][mU][mA][mA][mU][mA][mG][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC]	UAGAAAAACAAGA UAAUAGAGCAGCC GAAAGGCUGC (SEQ ID NO:73)

	GaINAc][adem-GaINAc]MMMMMM	(SEQ ID NO:204)	
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fCs][fUs][fA][fU][mU][fA][mU][mC][fU][mU][mG][mU][fU][mU][mU][mU][mC][mU][mAs][mGs][mG] (SEQ ID NO:205)	UCUAUUUAUCUUGU UUUUUCUAGG (SEQ ID NO:74)
DP1506 7P:DP1 5066G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GaINAc][adem- GaINAc][adem- GaINAc]MMMMMM	[mAs][mG][mA][mA][mA][mA][mA][fC][fA][fA][fG][mA][mU][mA][mA][mU][mA][mG][mC][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GaINAc][ademA-GaINAc][ademA- GaINAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:206)	AGAAAAACAAGUAU AUAGCAGCAGCCG AAAGGCUGC (SEQ ID NO:75)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fGs][fCs][fU][fA][mU][fU][mA][mU][fC][mU][mU][mG][fU][mU][mU][mU][mU][mC][mUs][mGs][mG] (SEQ ID NO:207)	UGCUAUUAUCUUG UUUUUCUGG (SEQ ID NO:76)
DP1506 9P:DP1 5068G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GaINAc][adem- GaINAc][adem- GaINAc]MMMMMM	[mAs][mA][mA][mA][mA][mC][mA][fA][fG][fA][fU][mA][mA][mU][mA][mG][mC][mA][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GaINAc][ademA-GaINAc][ademA- GaINAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:208)	AAAAACAAGUAUAAU AGCAUAGCAGCCG AAAGGCUGC (SEQ ID NO:77)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fUs][fG][fC][mU][fA][mU][mU][fA][mU][mC][mU][fU][mG][mU][mU][mU][mU][mUs][mGs][mG] (SEQ ID NO:209)	UAUGCUAUUAUCU UGUUUUUGG (SEQ ID NO:78)
DP1507 1P:DP1 5070G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GaINAc][adem- GaINAc][adem- GaINAc]MMMMMM	[mAs][mA][mC][mA][mG][mC][mA][fU][fA][fG][fU][mC][mA][mA][mA][mU][mA][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GaINAc][ademA-GaINAc][ademA- GaINAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:210)	AACAGCAUAGUCA AAUAAAAGCAGCC GAAAGGCUGC (SEQ ID NO:79)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fUs][fU][fA][mU][fU][mU][mG][fA][mC][mU][mA][fU][mG][mC][mU][mG][mU][mUs][mGs][mG] (SEQ ID NO:211)	UUUUUUUUGACUA UGCUGUUGG (SEQ ID NO:80)
DP1507 3P:DP1 5072G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem-	[mAs][mC][mA][mG][mA][mA][mA][fU][fU][fU][fC][mU][mC][mU][mA][mU][mC][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA-	ACAGAAAUUCUC UAUCUUAGCAGCC GAAAGGCUGC

	GaINAc][adem-GaINAc][adem-GaINAc]MMMMMM	GaINAc][ademA-GaINAc][ademA-GaINAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:212)	(SEQ ID NO:81)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fG][fA][mU][fA][mG][mA][fG][mA][m A][mA][fU][mU][mU][mC][mU][mG][mUs][mGs][m G] (SEQ ID NO:213)	UAAGAUAGAGAAA UUUCUGUGG (SEQ ID NO:82)
DP1507 5P:DP1 5074G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GaINAc][adem- GaINAc][adem- GaINAc]MMMMMM	[mUs][mG][mA][mA][mU][mG][mA][fA][fA][fU][fA][mA][mG][mA][mA][mA][mU][mG][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GaINAc][ademA-GaINAc][ademA- GaINAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:214)	UGAAUGAAUAAG AAAUGUAGCAGCC GAAAGGCUGC (SEQ ID NO:83)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fCs][fA][fU][mU][fU][mC][mU][fU][mA][m U][mU][fU][mC][mA][mU][mU][mC][mAs][mGs][m G] (SEQ ID NO:215)	UACAUUUUCUUAUU UCAUUCAGG (SEQ ID NO:84)
DP1507 7P:DP1 5076G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GaINAc][adem- GaINAc][adem- GaINAc]MMMMMM	[mAs][mC][mC][mC][mA][mG][mC][fA][fA][fC][fU][mC][mU][mC][mA][mA][mG][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GaINAc][ademA-GaINAc][ademA- GaINAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:216)	ACCCAGCAACUCU CAAGUUAGCAGCC GAAAGGCUGC (SEQ ID NO:85)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fC][fU][mU][fG][mA][mG][fA][mG][mU][mU][fG][mC][mU][mG][mG][mG][mUs][mGs][mG] (SEQ ID NO:217)	UAACUUGAGAGUU GCUGGGUGG (SEQ ID NO:86)
DP1507 9P:DP1 5078G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GaINAc][adem- GaINAc][adem- GaINAc]MMMMMM	[mCs][mC][mC][mA][mG][mC][mA][fA][fC][fU][fC][mU][mC][mA][mA][mG][mU][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GaINAc][ademA-GaINAc][ademA- GaINAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:218)	CCCAGCAACUCUC AAGUUUAGCAGCC GAAAGGCUGC (SEQ ID NO:87)
	M{MS}{MS}MMMMMM FMMMFMMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fA][fC][mU][fU][mG][mA][fG][mA][m G][mU][fU][mG][mC][mU][mG][mG][mGs][mGs][m G] (SEQ ID NO:219)	UAAACUUGAGAGU UGCUGGGGG (SEQ ID NO:88)

DP1508 1P:DP1 5080G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mCs][mC][mA][mG][mC][mA][mA][fC][fU][fC][fU][mC][mA][mA][mG][mU][mU][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:220)	CCAGCAACUCUCA AGUUUUAGCAGCC GAAAGGCUGC (SEQ ID NO:89)
	M{MS}{MS}MMMMM FMMMFMMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fA][fA][mC][fU][mU][mG][fA][mG][m A][mG][fU][mU][mG][mC][mU][mG][mGs][mGs][m G] (SEQ ID NO:221)	UAAAACUUGAGAG UUGCUGGGG (SEQ ID NO:90)
DP1508 3P:DP1 5082G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mA][mG][mA][mU][mA][mU][fA][fC][fU][fC][mC][mA][mU][mA][mG][mU][mG][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:222)	AAGAUUACUCCA UAGUGAAGCAGCC GAAAGGCUGC (SEQ ID NO:91)
	M{MS}{MS}MMMMM FMMMFMMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fCs][fA][fC][mU][fA][mU][mG][fG][mA][mG][mU][fA][mU][mA][mU][mC][mU][mUs][mGs][mG] (SEQ ID NO:223)	UUCACUAUGGAGU AUAUCUUGG (SEQ ID NO:92)
DP1508 5P:DP1 5084G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mU][mA][mU][mA][mC][mU][fC][fC][fA][fU][mA][mG][mU][mG][mA][mA][mG][mC][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:224)	AUAUACUCCAUAG UGAAGCAGCAGCC GAAAGGCUGC (SEQ ID NO:93)
	M{MS}{MS}MMMMM FMMMFMMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fGs][fCs][fU][fU][mC][fA][mC][mU][fA][mU][mG][mG][fA][mG][mU][mA][mU][mA][mUs][mGs][mG] (SEQ ID NO:225)	UGCUUCACUAUGG AGUAUAUGG (SEQ ID NO:94)
DP1508 7P:DP1 5086G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mUs][mA][mC][mU][mC][mC][mA][fU][fA][fG][fU][mG][mA][mA][mG][mC][mA][mA][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:226)	UACUCCAUAGUGA AGCAAUAGCAGCC GAAAGGCUGC (SEQ ID NO:95)
	M{MS}{MS}MMMMM FMMMFMMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fUs][fU][fG][mC][fU][mU][mC][fA][mC][mU][mA][fU][mG][mG][mA][mG][mU][mAs][mGs][mG] (SEQ ID NO:227)	UAUUGCUUCACUA UGGAGUAGG (SEQ ID NO:96)

DP1508 9P:DP1 5088G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mU][mA][mG][mU][mG][mA][fA][fG][fC][fA] mA][mU][mC][mU][mA][mA][mU][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:228)	AUAGUGAAGCAAU CUAAUUAGCAGCC GAAAGGCUGC (SEQ ID NO:97)
	M{MS}{MS}MMMMM FMMFMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fAs][fU][fU][mA][fG][mA][mU][fU][mG][mC][mU][fU][mC][mA][mC][mU][mA][mUs][mGs][mG] (SEQ ID NO:229)	UAAUUAGAUAUGCU UCACUAUGG (SEQ ID NO:98)
DP1509 1P:DP1 5090G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mUs][mC][mA][mA][mA][mA][mU][fG][fG][fA][fA] mG][mG][mU][mU][mA][mU][mA][mC][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:230)	UCAAAAUGGAAGG UUAUACAGCAGCC GAAAGGCUGC (SEQ ID NO:99)
	M{MS}{MS}MMMMM FMMFMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fGs][fUs][fA][fU][mA][fA][mC][mC][fU][mU][mC][mC][fA][mU][mU][mU][mU][mG][mAs][mGs][mG] (SEQ ID NO:231)	UGUAUAACCUUCC AUUUUGAGG (SEQ ID NO:100)
DP1509 3P:DP1 5092G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mA][mA][mU][mG][mG][mA][fA][fG][fG][fU] mU][mA][mU][mA][mC][mU][mC][mU][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:232)	AAAUGGAAGGUUA UACUCUAGCAGCC GAAAGGCUGC (SEQ ID NO:101)
	M{MS}{MS}MMMMM FMMFMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fGs][fA][fG][mU][fA][mU][mA][fA][mC][m C][mU][fU][mC][mC][mA][mU][mU][mUs][mGs][m G] (SEQ ID NO:233)	UAGAGUAUAACCU UCCAUUUGG (SEQ ID NO:102)
DP1509 5P:DP1 5094G	{MS}MMMMMMFFFF MMMMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mGs][mA][mA][mG][mG][mU][mU][fA][fU][fA][fC] mU][mC][mU][mA][mU][mA][mA][mA][mA][mG][m C][mA][mG][mC][mC][mG][ademA- GalNAc][ademA-GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:234)	GAAGGUUAUACUC UAUAAAAGCAGCC GAAAGGCUGC (SEQ ID NO:103)
	M{MS}{MS}MMMMM FMMFMFMFMFF{FS }{FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fUs][fU][fA][mU][fA][mG][mA][fG][mU][	UUUUAUAGAGUAU AACCUUCGG (SEQ ID NO:104)

		mA][mU][fA][mA][mC][mC][mU][mU][mCs][mGs][mG] (SEQ ID NO:235)	
DP1509 7P:DP1 5096G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mA][mG][mG][mU][mU][mA][fU][fA][fC][fU][mC][mU][mA][mU][mA][mA][mA][mA][mG][mC][mA][mG][mC][mC][mG][ademA-GalNAc][ademA-GalNAc][ademA-GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:236)	AAGGUUAUACUCU AUAAAAAGCAGCC GAAAGGCUGC (SEQ ID NO:105)
	M{MS}{MS}MMMMMM FMMFMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O-mUs][fUs][fUs][fU][fU][mA][fU][mA][mG][fA][mG][mU][mA][fU][mA][mA][mC][mC][mU][mUs][mGs][mG] (SEQ ID NO:237)	UUUUUAUAGAGUA UAACCUUGG (SEQ ID NO:106)
DP1509 9P:DP1 5098G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mG][mG][mU][mU][mA][mU][fA][fC][fU][fC][mU][mA][mU][mA][mA][mA][mA][mU][mA][mG][mC][mA][mG][mC][mC][mG][ademA-GalNAc][ademA-GalNAc][ademA-GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:238)	AGGUUAUACUCUA UAAAAUAGCAGCC GAAAGGCUGC (SEQ ID NO:107)
	M{MS}{MS}MMMMMM FMMFMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O-mUs][fAs][fUs][fU][fU][mU][fA][mU][mA][fG][mA][mG][mU][fA][mU][mA][mA][mC][mC][mUs][mGs][mG] (SEQ ID NO:239)	UAUUUAUAGAGU AUAACCUUGG (SEQ ID NO:108)
DP1510 1P:DP1 5100G	{MS}MMMMMMFFFF MMMMMMMMMMMM MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mU][mU][mC][mA][mG][mA][fA][fA][fG][fC][mU][mU][mU][mG][mA][mA][mU][mG][mA][mG][mC][mA][mG][mC][mC][mG][ademA-GalNAc][ademA-GalNAc][ademA-GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:240)	AUUCAGAAAGCUU UGAAUGAGCAGCC GAAAGGCUGC (SEQ ID NO:109)
	M{MS}{MS}MMMMMM FMMFMFMFF{FS} {FS}{Px-MS}	[MePhosphonate-4O-mUs][fCs][fAs][fU][fU][mC][fA][mA][mA][fG][mC][mU][mU][fU][mC][mU][mG][mA][mA][mUs][mGs][mG] (SEQ ID NO:241)	UCAUCAAAGCUU UCUGAAUGG (SEQ ID NO:110)
DP1343 9P:DP1 3438G	{MS}MFMFMFMFMF MFMFMFMFMFMF MMMMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMMMM	[mAs][mA][fA][mU][mC][mA][mA][fG][mA][fU][mU][fU][fG][mC][mU][mA][fU][mG][mU][mA][mG][mC][mA][mG][mC][mC][mG][ademA-GalNAc][ademA-GalNAc][ademA-GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:242)	AAAUCAAGAUUUG CUAUGUAGCAGCC GAAAGGCUGC (SEQ ID NO:111)

	M{MS}{MS}FMMFMF MFMMMFMFM{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fCs][mA][fU][mA][fG][mC][mA][mA] [fU][mC][fU][mU][fG][mA][mU][fU][mUs][mGs][mG] (SEQ ID NO:243)	UACAUAGCAAUUC UUGAUUUUGG (SEQ ID NO:112)
DP1344 3P:DP1 3442G	{MS}MFMMMFMF MFMMMFMFM MMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMM	[mCs][mU][fC][mA][mA][mC][mA][fU][mA][fU][mU] [fU][fG][mA][mU][mC][fA][mG][mU][mA][mG][mC] mA][mG][mC][mC][mG][ademA-GalNAc][ademA- GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:244)	CUCAACAUUUUG AUCAGUAGCAGCC GAAAGGCUGC (SEQ ID NO:113)
	M{MS}{MS}FMMFMF MFMMMFMFM{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fAs][fCs][mU][fG][mA][fU][mC][mA][mA] [fU][mA][fU][mG][fU][mU][mG][fA][mGs][mGs][mG] (SEQ ID NO:245)	UACUGAUCAAUA UGUUGAGGG (SEQ ID NO:114)
DP1346 5P:DP1 3464G	{MS}MFMMMFMF MFMMMFMFM MMMM[adem- GalNAc][adem- GalNAc][adem- GalNAc]MMMM	[mGs][mU][fG][mG][mA][mG][mA][fA][mA][fA][mC] [fA][fA][mC][mC][mU][fA][mA][mA][mA][mG][mC] mA][mG][mC][mC][mG][ademA-GalNAc][ademA- GalNAc][ademA- GalNAc][mG][mG][mC][mU][mG][mC] (SEQ ID NO:246)	GUGGAGAAAACAA CCUAAAAGCAGCC GAAAGGCUGC (SEQ ID NO:115)
	M{MS}{MS}FMMFMF MFMMMFMFM{FS} {FS}{Px-MS}	[MePhosphonate-4O- mUs][fUs][fUs][mU][fA][mG][fG][mU][mU][mG][mU] [fU][mU][fU][mC][fU][mC][mC][fA][mCs][mGs][mG] (SEQ ID NO:247)	UUUUAGGUUGUUU UCUCCACGG (SEQ ID NO:116)

In the modification patterns of Table A:

“M” refers to a 2'-OMe modified nucleotide;

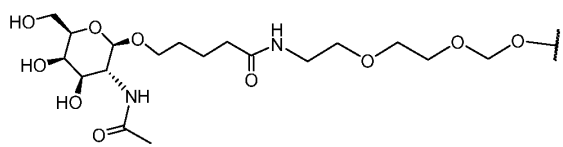
“F” refers to a 2'-F modified nucleotide;

“S” refers to a nucleotide with a 3'-phosphorothioate linkage;

“{MS}” refers to a 2'-OMe modified nucleotide with a 3'-phosphorothioate linkage;

“{FS}” refers to a 2'-F modified nucleotide with a 3'-phosphorothioate linkage;

“[adem-GalNAc]” refers to a nucleotide having a 2'-GalNAc conjugate:



“{Px-MS}” refers to a 2'-OMe modified nucleotide with a 3'-phosphorothioate linkage, and 5' phosphonate.

In the modified sequences of Table A:

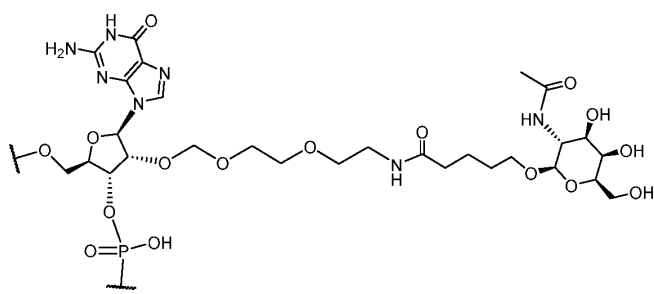
“[mN]” refers to a 2'-OMe modified nucleotide;

“[fN]” refers to a 2'-F modified nucleotide;

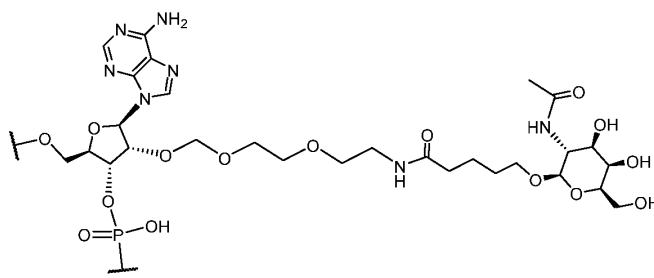
“[mNs]” refers to a 2'-OMe modified nucleotide with a 3'-phosphorothioate linkage;

“[fNs]” refers to a 2'-F modified nucleotide with a 3'-phosphorothioate linkage;

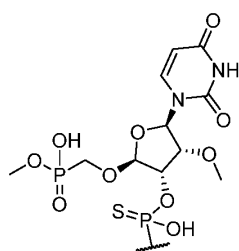
“[ademG-GalNAc]” refers to a G nucleotide having a 2'-GalNAc conjugate:



“[ademA-GalNAc]” refers to an A nucleotide having a 2'-GalNAc conjugate:



“[MePhosphonate-4O-mUs]” refers to a 5'-phosphonate-4'-Oxy-2'-OMe uridine with a 3'-phosphorothioate linkage:



[00227] *Non-Human Primate (NHP) Studies*

[00228] The GalNAc-conjugated ANGPTL3 oligonucleotides listed in **Table 4** are evaluated in cynomolgus monkeys (*Macaca fascicularis*). In this study, the NHPs are grouped so that their mean body weights (about 5.4 kg) are comparable between the control and experimental

groups. Each cohort contains two male and three female subjects. The GalNAc-conjugated ANGPTL3 oligonucleotides are administered subcutaneously on Study Day 0. Blood samples are collected on Study Days -8, -5 and 0, and weekly after dosing. Ultrasound-guided core needle liver biopsies are collected on Study Days 28, 56 and 84. At each time point, total RNA derived from the liver biopsy samples is subjected to qRT-PCR analysis to measure ANGPTL3 mRNA in oligonucleotide-treated NHPs relative to NHPs treated with a comparable volume of PBS. To normalize the data, the measurements are made relative to the geometric mean of two reference genes, PP1B and 18S rRNA. As shown in **FIG. 5A** (Day 28), **FIG. 5B** (Day 56), and **FIG. 5C** (Day 84), treating NHPs with the GalNAc-conjugated ANGPTL3 oligonucleotides listed in **Table 4** inhibits ANGPTL3 expression in the liver, as determined by a reduced amount of ANGPTL3 mRNA in liver samples from oligonucleotide-treated NHPs relative to NHPs treated with PBS. The mean percent reduction of ANGPTL3 mRNA in the liver samples of treated NHPs is indicated above the set of data points for each treatment group and a plot of the mean values over times is shown in **FIG. 6**. For all time points evaluated, ANGPTL3-1412 inhibits ANGPTL3 expression to a greater extent than the benchmark GalNAc-conjugated ANGPTL3 oligonucleotide ANGPTL3-0327. From the same NHP study, inhibition of ANGPTL3 expression is also determined by measuring ANGPTL3 protein in serum prepared from the pre-dose and weekly blood samples by ELISA. As shown in **FIG. 7**, a significant reduction in serum ANGPTL3 protein is observed in NHPs treated with GalNAc-conjugated ANGPTL3 oligonucleotides compared to NHPs treated with PBS. Values from three pre-dose samples are averaged and set to 100%, and data are reported as relative values compared to the pre-dose average. Taken together, these results demonstrate that treating NHPs with GalNAc-conjugated ANGPTL3 oligonucleotides reduces the amount of ANGPTL3 mRNA in the liver and concomitantly reduces the amount of ANGPTL3 protein in the serum.

[1] Taken together, these results show that GalNAc-conjugated ANGPTL3 oligonucleotides designed to target human ANGPTL3 mRNA inhibit ANGPTL3 expression *in vivo* (as determined by the reduction of the amount of ANGPTL3 mRNA and ANGPTL3 protein in treated animals).

SEQUENCE LISTING

[00229] The following nucleic and/or amino acid sequences are referred to in the disclosure above and are provided below for reference.

[00230] **Table 5:** ANGPTL3 Oligonucleotide Sequences (Unmodified)

Oligonucleotide	DP#	Sequence (Sense Strand)	SEQ ID NO	Sequence (Antisense Strand)	SEQ ID NO
ANGPTL3-0099	DP14993P: DP14992G	AUAAAAUGUUCACAAU UAAGCAGCCGAAAGGCU GC	1	UUAAUUGUGAACAUUUU UAUGG	2
ANGPTL3-0108	DP14995P: DP14994G	UUCACAAUUAAGCUCCU UCAGCAGCCGAAAGGCU GC	3	UGAAGGAGCUUAAUUGU GAAGG	4
ANGPTL3-0111	DP14997P: DP14996G	ACAAUUAAGCUCCUUCU UUAGCAGCCGAAAGGCU GC	5	UAAAGAAGGAGCUUAAU UGUGG	6
ANGPTL3-0112	DP14999P: DP14998G	CAAUUAAGCUCCUUCUU UUAGCAGCCGAAAGGCU GC	7	UAAAAGAAGGAGCUUAA UUGGG	8
ANGPTL3-0143	DP15001P: DP15000G	AGUUUUUCCUCCAGAA UUAGCAGCCGAAAGGCU GC	9	UAAUUCUGGAGGAAAUA ACUGG	10
ANGPTL3-0165	DP15003P: DP15002G	CAAGACAAUUCAUCAUU UGAGCAGCCGAAAGGCU GC	11	UCAAAUGAUGAAUUGUC UUGGG	12
ANGPTL3-0167	DP15005P: DP15004G	AGACAAUUCAUCAUUUG AUAGCAGCCGAAAGGCU GC	13	UAUCAAAUGAUGAAUUG UCUGG	14
ANGPTL3-0170	DP15007P: DP15006G	CAAUUCAUCAAUUGAUU CUAGCAGCCGAAAGGCU GC	15	UAGAAUCAAUGAUGAA UUGGG	16
ANGPTL3-0196	DP15009P: DP15008G	CAGAGCCAAAUCAAGA UUAGCAGCCGAAAGGCU GC	17	UAAUCUUGAUUUUUGGCU CUGGG	18
ANGPTL3-0197	DP15011P: DP15010G	AGAGCCAAAUCAAGAU UUAGCAGCCGAAAGGCU GC	19	UAAUCUUGAUUUUUGGC UCUGG	20
ANGPTL3-0198	DP15013P: DP15012G	GAGCCAAAUCAAGAUU UGAGCAGCCGAAAGGCU GC	21	UCAAUCUUGAUUUUUGG CUCGG	22
ANGPTL3-0201	DP15015P: DP15014G	CCAAAUCAAGAUUUGC UAAGCAGCCGAAAGGCU GC	23	UUAGCAAAUCUUGAUUU UGGGG	24
ANGPTL3-0202	DP15017P: DP15016G	CAAAAUCAAGAUUUGCU AUAGCAGCCGAAAGGCU GC	25	UAUAGCAAAUCUUGAUU UUGGG	26

ANGPTL3-0203	DP15019P: DP15018G	AAAAUCAAGAUUUGCUA UGAGCAGCCGAAAGGCU GC	27	UCAUAGCAAAUCUUGAU UUUGG	28
ANGPTL3-0212	DP15021P: DP15020G	AUUUGCUAUGUUAGACG AUAGCAGCCGAAAGGCU GC	29	UAUCGUCUACAUAAGCA AAUGG	30
ANGPTL3-0303	DP15023P: DP15022G	CAAAUUAUUGACAUUU UCAGCAGCCGAAAGGCU GC	31	UGAAAUAUGUCAUUAAU UUGGG	32
ANGPTL3-0310	DP15025P: DP15024G	AUGACAUUUUCAA CUAGCAGCCGAAAGGCU GC	33	UAGUUUUUGAAAUAUGU CAUGG	34
ANGPTL3-0330	DP15027P: DP15026G	AACAUUUUGAUCAGUC UUAGCAGCCGAAAGGCU GC	35	UAAGACUGAUCAAAUAU GUUGG	36
ANGPTL3-0332	DP15029P: DP15028G	CAUAAUUGAUCAGUCU UUAGCAGCCGAAAGGCU GC	37	UAAAAGACUGAUCAAAU AUGGG	38
ANGPTL3-0333	DP15031P: DP15030G	AUAUUUGAUCAGUCUU UUAGCAGCCGAAAGGCU GC	39	UAAAAGACUGAUCAAA UAUGG	40
ANGPTL3-0337	DP15033P: DP15032G	UUGAUCAGUCUUUUUAU GAAGCAGCCGAAAGGCU GC	41	UUCAUAAAAGACUGAU CAAGG	42
ANGPTL3-0394	DP15035P: DP15034G	AGGAACUGAGAAGAACU ACAGCAGCCGAAAGGCU GC	43	UGUAGUUCUUCUCAGUU CCUGG	44
ANGPTL3-0396	DP15037P: DP15036G	GAACUGAGAAGAACUAC AUAGCAGCCGAAAGGCU GC	45	UAUGUAGUUCUUCUCAG UUCGG	46
ANGPTL3-0400	DP15039P: DP15038G	UGAGAAGAACUACAUAU AAAGCAGCCGAAAGGCU GC	47	UUUAUAUGUAGUUCUUC UCAGG	48
ANGPTL3-0401	DP15041P: DP15040G	GAGAAGAACUACAUUA AAAGCAGCCGAAAGGCU GC	49	UUUAUAUGUAGUUCUUC CUCGG	50
ANGPTL3-0437	DP15043P: DP15042G	AGAGGUAAAGAAUAUGU CAAGCAGCCGAAAGGCU GC	51	UUGACAUUUCUUUACC UCUGG	52
ANGPTL3-0447	DP15045P: DP15044G	AAUAUGUCACUUGAACU CAAGCAGCCGAAAGGCU GC	53	UUGAGUUCAAGUGACAU AUUGG	54
ANGPTL3-0517	DP15047P: DP15046G	UGAAAUUUUAGAAGAG CAAGCAGCCGAAAGGCU GC	55	UUGCUCUUCUAAAUAU UCAGG	56
ANGPTL3-0518	DP15049P: DP15048G	GAAAUUUUAGAAGAGC AAAGCAGCCGAAAGGCU GC	57	UUUGCUCUUCUAAAUAU UUCGG	58

ANGPTL3-0532	DP15051P: DP15050G	AGCAACUAACUAACUUA AUAGCAGCCGAAAGGCU GC	59	UAUUAAGUUAGUUAGUU GCUGG	60
ANGPTL3-0541	DP15053P: DP15052G	CUAACUUAUUUCAAU CAAGCAGCCGAAAGGCU GC	61	UUGAUUUUGAAUUAAGU UAGGG	62
ANGPTL3-0582	DP15055P: DP15054G	GAAGUAACUUCACUAA AAAGCAGCCGAAAGGCU GC	63	UUUUUAAGUGAAGUUAC UUCGG	64
ANGPTL3-0602	DP15057P: DP15056G	UUUUGUAGAAAAACAAG AUAGCAGCCGAAAGGCU GC	65	UAUCUUGUUUUUCUACA AAAGG	66
ANGPTL3-0603	DP15059P: DP15058G	UUUGUAGAAAAACAAGA UAAGCAGCCGAAAGGCU GC	67	UUAUCUUGUUUUUCUAC AAAGG	68
ANGPTL3-0604	DP15061P: DP15060G	UUGUAGAAAAACAAGAU AAAGCAGCCGAAAGGCU GC	69	UUUAUCUUGUUUUUCUA CAAGG	70
ANGPTL3-0606	DP15063P: DP15062G	GUAGAAAAACAAGAUAA UAAGCAGCCGAAAGGCU GC	71	UUAUUAUCUUGUUUUUC UACGG	72
ANGPTL3-0607	DP15065P: DP15064G	UAGAAAAACAAGAUAAU AGAGCAGCCGAAAGGCU GC	73	UCUAUUAUCUUGUUUUU CUAGG	74
ANGPTL3-0608	DP15067P: DP15066G	AGAAAAACAAGAUAAUA GCAGCAGCCGAAAGGCU GC	75	UGCUAUUAUCUUGUUUU UCUGG	76
ANGPTL3-0610	DP15069P: DP15068G	AAAAACAAGAUAAUAGC AUAGCAGCCGAAAGGCU GC	77	UAUGCUAUUAUCUUGUU UUUGG	78
ANGPTL3-0676	DP15071P: DP15070G	AACAGCAUAGUCAAAUA AAAGCAGCCGAAAGGCU GC	79	UUUUUUUUGACUAUGCU GUUGG	80
ANGPTL3-0738	DP15073P: DP15072G	ACAGAAUUAUCUCUAUC UUAGCAGCCGAAAGGCU GC	81	UAAGAUAGAGAAAUUUC UGUGG	82
ANGPTL3-0796	DP15075P: DP15074G	UGAAUGAAAUAGAAAU GUAGCAGCCGAAAGGCU GC	83	UACAUUUCUUAUUUCAU UCAGG	84
ANGPTL3-0893	DP15077P: DP15076G	ACCCAGCAACUCUCAAG UUAGCAGCCGAAAGGCU GC	85	UAACUUGAGAGUUGCUG GGUGG	86
ANGPTL3-0894	DP15079P: DP15078G	CCCAGCAACUCUCAAGU UUAGCAGCCGAAAGGCU GC	87	UAAACUUGAGAGUUGCU GGGGG	88
ANGPTL3-0895	DP15081P: DP15080G	CCAGCAACUCUCAAGUU UUAGCAGCCGAAAGGCU GC	89	UAAAACUUGAGAGUUGC UGGGG	90

ANGPTL3-1059	DP15083P: DP15082G	AAGAUUACUCCAUAGU GAAGCAGCCGAAAGGCU GC	91	UUCACUAUGGAGUAUUAU CUUGG	92
ANGPTL3-1062	DP15085P: DP15084G	AUAUACUCCAUAGUGAA GCAGCAGCCGAAAGGCU GC	93	UGCUCACUAUGGAGUA UAUGG	94
ANGPTL3-1065	DP15087P: DP15086G	UACUCCAUAGUGAAGCA AUAGCAGCCGAAAGGCU GC	95	UAUUGCUUCACUAUGGA GUAGG	96
ANGPTL3-1071	DP15089P: DP15088G	AUAGUGAAGCAAUCUAA UUAGCAGCCGAAAGGCU GC	97	UAAUUAGAUUGCUUCAC UAUGG	98
ANGPTL3-1412	DP15091P: DP15090G	UAAAAUGGAAGGUUAU ACAGCAGCCGAAAGGCU GC	99	UGUAUAACCUUCCAUUU UGAGG	100
ANGPTL3-1415	DP15093P: DP15092G	AAAUGGAAGGUUAUACU CUAGCAGCCGAAAGGCU GC	101	UAGAGUAUAACCUUCCA UUUGG	102
ANGPTL3-1420	DP15095P: DP15094G	GAAGGUUAUACUCUAUA AAAGCAGCCGAAAGGCU GC	103	UUUUUAUAGAGUAUAACC UUCGG	104
ANGPTL3-1421	DP15097P: DP15096G	AAGGUUAUACUCUAUAA AAAGCAGCCGAAAGGCU GC	105	UUUUUAUAGAGUAUAAC CUUGG	106
ANGPTL3-1422	DP15099P: DP15098G	AGGUUAUACUCUAUAAA AUAGCAGCCGAAAGGCU GC	107	UAUUUAUAGAGUAUAA CCUGG	108
ANGPTL3-1468	DP15101P: DP15100G	AUUCAGAAAGCUUUGAA UGAGCAGCCGAAAGGCU GC	109	UCAUUCAAAGCUUUCUG AAUGG	110
ANGPTL3-0204	DP13439P: DP13438G	AAAUCAAGAUUUGCUAU GUAGCAGCCGAAAGGCU GC	111	UACAUAGCAAAUCUUGA UUUGG	112
ANGPTL3-0327	DP13443P: DP13442G	CUCAACAUUUUGAUCA GUAGCAGCCGAAAGGCU GC	113	UACUGAUCAAAUAUGUU GAGGG	114
ANGPTL3-1327	DP13465P: DP13464G	GUGGAGAAAACAACCUA AAAGCAGCCGAAAGGCU GC	115	UUUUAGGUUGUUUUCUC CACGG	116