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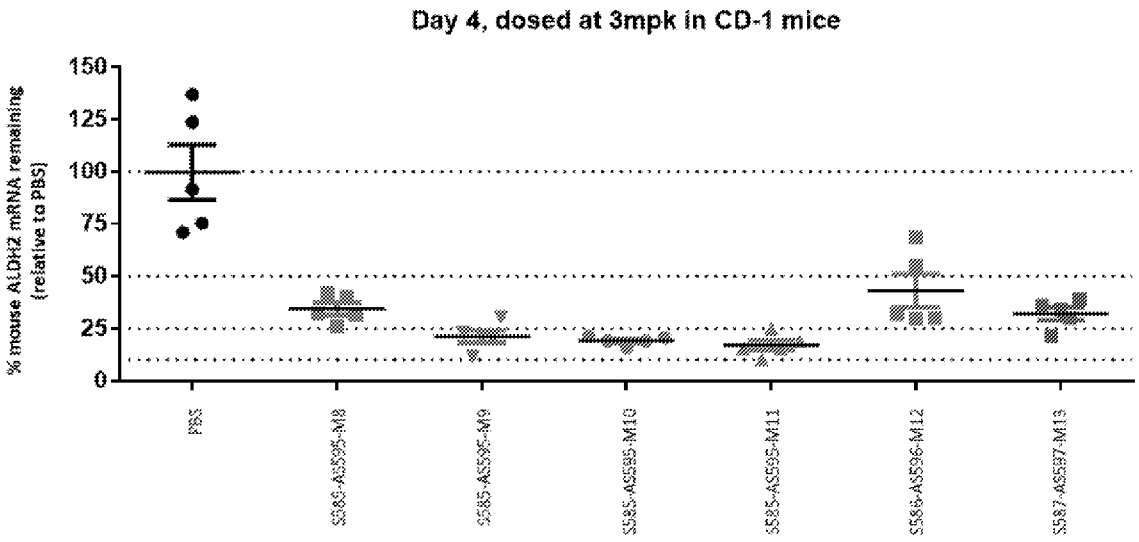
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(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING ALDH2 EXPRESSION

FIG. 7



(57) Abstract: This disclosure relates to oligonucleotides, compositions and methods useful for reducing ALDH2 expression, particularly in hepatocytes. Disclosed oligonucleotides for the reduction of ALDH2 expression may be double-stranded or single-stranded, and may be modified for improved characteristics such as stronger resistance to nucleases and lower immunogenicity. Disclosed oligonucleotides for the reduction of ALDH2 expression may also include targeting ligands to target a particular cell or organ, such as the hepatocytes of the liver, and may be used to treat alcoholism and related conditions.

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COMPOSITIONS AND METHODS FOR INHIBITING ALDH2 EXPRESSION

RELATED APPLICATION

[0001] This application claims the benefit under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/617692, filed January 16, 2018, and entitled "COMPOSITIONS AND METHODS FOR INHIBITING ALDH2 EXPRESSION," the entire contents of which is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present application relates to oligonucleotides and uses thereof, particularly uses relating to the treatment of alcoholism and associated conditions.

REFERENCE TO THE SEQUENCE LISTING

[0003] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled D0800.70010WO00-SEQ.txt created on January 15, 2019 which is 128 kilobytes in size. The information in electronic format of the sequence listing is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0004] Acetaldehyde is an intermediary in the oxidation of alcohol by the body. When acetaldehyde metabolism is inhibited, acetaldehyde accumulates, resulting in the occurrence of toxic symptoms and great discomfort. Mitochondrial aldehyde dehydrogenase (ALDH2) is an enzyme that has a major role in detoxification of acetaldehyde in the body. Genetic polymorphisms of human ALDH2 have been well surveyed among a wide range of ethnic groups. Individuals heterozygous or homozygous for the abnormal gene have lower ALDH2 enzymatic activity, and this deficiency is manifested by facial flushing, nausea, and cardiac palpitations following alcohol consumption. Studies have revealed a reduced prevalence of alcoholism among these individuals, which has been attributed to these unpleasant effects. Therefore, interference with ALDH2 activity can decrease a human's alcohol tolerance and desire to consume alcohol. Pharmacological compounds targeting ALDH2 have been used to induce alcohol aversion in humans.

[0005] For example, disulfiram is a compound which interferes with the metabolism of acetaldehyde in vivo by inhibiting ALDH2 enzyme. Alcohol consumption within 12 hours

of disulfiram administration can produce facial flushing, throbbing in head and neck, nausea, vomiting, sweating, and dizziness, among other symptoms. Disulfiram has clinical limitations, however, due to a range of side-effects, such as drowsiness, headache and, less often, neurotoxicity. Moreover, because daily administration is typically required for disulfiram and similar therapeutics to be effective, these drugs suffer from extremely poor patient compliance.

BRIEF SUMMARY OF THE INVENTION

[0006] Aspects of the disclosure relate to oligonucleotides and related methods for treating alcoholism in a subject. In some embodiments, potent RNAi oligonucleotides have been developed for selectively inhibiting ALDH2 expression in a subject. In some embodiments, the RNAi oligonucleotides are useful for reducing ALDH2 activity, and thereby decreasing alcohol tolerance and/or the desire to consume alcohol. In some embodiments, key regions of ALDH2 mRNA (referred to as hotspots) have been identified herein that are particularly amenable to targeting using such oligonucleotide-based approaches (*See* Example 1). In some embodiments, RNAi oligonucleotides provided herein incorporate modified phosphates, nicked tetraloop structures, and/or other modifications that improve activity, bioavailability and/or minimize the extent of enzymatic degradation after *in vivo* administration. In some embodiments, because the RNAi oligonucleotides provided herein can produce persistent reductions in ALDH2 activity, they overcome compliance issues associated with daily administration of existing small molecule inhibitors of ALDH2.

[0007] One aspect of the present disclosure provides oligonucleotides for reducing expression of ALDH2. In some embodiments, he oligonucleotides comprise an antisense strand comprising a sequence as set forth in any one of SEQ ID NOs: 591-600. In some embodiments, the oligonucleotides further comprise a sense strand that comprises a sequence as set forth in any one of SEQ ID NOs: 581-590. In some embodiments, the antisense strand consists of a sequence as set forth in any one of SEQ ID NOs: 591-600. In some embodiments, the sense strand consists of a sequence as set forth in any one of SEQ ID NOs: 581-590.

[0008] One aspect of the present disclosure provides oligonucleotides for reducing expression of ALDH2, in which the oligonucleotides comprise an antisense strand of 15 to 30 nucleotides in length. In some embodiments, the antisense strand has a region of complementarity to a target sequence of ALDH2 as set forth in any one of SEQ ID NOs: 601-607. In some embodiments, the region of complementarity is at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleotides in length. In some

embodiments, the region of complementarity is fully complementary to the target sequence of ALDH2. In some embodiments, the antisense strand is 19 to 27 nucleotides in length. In some embodiments, the antisense strand is 21 to 27 nucleotides in length. In some embodiments, the oligonucleotide further comprises a sense strand of 15 to 40 nucleotides in length, in which the sense strand forms a duplex region with the antisense strand. In some embodiments, the sense strand is 19 to 40 nucleotides in length. In some embodiments, the duplex region is 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, the region of complementarity to ALDH2 is at least 19 contiguous nucleotides in length. In some embodiments, the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 581-590. In some embodiments, the sense strand consists of a sequence as set forth in any one of SEQ ID NOs: 581-590. In some embodiments, the antisense strand comprises a sequence as set forth in any one of SEQ ID NOs: 591-600. In some embodiments, the antisense strand consists of a sequence as set forth in any one of SEQ ID NOs: 591-600. In some embodiments, the sense strand comprises 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 3 to 5 nucleotides in length.

[0009] Another aspect of the present disclosure provides an oligonucleotide for reducing expression of ALDH2, the oligonucleotide comprising an antisense strand and a sense strand, in which the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to ALDH2, in which the sense strand comprises 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 3 to 5 nucleotides in length, and in which the antisense strand and the sense strand form a duplex structure of at least 19 nucleotides in length but are not covalently linked. In some embodiments, the region of complementarity is fully complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleotides of ALDH2 mRNA. In some embodiments, L is a tetraloop. In some embodiments, L is 4 nucleotides in length. In some embodiments, L comprises a sequence set forth as GAAA. In some embodiments, the antisense strand is 27 nucleotides in length and the sense strand is 25 nucleotides in length. In some embodiments, the antisense strand and sense strand form a duplex region of 25 nucleotides in length.

[00010] In some embodiments, an oligonucleotide further comprises a 3′-overhang sequence on the antisense strand of two nucleotides in length. In some embodiments, an oligonucleotide comprises an antisense strand and a sense strand that are each in a range of 21 to 23 nucleotides in length. In some embodiments, an oligonucleotide comprises a duplex

structure in a range of 19 to 21 nucleotides in length. In some embodiments, an oligonucleotide comprises a 3′-overhang sequence of one or more nucleotides in length, in which the 3′-overhang sequence is present on the antisense strand, the sense strand, or the antisense strand and sense strand. In some embodiments, an oligonucleotide comprises a 3′-overhang sequence of two nucleotides in length, in which the 3′-overhang sequence is present on the antisense strand, and in which 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.

[00011] In some embodiments, an oligonucleotide comprises at least one modified nucleotide. In some embodiments, the modified nucleotide comprises a 2′-modification. In some embodiments, the 2′-modification is a modification selected from: 2′-aminoethyl, 2′-fluoro, 2′-O-methyl, 2′-O-methoxyethyl, and 2′-deoxy-2′-fluoro-β-d-arabinonucleic acid. In some embodiments, all of the nucleotides of an oligonucleotide are modified.

[00012] In some embodiments, an oligonucleotide comprises at least one modified internucleotide linkage. In some embodiments, the at least one modified internucleotide linkage is a phosphorothioate linkage. In some embodiments, the 4′-carbon of the sugar of the 5′-nucleotide of the antisense strand comprises a phosphate analog. In some embodiments, the phosphate analog is oxymethylphosphonate, vinylphosphonate, or malonylphosphonate.

[00013] In some embodiments, at least one nucleotide of an oligonucleotide is conjugated to one or more targeting ligands. In some embodiments, each targeting ligand comprises a carbohydrate, amino sugar, cholesterol, polypeptide or lipid. In some embodiments, each targeting ligand comprises a N-acetylgalactosamine (GalNAc) moiety. In some embodiments, the GalNac moiety is a monovalent GalNAc moiety, a bivalent GalNAc moiety, a trivalent GalNAc moiety, or a tetravalent GalNAc moiety. In some embodiments, up to 4 nucleotides of L of the stem-loop are each conjugated to a monovalent GalNAc moiety. In some embodiments, the targeting ligand comprises an aptamer.

[00014] Another aspect of the present disclosure provides a composition comprising an oligonucleotide of the present disclosure and an excipient. Another aspect of the present disclosure provides a method comprising administering a composition of the present disclosure to a subject. In some embodiments, the method results in a decreased ethanol tolerance in a subject. In some embodiments, the method results in a inhibition of ethanol intake by a subject. In some embodiments, the method results in a decreased desire of a subject to consume ethanol. In some embodiments, the subject to be treated suffers from alcoholism.

[00015] Another aspect of the present disclosure provides an oligonucleotide for

reducing expression of ALDH2, the oligonucleotide comprising a sense strand of 15 to 40 nucleotides in length and an antisense strand of 15 to 30 nucleotides in length, in which the sense strand forms a duplex region with the antisense strand, in which the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 581-590 and results in a the antisense strand comprises a complementary sequence selected from SEQ ID NOs: 591-600.

[00016] In some embodiments, the oligonucleotide comprises a pair of sense and antisense strands selected from a row of the table set forth in Table 4.

BRIEF DESCRIPTION OF THE DRAWINGS

[00017] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate certain embodiments, and together with the written description, serve to provide non-limiting examples of certain aspects of the compositions and methods disclosed herein.

[00018] FIG. 1 is a flowchart depicting the experimental design used to select compounds for testing in cell and animal models and to develop double-stranded oligonucleotides for reducing expression of ALDH2.

[00019] FIG. 2 is a graph showing the percent of ALDH2 mRNA remaining after a screen of 288 ALDH2 oligonucleotides in HepG2 cells. The nucleotide position in NM_000690.3 that corresponds to the 3' end of the sense strand of each siRNA is indicated on the x-axis.

[00020] FIGs. 3A-3D are a set of graphs showing the percentage of mRNA remaining after ALDH2 oligonucleotide screening of 96 ALDH2 oligonucleotides at three different concentrations (1 nM, 0.1nM and 0.01nM) in HepG2 cells.

[00021] FIG. 4 is a schematic showing a non-limiting example of a double-stranded oligonucleotide with a nicked tetraloop structure that has been conjugated to four GalNAc moieties (diamond shapes).

[00022] FIGs. 5A-5B are a set of graphs showing the results of screening in HepG2 cells using ALDH2 oligonucleotides of different base sequences in the nicked tetraloop structure, adapted to different modification patterns, and at three different concentrations (1 nM, 0.1nM and 0.01nM).

[00023] FIG. 6 is a graph showing the results of screening in Hepa1-6 cells using ALDH2 oligonucleotides of different base sequences in the nicked tetraloop structure, adapted to different modification patterns, and at three different concentrations (1 nM, 0.1nM and 0.01nM).

[00024] FIG. 7 is a graph showing an in vivo activity evaluation of GalNAc-conjugated ALDH2 oligonucleotides in a nicked tetraloop structure. Three different oligonucleotide sequences were tested. Oligonucleotides were subcutaneously administered to mice at 3 mg/kg. The data show the amount of ALDH2 mRNA remaining at day 4 following administration normalized to PBS control.

[00025] FIG. 8 is a graph showing the results of a duration study of GalNAc-conjugated ALDH2 oligonucleotides with different modification patterns in non-human primates (NHP). A single dose (3 mg/kg) of the oligonucleotides was subcutaneously administered to non-human primates. The data show the amount of ALDH2 mRNA remaining 4, 12, and 16 weeks following administration, relative to the amount of ALDH2 mRNA prior to administration. "*" means one non-human primate was euthanized and not included in the 16-week analysis.

[00026] FIG. 9 is a graph showing the result of an in vivo assay screening GalNAc-conjugated ALDH2 oligonucleotides with different modification patterns to identify the modification pattern(s) that enhance the activity of the oligonucleotides in reducing ALDH2 mRNA level in mice.

[00027] FIG. 10 a graph showing the comparison of the activities of GalNac-conjugated ALDH2 oligonucleotides with different modification patterns in reducing ALDH2 mRNA level in mice. Oligonucleotides were subcutaneously administered to mice at 0.5 mg/kg. The data was normalized to PBS control and showed the amount of ALDH2 mRNA remaining at day 4 following administration.

[00028] FIG. 11 is a graph showing the results of a dose titration study of the indicated GalNac-conjugated ALDH2 oligonucleotides in CD-1 mice. Oligonucleotides were subcutaneously administered to mice at 0.1, 0.3, or 0.5 mg/kg. The data was normalized to PBS control and showed the amount of ALDH2 mRNA remaining 72 hours after administration of the oligonucleotides.

[00029] FIG. 12 is a graph showing the comparison of ALDH2 mRNA suppression activities of a GalNac-conjugated ALDH2 oligonucleotide with different modification patterns. Oligonucleotides were subcutaneously administered to mice at 0.5 mg/kg. The data was normalized to PBS control and showed the amount of ALDH2 mRNA remaining at day 4 following administration.

[00030] FIG. 13 is a graph showing the results of a duration study of the indicated GalNac-conjugated ALDH2 oligonucleotides in mice. Oligonucleotides were subcutaneously administered to mice at 3 mg/kg. The data was normalized to PBS control and showed the

amount of ALDH2 mRNA remaining up to day 35 following administration.

DETAILED DESCRIPTION OF THE INVENTION

[00031] According to some aspects, the disclosure provides oligonucleotides targeting ALDH2 mRNA that are effective for reducing ALDH2 expression in cells, particularly liver cells (e.g., hepatocytes) for the treatment of alcoholism. Accordingly, in related aspects, the disclosure provided methods of treating alcoholism that involve selectively reducing ALDH2 gene expression in liver. In certain embodiments, ALDH2 targeting oligonucleotides provided herein are designed for delivery to selected cells of target tissues (*e.g.*, liver hepatocytes) to treat alcoholism in a subject.

[00032] Further aspects of the disclosure, including a description of defined terms, are provided below.

I. Definitions

[00033] **Alcoholism**: As used herein, the term, "alcoholism" refers to repeated use of ethanol by an individual despite recurrent adverse consequences, which may or may not be combined with tolerance, withdrawal, and/or an uncontrollable drive to consume alcohol. Alcoholism may be classified as alcohol abuse, alcohol use disorder or alcohol dependence. A variety of approaches may be used to identify an individual suffering from alcoholism. For example, the World Health Organization has established the Alcohol Use Disorders Identification Test (AUDIT) as a tool for identifying potential alcohol misuse, including dependence and other similar tests have been developed, including the Michigan Alcohol Screening Test (MAST). Laboratory tests may be used to evaluate blood markers for detecting chronic use and/or relapse in alcohol drinking, including tests to detect levels of gamma-glutamyl transferase (GGT), mean corpuscular volume (red blood cell size), aspartate aminotransferase (AST), alanine aminotransferase (ALT), carbohydrate-deficient transferring (CDT), ethyl glucuronide (EtG), ethyl sulfate (EtS), and/or phosphatidylethanol (PEth). Animal models (e.g., mouse models) of alcoholism have been established (*see*, *e.g.*, Rijk H, Crabbe JC, Rigter H. A mouse model of alcoholism. Physiol Behav. 1982 Nov;29(5):833-9; Elizabeth Brandon-Warner, et al., Rodent Models of Alcoholic Liver Disease: Of Mice and Men. Alcohol. 2012 Dec; 46(8): 715–725; and Adeline Bertola, et al., Mouse model of chronic and binge ethanol feeding (the NIAAA model). Nature Protocols 8, 627–637 (2013).)

[00034] **ALDH2:** As used herein, the term, "ALDH2" refers to the aldehyde dehydrogenase 2 family (mitochondrial) gene. ALDH2 encodes proteins that belong to the

aldehyde dehydrogenase family of proteins and that function as the second enzyme of the oxidative pathway of alcohol metabolism that synthesizes acetate (acetic acid) from ethanol. Homologs of ALDH2 are conserved across a range of species, including human, mouse, rat, non-human primate species, and others (*see, e.g.*, NCBI HomoloGene:55480.) ALDH2 also has homology with other aldehyde dehydrogenase encoding genes, including, for example, ALDH1A1. In humans, ALDH2 encodes at least two transcripts, namely NM_000690.3 (variant 1) and NM_001204889.1 (variant 2), each encoding a different isoform, NP_000681.2 (isoform 1) and NP_001191818.1 (isoform 2), respectively. Transcript variant 2 lacks an in-frame exon in the 5' coding region, compared to transcript variant 1, and encodes a shorter isoform (2), compared to isoform 1. Polymorphisms in ALDH2 have been identified (*see, e.g.*, Chang JS, Hsiao JR, Chen CH. ALDH2 polymorphism and alcohol-related cancers in Asians: a public health perspective. J Biomed Sci. 2017 Mar 3;24(1):19. Review.)

[00035] **Approximately:** As used herein, the term "approximately" or "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, the term "approximately" or "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).

[00036] **Administering**: As used herein, the terms "administering" or "administration" means to provide 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).

[00037] **Asialoglycoprotein receptor (ASGPR)**: As used herein, the term "Asialoglycoprotein receptor" or "ASGPR" refers to a bipartite C-type lectin formed by a major 48 kDa (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, internalization, and subsequent clearance of circulating glycoproteins that contain terminal galactose or N-acetylgalactosamine residues (asialoglycoproteins).

[00038] **Complementary:** As used herein, the term "complementary" refers to a structural relationship between nucleotides (*e.g.*, two nucleotide on opposing nucleic acids or on opposing regions of a single nucleic acid strand) that permits the 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 nucleotide sequences that are complementary to each other so as to form regions of complementarity, as described herein.

[00039] **Deoxyribonucleotide:** As used herein, the term "deoxyribonucleotide" refers to a nucleotide having a hydrogen at the 2′ position of its pentose sugar as 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.

[00040] **Double-stranded oligonucleotide:** As used herein, the term "double-stranded oligonucleotide" refers to an oligonucleotide that is substantially in a duplex form. In some embodiments, complementary base-pairing of duplex region(s) of a double-stranded 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 double-stranded 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 double-stranded oligonucleotide is formed from a 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 double-stranded oligonucleotide comprises two covalently separate nucleic acid strands that are fully duplexed with one another. However, in some embodiments, a double-stranded 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 double-stranded oligonucleotide comprises antiparallel sequences of nucleotides that are partially complementary, and thus, may have one or more mismatches, which may include internal mismatches or end mismatches.

[00041] **Duplex:** As used herein, the term "duplex," in reference to nucleic acids (*e.g.*, oligonucleotides), refers to a structure formed through complementary base-pairing of two antiparallel sequences of nucleotides.

[00042] **Excipient:** As used herein, the term "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.

[00043] **Hepatocyte**: As used herein, the term "hepatocyte" or "hepatocytes" refers to cells of the parenchymal tissues of the liver. These cells make up approximately 70-85% of

the liver's mass and manufacture serum albumin, fibrinogen, and the prothrombin group of clotting factors (except for Factors 3 and 4). Markers for hepatocyte lineage cells may 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(7436): 247-250, the contents of which relating to hepatocyte markers is incorporated herein by reference.

[00044] **Loop:** As used herein, the term "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").

[00045] **Modified Internucleotide Linkage:** As used herein, the term "modified internucleotide linkage" refers to an internucleotide linkage having one or more chemical modifications 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.*

[00046] **Modified Nucleotide:** As used herein, the term "modified nucleotide" refers to a nucleotide having one or more chemical modifications 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 modifications 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*. In certain embodiments, a modified nucleotide comprises a 2'-O-methyl or a 2'-F substitution at the 2' position of the ribose ring.

[00047] **Nicked Tetraloop Structure**: A "nicked tetraloop structure" is a structure of a RNAi oligonucleotide characterized by the presence of separate sense (passenger) and antisense (guide) strands, in which the sense strand has a region of complementarity to the antisense strand such that the two strands form a duplex, and in which at least one of the strands, generally the sense strand, extends from the duplex in which the extension contains a tetraloop and two self-complementary sequences forming a stem region adjacent to the tetraloop, in which the tetraloop is configured to stabilize the adjacent stem region formed by the self-complementary sequences of the at least one strand.

[00048] **Oligonucleotide:** As used herein, the term "oligonucleotide" refers to a short nucleic acid, *e.g.*, of less than 100 nucleotides in length. An oligonucleotide can comprise ribonucleotides, deoxyribonucleotides, and/or modified nucleotides including, for example, modified ribonucleotides. An oligonucleotide may be single-stranded or double-stranded. 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 single-stranded siRNA. In some embodiments, a double-stranded oligonucleotide is an RNAi oligonucleotide.

[00049] **Overhang:** As used herein, the term "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 double-stranded oligonucleotide. In certain embodiments, the overhang is a 3′ or 5′ overhang on the antisense strand or sense strand of a double-stranded oligonucleotide.

[00050] **Phosphate analog:** As used herein, the term "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 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, for example, International Patent Application PCT/US2017/049909, filed on September 1, 2017, U.S. Provisional Application numbers 62/383,207, filed on September 2, 2016, and 62/393,401, filed on September 12, 2016, the contents of each of which relating to phosphate analogs are incorporated herein by reference. Other modifications have been developed for the 5′ end of oligonucleotides (see, *e.g.*, WO 2011/133871; U.S. Patent No. 8,927,513; and Prakash *et al.* (2015), Nucleic Acids Res., 43(6):2993-3011, the contents of each of which relating to phosphate analogs are incorporated herein by reference).

[00051] **Reduced expression:** As used herein, the term "reduced expression" of a gene refers to a decrease in the amount of RNA transcript or protein encoded by the gene and/or a decrease in the amount of activity of the gene in a cell or subject, as compared to an appropriate reference cell or subject. For example, the act of treating a cell with a double-stranded oligonucleotide (*e.g.*, one having an antisense strand that is complementary to ALDH2 mRNA sequence) may result in a decrease in the amount of RNA transcript, protein and/or enzymatic activity (*e.g.*, encoded by the ALDH2 gene) compared to a cell that is not treated with the double-stranded oligonucleotide. Similarly, "reducing expression" as used herein refers to an act that results in reduced expression of a gene (*e.g.*, ALDH2).

[00052] **Region of Complementarity:** As used herein, the term "region of complementarity" refers to a sequence of nucleotides of a nucleic acid (*e.g.*, a double-stranded oligonucleotide) that is sufficiently complementary to an antiparallel sequence of nucleotides (e.g., a target nucleotide sequence within an mRNA) to permit hybridization between the two sequences of nucleotides under appropriate hybridization conditions, *e.g.*, in a phosphate buffer, in a cell, *etc.* A region of complementarity may be fully complementary to a nucleotide sequence (e.g., a target nucleotide sequence present within an mRNA or portion thereof). For example, a region of complementary that is fully complementary to a nucleotide sequence present in an mRNA has a contiguous sequence of nucleotides that is complementary, without any mismatches or gaps, to a corresponding sequence in the mRNA. Alternatively, a region of complementarity may be partially complementary to a nucleotide sequence (e.g., a nucleotide sequence present in an mRNA or portion thereof). For example, a region of complementary that is partially complementary to a nucleotide sequence present in an mRNA has a contiguous sequence of nucleotides that is complementary to a corresponding sequence in the mRNA but that contains one or more mismatches or gaps (e.g., 1, 2, 3, or more mismatches or gaps) compared with the corresponding sequence in the mRNA, provided that the region of

complementarity remains capable of hybridizing with the mRNA under appropriate hybridization conditions.

[00053] **Ribonucleotide:** As used herein, the term "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.

[00054] **RNAi Oligonucleotide:** As used herein, the term "RNAi oligonucleotide" refers to either (a) a double stranded 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 single stranded 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.

[00055] **Strand:** As used herein, the term "strand" refers to a single contiguous sequence of nucleotides linked together through internucleotide linkages (*e.g.*, phosphodiester linkages, phosphorothioate linkages). In some embodiments, a strand has two free ends, *e.g.*, a 5′-end and a 3′-end.

[00056] **Subject:** As used herein, the term "subject" means any mammal, including mice, rabbits, and humans. In one embodiment, the subject is a human or non-human primate. The terms "individual" or "patient" may be used interchangeably with "subject."

[00057] **Synthetic:** As used herein, the term "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.

[00058] **Targeting ligand**: As used herein, the term "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.

[00059] **Tetraloop**: As used herein, the term "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 melting temperature of at least 50 °C, at least 55 °C., at least 56 °C, at least 58 °C, at least 60 °C, at least 65 °C or at least 75 °C in 10 mM $NaHPO_4$ to a hairpin comprising a duplex of at least 2 base pairs in length. In some embodiments, a tetraloop may stabilize a base pair 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.*, Nature 1990 Aug. 16; 346(6285):680-2; Heus and Pardi, Science 1991 Jul. 12; 253(5016):191-4). 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 three, four, five, or six 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 four 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) Nucl. 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 UNCG family of tetraloops (*e.g.*, UUCG), the GNRA family of tetraloops (*e.g.*, GAAA), and the CUUG tetraloop (Woese *et al.*, Proc Natl Acad Sci USA. 1990 November; 87(21):8467-71; Antao *et al.*, Nucleic Acids Res. 1991 Nov. 11; 19(21):5901-5). 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, for example: Nakano *et al.* Biochemistry, 41 (48), 14281-14292, 2002. SHINJI *et al.* Nippon Kagakkai Koen Yokoshu VOL. 78th; NO. 2; PAGE. 731 (2000), which are incorporated by reference herein for their relevant disclosures. In some

embodiments, the tetraloop is contained within a nicked tetraloop structure.

[00060] **Treat**: As used herein, the term "treat" refers to the act of providing care to a subject in need thereof, *e.g.*, through the administration a therapeutic agent (*e.g.*, an oligonucleotide) 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.

II. Oligonucleotide-Based Inhibitors

i. ALDH2 Targeting Oligonucleotides

[00061] Potent oligonucleotides have been identified herein through examination of the ALDH2 mRNA, including mRNAs of multiple different species (human, cynomolgus monkey, and mouse (*see*, *e.g.*, Example 1)) and *in vitro* and *in vivo* testing. Such oligonucleotides can be used to achieve therapeutic benefit for alcoholic subjects by reducing ALDH2 activity, and consequently, by decreasing alcohol tolerance and/or the desire to consume alcohol. For example, potent RNAi oligonucleotides are provided herein that have a sense strand comprising, or consisting of, a sequence as set forth in any one of SEQ ID NO: 581-590 and an antisense strand comprising, or consisting of, a complementary sequence selected from SEQ ID NO: 591-600, as is also arranged the table provided in Table 4 (*e.g.*, a sense strand comprising a sequence as set forth in SEQ ID NO: 581 and an antisense strand comprising a sequence as set forth in SEQ ID NO: 591).

[00062] The sequences can be put into multiple different oligonucleotide structures (or formats). For example, in some embodiments, the sequences can be incorporated into oligonucleotides that comprise sense and antisense strands that are both in the range of 17 to 36 nucleotides in length. In some embodiments, oligonucleotides incorporating such sequences are provided that have a tetraloop structure within a 3' extension of their sense strand, and two terminal overhang nucleotides at the 3' end of its antisense strand. In some embodiments, the two terminal overhang nucleotides are GG. Typically, one or both of the two terminal GG nucleotides of the antisense strand is or are not complementary to the target.

[00063] In some embodiments, oligonucleotides incorporating such sequences are provided that have sense and antisense strands that are both in the range of 21 to 23 nucleotides in length. In some embodiments, a 3' overhang is provided on the sense, antisense, or both sense and antisense strands that is 1 or 2 nucleotides in length. In some embodiments, an

oligonucleotide has a guide strand of 23 nucleotides and a passenger strand of 21 nucleotides, in which the 3′-end of passenger strand and 5′-end of guide strand form a blunt end and where the guide strand has a two nucleotide 3′ overhang.

[00064] In some embodiments, it has been discovered that certain regions of ALDH2 mRNA are hotspots for targeting because they are more amenable than other regions to oligonucleotide-based inhibition. In some embodiments, a hotspot region of ALDH2 comprises, or consists of, a sequence as forth in any one of SEQ ID NOs:601-607. These regions of ALDH2 mRNA may be targeted using oligonucleotides as discussed herein for purposes of inhibiting ALDH2 mRNA expression.

[00065] Accordingly, in some embodiments, oligonucleotides provided herein are designed so as to have regions of complementarity to ALDH2 mRNA (e.g., within a hotspot of ALDH2 mRNA) for purposes of targeting the mRNA in cells and inhibiting its expression. The region of complementarity is generally of a suitable length and base content to enable annealing of the oligonucleotide (or a strand thereof) to ALDH2 mRNA for purposes of inhibiting its expression.

[00066] In some embodiments, an oligonucleotide disclosed herein comprises a region of complementarity (*e.g.*, on an antisense strand of a double-stranded oligonucleotide) that is at least partially complementary to a sequence as set forth in SEQ ID NOs: 1-14 and 17-290, which include sequences mapping to within hotspot regions of ALDH2 mRNA. In some embodiments, an oligonucleotide disclosed herein comprises a region of complementarity (*e.g.*, on an antisense strand of a double-stranded oligonucleotide) that is fully complementary to a sequence as set forth in SEQ ID NOs: 1-14 and 17-290. In some embodiments, a region of complementarity of an oligonucleotide that is complementary to contiguous nucleotides of a sequence as set forth in SEQ ID NOs: 1-14 and 17-290 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: 1-14 and 17-290 spans a portion of the entire length of an antisense strand (e.g., all but two nucleotides at the 3' end of the antisense strand). In some embodiments, an oligonucleotide disclosed herein comprises a region of complementarity (*e.g.*, on an antisense strand of a double-stranded oligonucleotide) that is at least partially (*e.g.*, fully) complementary to a contiguous stretch of nucleotides spanning nucleotides 1-19 of a sequence as set forth in SEQ ID NOs: 581-590.

[00067] In some embodiments, the region of complementarity is 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, at least 23, at least 24, at least 25 nucleotides in length. In some embodiments, an oligonucleotide provided herein has a region of complementarity to ALDH2 that is in the range of 12 to 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, an oligonucleotide provided herein has a region of complementarity to ALDH2 that is 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides in length.

[00068] In some embodiments, a region of complementarity to ALDH2 may have one or more mismatches compared with a corresponding sequence of ALDH2 mRNA. A region of complementarity on an oligonucleotide may have up to 1, up to 2, up to 3, up to 4, up to 5, *etc.* mismatches provided that it maintains the ability to form complementary base pairs with ALDH2 mRNA under appropriate hybridization conditions. Alternatively, a region of complementarity on an oligonucleotide may have no more than 1, no more than 2, no more than 3, no more than 4, or no more than 5 mismatches provided that it maintains the ability to form complementary base pairs with ALDH2 mRNA under appropriate hybridization conditions. In some embodiments, if there are more than one mismatches in a region of complementarity, they may be positioned consecutively (*e.g.*, 2, 3, 4, or more in a row), or interspersed throughout the region of complementarity provided that the oligonucleotide maintains the ability to form complementary base pairs with ALDH2 mRNA under appropriate hybridization conditions.

[00069] Still, in some embodiments, double-stranded oligonucleotides provided herein comprise, of consist of, a sense strand having a sequence as set forth in any one of SEQ ID NO: 1-14 and 17-290 and an antisense strand comprising a complementary sequence selected from SEQ ID NO: 291-304 and 307-580, as is arranged in the table provided in Table 4 (*e.g.*, a sense strand comprising a sequence as set forth in SEQ ID NO: 1 and an antisense strand comprising a sequence as set forth in SEQ ID NO: 291).

ii. Oligonucleotide Structures

[00070] There are a variety of structures of oligonucleotides that are useful for targeting ALDH2 in the methods of the present disclosure, including RNAi, miRNA, *etc.* Any of the structures described herein or elsewhere may be used as a framework to incorporate or target a sequence described herein (*e.g.*, a hotpot sequence of ALDH2 such as those illustrated in SEQ ID NOs: 601-607). Double-stranded oligonucleotides for targeting ALDH2 expression (e.g., via the RNAi pathway) generally have a sense strand and an antisense strand that form a duplex with one another. In some embodiments, the sense and antisense strands are not

covalently linked. However, in some embodiments, the sense and antisense strands are covalently linked.

[00071] In some embodiments, double-stranded oligonucleotides for reducing the expression of ALDH2 expression engage RNA interference (RNAi). For example, RNAi oligonucleotides have been developed with each strand having sizes of 19-25 nucleotides with at least one 3' overhang of 1 to 5 nucleotides (see, *e.g.*, U.S. Patent No. 8,372,968). Longer oligonucleotides have also been developed that are processed by Dicer to generate active RNAi products (see, *e.g.*, U.S. Patent No. 8,883,996). Further work produced extended double-stranded 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.*, U.S. Patent Nos. 8,513,207 and 8,927,705, as well as WO2010033225, which are incorporated by reference herein for their disclosure of these oligonucleotides). Such structures may include single-stranded extensions (on one or both sides of the molecule) as well as double-stranded extensions.

[00072] In some embodiments, oligonucleotides may be in the range of 21 to 23 nucleotides in length. In some embodiments, oligonucleotides may have an overhang (*e.g.*, of 1, 2, or 3 nucleotides in length) in the 3' end of the sense and/or antisense strands. In some embodiments, oligonucleotides (*e.g.*, siRNAs) may comprise 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. *See*, for example, US9012138, US9012621, and US9193753, the contents of each of which are incorporated herein for their relevant disclosures.

[00073] In some embodiments, an oligonucleotide of the invention has a 36 nucleotide sense strand that comprises an region extending beyond the antisense-sense duplex, where the extension region has a stem-tetraloop structure where the stem is a six base pair duplex and where the tetraloop has four nucleotides. In certain of those embodiments, three or four of the tetraloop nucleotides are each conjugated to a monovalent GalNac ligand.

[00074] In some embodiments, an oligonucleotide of the invention 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.

[00075] Other oligonucleotides designs for use with the compositions and methods disclosed 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.* Methods Mol. Biol. 2010; 629:141-158), blunt siRNAs (*e.g.*, of

19 bps in length; see: *e.g.*, Kraynack and Baker, RNA Vol. 12, p163-176 (2006)), asymmetrical siRNAs (aiRNA; see, *e.g.*, Sun *et al.*, Nat. Biotechnol. 26, 1379–1382 (2008)), asymmetric shorter-duplex siRNA (see, *e.g.*, Chang *et al.*, Mol Ther. 2009 Apr; 17(4): 725-32), fork siRNAs (see, *e.g.*, Hohjoh, FEBS Letters, Vol 557, issues 1-3; Jan 2004, p 193-198), single-stranded siRNAs (Elsner; Nature Biotechnology 30, 1063 (2012)), dumbbell-shaped circular siRNAs (see, *e.g.*, Abe *et al.* J Am Chem Soc 129: 15108-15109 (2007)), and small internally segmented interfering RNA (sisiRNA; see, *e.g.*, Bramsen *et al.*, Nucleic Acids Res. 2007 Sep; 35(17): 5886–5897). Each of the foregoing references is incorporated by reference in its entirety for the related disclosures therein. Further non-limiting examples of an oligonucleotide structures that may be used in some embodiments to reduce or inhibit the expression of ALDH2 are microRNA (miRNA), short hairpin RNA (shRNA), and short siRNA (see, *e.g.*, Hamilton *et al.*, Embo J., 2002, 21(17): 4671-4679; see also U.S. Application No. 20090099115).

a. Antisense Strands

[00076] In some embodiments, an oligonucleotide disclosed herein for targeting ALDH2 comprises an antisense strand comprising or consisting of a sequence as set forth in any one of SEQ ID NOs: 291-304, 307-580,and 591-600. In some embodiments, an oligonucleotide comprises an antisense strand comprising or consisting of at least 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: 291-304, 307-580,and 591-600.

[00077] In some embodiments, a double-stranded oligonucleotide may have an antisense strand of up to 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 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 35, or at least 38 nucleotides in length). In some embodiments, an oligonucleotide may have an antisense strand in a range of 12 to 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 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.

[00078] 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 Argonaut protein, 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."

b. Sense Strands

[00079] In some embodiments, an oligonucleotide disclosed herein for targeting ALDH2 comprises or consists of a sense strand sequence as set forth in in any one of SEQ ID NOs: 1-14, 17-290, and 581-590. In some embodiments, an oligonucleotide has a sense strand that comprises or consists of at least 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: 1-14, 17-290, and 581-590.

[00080] In some embodiments, an oligonucleotide may have a sense strand (or passenger strand) of up to 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 a sense strand of at least 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 35, or at least 38 nucleotides in length). In some embodiments, an oligonucleotide may have a sense strand in a range of 12 to 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.

[00081] 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 nucleotides in length. In some embodiments, a stem-loop provides the molecule better 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 provided herein in which the sense strand comprises (*e.g.*, at its 3′-end) a stem-loop set forth as: S_1-L-S_2, in

which S_1 is complementary to S_2, and in which L forms a loop between S_1 and S_2 of up to 10 nucleotides in length (*e.g.*, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length).

[00082] In some embodiments, a loop (L) 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.

c. Duplex Length

[00083] In some embodiments, a duplex formed between a sense and antisense strand is at least 12 (*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, a duplex formed between a sense and antisense strand is in the range of 12-30 nucleotides in length (*e.g.*, 12 to 30, 12 to 27, 12 to 22, 15 to 25, 18 to 30, 18 to 22, 18 to 25, 18 to 27, 18 to 30, 19 to 30 or 21 to 30 nucleotides in length). In some embodiments, a duplex formed between a sense and antisense strand 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 a duplex formed between a sense and antisense strand does not span the entire length of the sense strand and/or antisense strand. In some embodiments, a duplex between a sense and antisense strand spans the entire length of either the sense or antisense strands. In certain embodiments, a duplex between a sense and antisense strand spans the entire length of both the sense strand and the antisense strand.

d. Oligonucleotide Ends

[00084] In some embodiments, an oligonucleotide provided herein 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, oligonucleotides provided herein have one 5'end that is thermodynamically less stable compared to the other 5' end. In some embodiments, an asymmetric oligonucleotide is provided that includes a blunt end at the 3' end of a sense strand and an overhang at the 3' end of an antisense strand. In some embodiments, a 3' overhang on an antisense strand is 1-8 nucleotides in length (*e.g.*, 1, 2, 3, 4, 5, 6, 7 or 8 nucleotides in length).

[00085] 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 one and six nucleotides, optionally one to five, one to four, one to three, one to two, two to six, two to five, two to four, two to three, three to six, three to five, three to four, four to six, four to five, five to

six nucleotides, or one, two, three, four, five or six nucleotides. However, in some embodiments, the overhang is a 5′ overhang comprising a length of between one and six nucleotides, optionally one to five, one to four, one to three, one to two, two to six, two to five, two to four, two to three, three to six, three to five, three to four, four to six, four to five, five to six nucleotides, or one, two, three, four, five or six nucleotides.

[00086] In some embodiments, one or more (*e.g.*, 2, 3, 4) terminal nucleotides of the 3' end or 5' end of a sense and/or antisense strand are modified. For example, in some embodiments, one or two terminal nucleotides of the 3' end of an antisense strand are modified. In some embodiments, the last nucleotide at the 3' end of an antisense strand is modified, *e.g.*, comprises 2'-modification, *e.g.*, a 2'-O-methoxyethyl. In some embodiments, the last one or two terminal nucleotides at the 3' end of an antisense strand are complementary to the target. In some embodiments, the last one or two nucleotides at the 3' end of the antisense strand are not complementary to the target. In some embodiments, the 5' end and/or the 3' end of a sense or antisense strand has an inverted cap nucleotide.

e. Mismatches

[00087] In some embodiments, there is one or more (*e.g.*, 1, 2, 3, 4, 5) mismatches 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'-terminus of the sense strand contains one or more mismatches. In one embodiment, two mismatches are incorporated at the 3' terminus 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.

iii. Single-Stranded Oligonucleotides

[00088] In some embodiments, an oligonucleotide for reducing ALDH2 expression as described herein is single-stranded. Such structures may include, but are not limited to single-stranded RNAi oligonucleotides. Recent efforts have demonstrated the activity of single-stranded RNAi oligonucleotides (see, *e.g.*, Matsui *et al.* (May 2016), Molecular Therapy, Vol. 24(5), 946-955). However, in some embodiments, oligonucleotides provided herein are antisense oligonucleotides (ASOs). An antisense oligonucleotide is a single-stranded 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. Antisense oligonucleotides for use in the instant disclosure may be modified in any suitable manner known in the art including, for example, as shown in U.S. Patent No. 9,567,587, which is incorporated by reference herein for its disclosure regarding modification of antisense oligonucleotides (including, *e.g.*, length, sugar moieties of the nucleobase (pyrimidine, purine), and alterations of the heterocyclic portion of the nucleobase). Further, antisense molecules have been used for decades to reduce expression of specific target genes (see, *e.g.*, Bennett *et al.*; Pharmacology of Antisense Drugs, Annual Review of Pharmacology and Toxicology, Vol. 57: 81-105).

iv. Oligonucleotide Modifications

[00089] Oligonucleotides may be modified in various ways to improve or control specificity, stability, delivery, bioavailability, resistance from nuclease degradation, immunogenicity, base-paring properties, RNA distribution and cellular uptake and other features relevant to therapeutic or research use. See, *e.g.*, Bramsen *et al.*, Nucleic Acids Res., 2009, 37, 2867-2881; Bramsen and Kjems (Frontiers in Genetics, 3 (2012): 1-22). Accordingly, in some embodiments, oligonucleotides of the present disclosure may include one or more suitable modifications. In some embodiments, a modified nucleotide has a modification in its base (or nucleobase), the sugar (*e.g.*, ribose, deoxyribose), or the phosphate group.

[00090] The number of modifications on an oligonucleotide and the positions of those nucleotide modifications may influence the properties of an oligonucleotide. For example, oligonucleotides may be delivered *in vivo* by conjugating them to or encompassing them in a lipid nanoparticle (LNP) or similar carrier. However, when an oligonucleotide is not protected by an LNP or similar carrier (e.g., "naked delivery"), it may be advantageous for at least some of the its nucleotides to be modified. Accordingly, in certain embodiments of any of the oligonucleotides provided herein, all or substantially all of the nucleotides of an oligonucleotide are modified. In certain embodiments, more than half of the nucleotides are modified. In certain embodiments, less than half of the nucleotides are modified. Typically, with naked delivery, every sugar is modified at the 2′-position. These modifications may be reversible or irreversible. In some embodiments, an oligonucleotide as disclosed herein has a number and type of modified nucleotides sufficient to cause the desired characteristic (*e.g.*, protection from enzymatic degradation, capacity to target a desired cell after *in vivo*

administration, and/or thermodynamic stability).

a. Sugar Modifications

[00091] In some embodiments, a modified sugar (also referred to herein as a sugar analog) includes a modified deoxyribose or ribose moiety, *e.g.*, in which 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), Molecular Therapy – Nucleic Acids, 2, e103), and bridged nucleic acids ("BNA") (see, *e.g.*, Imanishi and Obika (2002), The Royal Society of Chemistry, Chem. Commun., 1653-1659). Koshkin *et al.*, Snead *et al.*, and Imanishi and Obika are incorporated by reference herein for their disclosures relating to sugar modifications.

[00092] In some embodiments, a nucleotide modification in a sugar comprises a 2′-modification. In certain embodiments, the 2′-modification may be 2′-aminoethyl, 2′-fluoro, 2′-O-methyl, 2′-O-methoxyethyl, or 2′-deoxy-2′-fluoro-β-d-arabinonucleic acid. Typically, the modification is 2′-fluoro, 2′-O-methyl, or 2′-O-methoxyethyl. However, a large variety of 2′ position modifications that have been developed for use in oligonucleotides can be employed in oligonucleotides disclosed herein. See, e.g., Bramsen et al., Nucleic Acids Res., 2009, 37, 2867-2881. 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 linkage between the 2'-carbon and a 1′-carbon or 4′-carbon of the sugar. For example, the linkage may comprise 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.

[00093] In some embodiments, the terminal 3′-end group (*e.g.*, a 3′-hydroxyl) is a phosphate group or other group, which can be used, for example, to attach linkers, adapters or labels or for the direct ligation of an oligonucleotide to another nucleic acid.

b. 5′ Terminal Phosphates

[00094] 5′-terminal phosphate groups of oligonucleotides may or in some circumstances enhance the interaction with Argonaut 2. 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 5′ end of an oligonucleotide strand is attached to a chemical moiety that mimics the electrostatic and steric properties of a natural 5′-phosphate group ("phosphate mimic") (see, *e.g.,* Prakash *et al.* (2015), Nucleic Acids Res., Nucleic Acids Res. 2015 Mar 31; 43(6): 2993–3011, the contents of which relating to phosphate analogs are incorporated herein by reference). Many phosphate mimics have been developed that can be attached to the 5′ end (see, *e.g.,* U.S. Patent No. 8,927,513, the contents of which relating to phosphate analogs are incorporated herein by reference). Other modifications have been developed for the 5′ end of oligonucleotides (see, *e.g.,* WO 2011/133871, the contents of which relating to phosphate analogs are incorporated herein by reference). In certain embodiments, a hydroxyl group is attached to the 5′ end of the oligonucleotide.

[00095] 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, for example, International Patent Application PCT/US2017/049909, filed on September 1, 2017, U.S. Provisional Application numbers 62/383,207, entitled *4′-Phosphate Analogs and Oligonucleotides Comprising the Same*, filed on September 2, 2016, and 62/393,401, filed on September 12, 2016, entitled *4′-Phosphate Analogs and Oligonucleotides Comprising the Same*, the contents of each of which relating to phosphate analogs are incorporated herein by reference. In some embodiments, an oligonucleotide provided 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 aminomethyl 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, CH_3, an alkyl group, CH_2CH_2CN, $CH_2OCOC(CH_3)_3$, $CH_2OCH_2CH_2Si(CH_3)_3$, or a protecting group. In certain embodiments, the alkyl group is CH_2CH_3. More typically, R is independently selected from H, CH_3, or CH_2CH_3.

c. Modified Internucleoside Linkages

[00096] In some embodiments, the oligonucleotide may comprise a modified internucleoside linkage. In some embodiments, phosphate modifications or substitutions may result in an oligonucleotide that comprises at least one (*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 1 to 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.

[00097] A modified internucleotide linkage may be a phosphorodithioate linkage, a phosphorothioate linkage, a phosphotriester linkage, a thionoalkylphosphonate linkage, a thionoalkylphosphotriester 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.

d. Base modifications

[00098] In some embodiments, oligonucleotides provided 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 a nitrogen atom. *See e.g.*, U.S. Published Patent Application No. 20080274462. In some embodiments, a modified nucleotide comprises a universal base. However, in certain embodiments, a modified nucleotide does not contain a nucleobase (abasic).

[00099] 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 the 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, 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.

[000100] Non-limiting examples of universal-binding nucleotides include inosine, 1-β-D-ribofuranosyl-5-nitroindole, and/or 1-β-D-ribofuranosyl-3-nitropyrrole (US Pat. Appl. Publ. No. 20070254362 to Quay *et al.*; Van Aerschot *et al.*, An acyclic 5-nitroindazole nucleoside analogue as ambiguous nucleoside. Nucleic Acids Res. 1995 Nov 11;23(21):4363-70; Loakes *et al.*, 3-Nitropyrrole and 5-nitroindole as universal bases in primers for DNA sequencing and PCR. Nucleic Acids Res. 1995 Jul 11;23(13):2361-6; Loakes and Brown, 5-Nitroindole as an universal base analogue. Nucleic Acids Res. 1994 Oct 11;22(20):4039-43. Each of the foregoing is incorporated by reference herein for their disclosures relating to base modifications).

e. Reversible Modifications

[000101] 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).

[000102] 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* U.S. Published Application No. 2011/0294869 originally assigned to Traversa Therapeutics, Inc. ("Traversa"), PCT Publication No. WO 2015/188197 to Solstice Biologics, Ltd. ("Solstice"), Meade *et al.*, *Nature Biotechnology*, 2014,32:1256-1263 ("Meade"), PCT Publication No. WO 2014/088920 to Merck Sharp & Dohme Corp, each of which are incorporated by reference for their disclosures of such modifications. 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 (Dellinger *et al. J. Am. Chem. Soc.* 2003,125:940-950).

[000103] 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 as 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.

[000104] 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 a 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.*, International Patent Application PCT/US2017/048239 and U.S. Prov. Appl. No. 62/378,635, entitled Compositions Comprising Reversibly Modified Oligonucleotides and Uses Thereof, which was filed on August 23, 2016, the contents of which are incorporated by reference herein for its relevant disclosures.

v. Targeting Ligands

[000105] In some embodiments, it may be desirable to target the oligonucleotides of the disclosure to one or more cells or one or more organs. Such a strategy may help to avoid undesirable effects in other organs, or may avoid undue loss of the oligonucleotide to cells, tissue or organs that would not benefit for the oligonucleotide. Accordingly, in some embodiments, oligonucleotides disclosed herein may be modified to facilitate targeting of a particular tissue, cell or organ , *e.g.*, to facilitate delivery of the oligonucleotide to the liver. In certain embodiments, oligonucleotides disclosed herein may be modified to facilitate delivery of the oligonucleotide to the hepatocytes of the liver. In some embodiments, an

oligonucleotide comprises a nucleotide that is conjugated to one or more targeting ligands.

[000106] A targeting ligand may comprise 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, a 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, transferrin, 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.

[000107] 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.*, 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, as described, for example, in International Patent Application Publication WO 2016/100401, which was published on June 23, 2016, the relevant contents of which are incorporated herein by reference.

[000108] In some embodiments, it is desirable to target an oligonucleotide that reduces the expression of ALDH2 to the hepatocytes of the liver of a subject. Any suitable hepatocyte targeting moiety may be used for this purpose.

[000109] GalNAc is a high affinity ligand for asialoglycoprotein receptor (ASGPR), which is primarily expressed on the sinusoidal surface of hepatocyte cells and has a major role in binding, internalization, and subsequent clearance of circulating glycoproteins that contain terminal galactose or N-acetylgalactosamine residues (asialoglycoproteins). Conjugation (either indirect or direct) of GalNAc moieties to oligonucleotides of the instant disclosure may be used to target these oligonucleotides to the ASGPR expressed on these hepatocyte cells.

[000110] 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 of the instant

disclosure is conjugated to one or more bivalent GalNAc, trivalent GalNAc, or tetravalent GalNAc moieties.

[000111] 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 the loop (L) of the stem-loop 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. 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 GalNAc moiety. In some embodiments, GalNAc moieties are conjugated to a nucleotide of the sense strand. For example, four GalNAc moieties can be conjugated to nucleotides in the tetraloop of the sense strand, where each GalNAc moiety is conjugated to one nucleotide.

[000112] 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 International Patent Application Publication Number WO2016100401 A1, which published on June 23, 2016, and the contents of which relating to such linkers are incorporated herein by reference. In some embodiments, the linker is a labile linker. However, in other embodiments, the linker is fairly stable. In some embodiments, a duplex extension (up to 3, 4, 5, or 6 base pairs in length) is provided between a targeting ligand (e.g., a GalNAc moiety) and a double-stranded oligonucleotide.

III. Formulations

[000113] 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, provided herein are compositions comprising oligonucleotides (*e.g.*, single-stranded or double-stranded oligonucleotides) to reduce the expression of ALDH2. Such compositions can be suitably formulated such that when administered to a subject, either into the immediate environment of a target cell or systemically, a sufficient portion of the oligonucleotides enter the cell to reduce

ALDH2 expression. Any of a variety of suitable oligonucleotide formulations can be used to deliver oligonucleotides for the reduction of ALDH2 as disclosed herein. In some embodiments, an oligonucleotide is formulated in buffer solutions such as phosphate-buffered saline solutions, liposomes, micellar structures, and capsids. In some embodiments, naked oligonucleotides or conjugates thereof are formulated in water or in an aqueous solution (e.g., water with pH adjustments). In some embodiments, naked oligonucleotides or conjugates thereof are formulated in basic buffered aqueous solutions (e.g., PBS)

[000114] 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.

[000115] 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).

[000116] In some embodiments, formulations as disclosed 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 polyvinyl pyrolidone), or a collapse temperature modifier (*e.g.*, dextran, ficoll, or gelatin).

[000117] 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, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Typically. the route of

administration is intravenous or subcutaneous.

[000118] 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 dispersions. For intravenous or subcutaneous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL.TM. (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, 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, and 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.

[000119] In some embodiments, a composition may contain at least about 0.1% of the therapeutic agent (*e.g.*, an oligonucleotide for reducing ALDH2 expression) or more, although the percentage of the active ingredient(s) may be between about 1% and 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.

[000120] Even though a number of embodiments are directed to liver-targeted delivery of any of the oligonucleotides disclosed herein, targeting of other tissues is also contemplated.

IV. Methods of Use

i. Reducing ALDH2 Expression in Cells

[000121] In some embodiments, methods are provided for delivering to a cell an effective amount any one of oligonucleotides disclosed herein for purposes of reducing expression of ALDH2 in the cell. Methods provided herein are useful in any appropriate cell type. In some embodiments, a cell is any cell that expresses ALDH2 (*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 that has been obtained from a subject and that may have undergone a limited number of a 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). In specific embodiments, methods are provided for delivering to a cell an effective amount any one of the oligonucleotides disclosed herein for purposes of reducing expression of ALDH2 solely in hepatocytes.

[000122] In some embodiments, oligonucleotides disclosed herein can be introduced using appropriate nucleic acid delivery methods including injection of a solution containing the oligonucleotides, bombardment by particles covered by the oligonucleotides, exposing the cell or organism 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.

[000123] The consequences of inhibition can be confirmed by an appropriate assay to evaluate one or more properties of a cell or subject, or by biochemical techniques that evaluate molecules indicative of ALDH2 expression (*e.g.*, RNA, protein). In some embodiments, the extent to which an oligonucleotide provided herein reduces levels of expression of ALDH2 is evaluated by comparing expression levels (*e.g.*, mRNA or protein levels of ALDH2 to an appropriate control (*e.g.*, a level of ALDH2 expression in a cell or population of cells to which an oligonucleotide has not been delivered or to which a negative control has been delivered). In some embodiments, an appropriate control level of ALDH2 expression 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.

[000124] In some embodiments, administration of an oligonucleotide as described herein results in a reduction in the level of ALDH2 expression in a cell. In some embodiments, the reduction in levels of ALDH2 expression may be a reduction to 1% or lower, 5% or lower, 10% or lower, 15% or lower, 20% or lower, 25% or lower, 30% or lower, 35% or lower, 40% or lower, 45% or lower, 50% or lower, 55% or lower, 60% or lower, 70% or lower, 80% or lower, or 90% or lower compared with an appropriate control level of ALDH2. The appropriate control level may be a level of ALDH2 expression in a cell or population of cells that has not been contacted with an oligonucleotide as described herein. In some embodiments, the effect of delivery of an oligonucleotide to a cell according to a method disclosed herein is assessed after a finite period of time. For example, levels of ALDH2 may be analyzed in a cell at least 8 hours, 12 hours, 18 hours, 24 hours; or at least one, two, three,

four, five, six, seven, or fourteen days after introduction of the oligonucleotide into the cell.

[000125] In some embodiments, an oligonucleotide is delivered in the form of a transgene that is engineered to express in a cell the oligonucleotides (*e.g.*, its sense and antisense strands). In some embodiments, an oligonucleotide is delivered using a transgene that is 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.

ii. Treatment Methods

[000126] Aspects of the disclosure relate to methods for reducing ALDH2 expression for the treatment of alcoholism in a subject. In some embodiments, the methods may comprise administering to a subject in need thereof an effective amount of any one of the oligonucleotides disclosed herein. Such treatments could be used, for example, to decrease ethanol tolerance in a subject, thereby inhibiting ethanol intake by the subject (*e.g.*, by decreasing the desire of the subject to consume ethanol). The present disclosure provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) alcoholism and/or a disease or disorder associated with alcoholism.

[000127] In certain aspects, the disclosure provides a method for preventing in a subject, a disease or disorder as described herein by administering to the subject a therapeutic agent (*e.g.*, an oligonucleotide or vector or transgene encoding same). In some embodiments, the subject to be treated is a subject who will benefit therapeutically from a reduction in the amount of ALDH2 protein, *e.g.*, in the liver.

[000128] Methods described herein typically involve administering to a subject 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 is capable of treating 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.

[000129] In some embodiments, a subject is administered any one of the compositions disclosed 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, intramuscular injection,), 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 disclosed herein are administered intravenously or subcutaneously.

[000130] In some embodiments, oligonucleotides are administered at a dose in a range of 0.1 mg/kg to 25 mg/kg (e.g., 1 mg/kg to 5mg/kg). In some embodiments, oligonucleotides are administered at a dose in a range of 0.1 mg/kg to 5 mg/kg or in a range of 0.5 mg/kg to 5 mg/kg.

[000131] As a non-limiting set of examples, the oligonucleotides of the instant disclosure would typically be administered once per year, twice per year, quarterly (once every three months), bi-monthly (once every two months), monthly, or weekly.

[000132] 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.

EXAMPLES

Example 1: Development of ALDH2 oligonucleotide inhibitors using human and mouse cell-based assays

[000133] FIG. 1 shows a workflow using human and mouse-based assays to develop candidate oligonucleotides for inhibition of ALDH2 expression. First, a computer-based algorithm was used to generate candidate oligonucleotide sequences (25-27-mer) for ALDH2 inhibition. Cell-based assays and PCR assays were then employed for evaluation of candidate oligonucleotides for their ability to reduce ALDH2 expression.

[000134] The computer-based algorithm provided oligonucleotides that were complementary to the human ALDH2 mRNA (SEQ ID NO: 608, Table 1), of which certain sequences were also complementary to the cynomolgus monkey ALDH2 mRNA (SEQ ID NO:609, Table 1) and/or the mouse ALDH2 mRNA (SEQ ID NO: 610, Table 1).

Table 1. Sequences of human, cynomolgus monkey and mouse ALDH2 mRNA

Species	**GenBank RefSeq #**	**SEQ ID NO.**
Human	NM_000690.3	608
Cynomolgus Monkey	XM_005572278.2	609
Mouse	NM_009656.4	610

[000135] Of the oligonucleotides that the algorithm provided, 288 oligonucleotides were selected as candidates for experimental evaluation in a HepG2 cell-based assay. In this assay, HepG2, human hepatoma cells expressing ALDH2 were transfected with the oligonucleotides. Cells were maintained for a period of time following transfection and then levels of remaining ALDH2 mRNA were interrogated using TAQMAN®-based qPCR assays. Two qPCR assays, a 3′ assay and a 5′ assay, were used to determine mRNA levels as measured by HEX and FAM probes, respectively. The results of the HepG2 cell-based assay with the 288 oligonucleotides are shown in FIG. 2. The percent mRNA remaining is shown for each of the 3′ assay (circle shapes) and the 5′ assay (diamond shapes). Oligonucleotides resulting in less than or equal to 25% mRNA remaining compared to negative controls were considered hits. Oligonucleotides with low complementarity to the human genome were used as negative controls.

[000136] Based on the activity and locations of these oligonucleotides, hotspots on the human ALDH2 mRNA were defined. A hotspot was identified as a stretch on the human ALDH2 mRNA sequence associated with at least one oligonucleotide resulting in mRNA levels that were less than or equal to 25% in either assay compared with controls. Accordingly, the following hotspots within the human ALDH2 mRNA sequence were identified: 181-273; 445-539; 646-696; 691-749; 1165-1235; 1770-1821; and 1824-1916.

[000137] The sequences of the hotspots are outlined in Table 2.

Table 2. Sequences of Hotspots

Hotspot Position In Human ALDH2 mRNA	**Sequence**	**SEQ ID NO.**
181-273	AACCAGCAGCCCGAGGTCTTCTGCAACCAGATTTTCAT AAACAATGAATGGCACGATGCCGTCAGCAGGAAAACAT TCCCCACCGTCAATCCG	601
445-539	ACCTACCTGGCGGCCTTGGAGACCCTGGACAATGGCAA GCCCTATGTCATCTCCTACCTGGTGGATTTGGACATGGT CCTCAAATGTCTCCGGTATTATGC	602
646-696	CCGTGGAATTTCCCGCTCCTGATGCAAGCATGGAAGCT GGGCCCAGCCTTG	603
691-749	GCCTTGGCAACTGGAAACGTGGTTGTGATGAAGGTAGC TGAGCAGACACCCCTCACCGC	604
1165-1235	GAGCAGGGGCCGCAGGTGGATGAAACTCAGTTTAAGAA GATCCTCGGCTACATCAACACGGGGAAGCAAGA	605

1770-1821	TCTCTTGGGTCAAGAAAGTTCTAGAATTTGAATTGATAA ACATGGTGGGTTG	606
1824-1916	TGAGGGTAAGAGTATATGAGGAACCTTTTAAACGACAA CAATACTGCTAGCTTTCAGGATGATTTTTAAAAAATAGA TTCAAATGTGTTATCC	607

Dose Response Analysis

[000138] Of the 288 oligonucleotides evaluated in the initial HepG2 cell-based assay, 96 particularly active oligonucleotides were selected as hits based on their ability to knock down ALDH2 levels and were subjected to a secondary screen.

[000139] In this secondary screen, the candidate oligonucleotides were tested using the same assay as in the primary screen, but at three different concentrations (1 nM, 0.1 nM and 0.01 nM) (FIGs. 3A-3D). The target mRNA levels were normalized based on splicing factor, arginine/serine-rich 9 (SFRS9), a housekeeping gene that provides a stable expression reference across samples, to generate the percent mRNA shown in FIGs. 3A-3D. The tested oligonucleotides in each of FIGs. 3A-3D are shown compared to negative control sequences (NC1, NC5, NC7, BCAT NC) and mock transfection. All 96 oligonucleotides had the same modification pattern, designated M1, which contains a combination of ribonucleotides, deoxyribonucleotides and 2′-O-methyl modified nucleotides. The sequences of the 96 oligonucleotides tested are provided in Table 3 (data for SEQ ID NOs. 15-16 and 305-306 not shown in FIGs. 3A-3D).

Table 3. Candidate oligonucleotide Sequences for HepG2 Cell-Based Assay

Hs	Cm	Mm	Sense SEQ ID NO.	Corresponding Antisense SEQ ID NO.
X	X	X	20, 27-30, 36, 37, 39-40,78-86, 88-89, 93-94, 96, 98-100, 102	310, 317-320, 326, 327, 329-330, 368-376, 378-379, 383-384, 386, 388-390, 392
X	X		45-46, 50-51, 115, 117, 119, 122-124, 161, 178, 181, 187, 189, 204-205, 208-209, 237, 239-240, 290	335-336, 340-341, 405, 407, 409, 412-414, 451, 468, 471, 477, 479, 494-495, 498-499, 527, 529-530, 580
X		X	1-2, 22-23	291-292, 312-313
X			3-4, 8, 10-14, 129, 140, 144, 162, 192, 194, 218-219, 222, 225, 227, 229-230, 233-234, 243, 245-246, 249-251, 253-254, 256-257, 259, 267-269, 274-275, 278-279, 281	293-294, 298, 300-304, 419, 430, 434, 452, 482, 484, 508-509, 512, 515, 517, 519-520, 523-524, 533, 535-536, 539-541, 543-544, 546-547, 549, 557-559, 564-565, 568-569,

				571

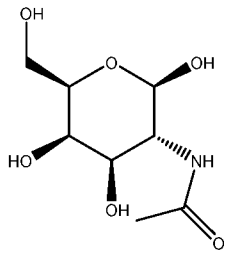
Hs: human, Cm: cynomolgus monkey, and Mm: mouse; the sense and antisense SEQ ID NO. columns provide the sense strand and respective antisense strand, in relative order, that are hybridized to make each oligonucleotide. For example, sense strand of SEQ ID NO: 1 hybridizes with antisense strand of SEQ ID NO: 291, and the sense strand of SEQ ID NO: 2 hybridizes with the antisense strand of SEQ ID NO: 293; each of the oligonucleotides tested had the same modification pattern.

[000140] At this stage, eight best performing oligonucleotides from the testing were selected for further testing. The selected oligonucleotides were converted to nicked tetraloop structure formats (a 36-mer passenger strand with a 22-mer guide strand). See FIG. 4 for a generic tetraloop structure. These oligonucleotides were then tested as before, evaluating each oligonucleotide at three concentrations for its ability to reduce ALDH2 mRNA expression in HepG2 cells. FIGs. 5A-5B show data for oligonucleotides made from different base sequences with nicked tetraloop structures, each adapted to six different modification patterns. The target mRNA levels were normalized as described above to generate the percent mRNA shown in FIGs. 5A-5B, and the tested oligonucleotides in each of FIGs. 5A-5B are shown compared to negative control sequences (BCAT, C121, NC1, NC7) and mock transfection. Data for SEQ ID NOs: 581-582 and SEQ ID NOs: 591-592 are not shown in FIGs. 5A-5B.

[000141] Certain tetraloop-modified oligonucleotides were further tested in Hepa1-6 cells using the same modification patterns for each compound (FIG. 6). The target mRNA levels were normalized based on hypoxanthine ribosyltransferase (HPRT), a housekeeping gene that provides a stable expression reference across samples. The tested oligonucleotides in FIG. 6 are shown compared to negative control sequences (BCAT NC, NC1, NC5, NC7) and mock transfection.

In vivo murine experimentation

[000142] Data from the above *in vitro* experiments were assessed to identify tetraloops and modification patterns that would improve delivery properties while maintaining activity for reduction of ALDH2 expression in the mouse hepatocytes. Based on this analysis, select oligonucleotides were then conjugated to GalNAc moieties. Four GalNAc moieties were conjugated to nucleotides in the tetraloop of the sense strand. Conjugation was performed using a click linker. The GalNAc used was as shown below:



N-Acetyl-b-D-galactosamine (CAS#: 14131-60-3)

[000143] A total of six highly potent GalNAc-conjugated ALDH2 oligonucleotides from three different base sequences and having different modification patterns with nicked tetraloop structures were subcutaneously administered to CD-1 mice at 3 mg/kg. Mice were euthanized on day 4 following administration. Liver samples were obtained and RNA was extracted to evaluate ALDH2 mRNA levels by RT-qPCR. The percent ALDH2 mRNA as compared to PBS control mRNA was determined based on these measurements and is shown in FIG. 7.

Example 2: Duration study of GalNAc-conjugated ALDH2 oligonucleotides in non-human primates (NHP)

[000144] This study was designed to evaluate pharmacodynamics of a single dose of GalNAc-conjugated ALDH2 oligonucleotides with different modification patterns (e.g., modification patterns that have different numbers of 2′-fluoro modifications and/or different numbers of phosphorothioate linkages in the anti-sense strand). The GalNAc-conjugated ALDH2 oligonucleotides tested in this study were: S585-AS595-M14, S585-AS595-M15, S585-AS595-M16, S585-AS595-M17, S587-AS597-M23, and S587-AS597-M24. A single dose of the GalNAc-conjugated ALDH2 oligonucleotides were subcutaneously administered to non-human primates (n=4 for each group) at 3 mg/kg. Animals fasted overnight and serum samples and liver biopsies were collected prior to feeding the next morning. One pre-dose biopsy was collected for each animal during acclimation and three biopsies were collected 4, 8, or 12, or 16 weeks post administration. The biopsies were divided into two sections, one was flash-frozen and stored at -80 °C and the other was processed in RNAlater (ThermoFisher Scientific) and stored at 4 °C for mRNA level analyses.

[000145] The amount of ALDH2 mRNA remaining 4, 8, 12, or 16 weeks following administration, relative to the amount of ALDH2 mRNA prior to administration were analyzed by quantitative PCR (qPCR) and the results showed that four out of the six GalNAc-conjugated ALDH2 oligonucleotides achieved about 50% ALDH2 mRNA suppression and the

effects maintained for three months after a single 3 mg/kg dose (FIG. 8). The results support a proposed dosing frequency of once-per-quarter or less in humans.

[000146] The serum samples were for stored liver function panel test, including Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) Lactate Dehydrogenase (LDH), Gamma Glutamyl Transferase (GGT).

Example 3: Improving the GalNAc-conjugated ALDH2 oligonucleotides using different modification patterns

[000147] The study was designed to evaluate the effect of different modification patterns on the activity of GalNAc-conjugated ALDH2 oligonucleotides in reducing ALDH2 mRNA level. As shown in FIG. 9, two GalNAc-conjugated ALDH2 oligonucleotides (S585-AS595 and S587-AS597) in an array of different modification patterns (M14-M40) were screened in an in vivo assay in mice for their activities in reducing ALDH2 mRNA level. Five GalNAc-conjugated ALDH2 oligonucleotides showed higher activities of reducing ALDH2 mRNA level (S585-AS595-M23, S585-AS595-M24, S585-AS595-M16, S585-AS595-M17, and S587-AS597-M23).

[000148] Several of the GalNAc-conjugated ALDH2 oligonucleotides tested in FIG. 9 were also tested for their in vivo activity in reducing ALDH2 mRNA level in mice. A single dose of the GalNAc-conjugated ALDH2 oligonucleotides were subcutaneously administered to mice at 0.5 mg/kg and the levels of ALDH2 mRNA in mice liver were evaluated by qPCR 4 days post administration. The results shows that modification patterns M22, M15, M24, M17, M26, M30, and M32 boosted the potency of the oligonucleotides tested compared to modification patterns M21, M14, M23, M16, M25, M29, and M31 (FIG. 10).

[000149] Next, the GalNAc-conjugated ALDH2 oligonucleotides that showed higher activities in reducing ALDH2 mRNA level in FIGs. 9 and 10 (S585-AS595-M15, S585-AS595-M16, S585-AS595-M17, S585-AS595-M24, S585-AS595-M26, S585-AS595-M31, S585-AS595-M32) were tested in a dose titration study in mice. The GalNAc-conjugated ALDH2 oligonucleotides were subcutaneously administered to mice at 0.1, 0.3, or 0.5 mg/kg and the levels of ALDH2 mRNA in mice liver were evaluated by qPCR 72 hours post administration. A dose-dependent response was observed for all the oligonucleotides tested (FIG. 11).

[000150] The effects of the numbers of phosphorothioate linkages on the activities of two GalNAc-conjugated ALDH2 oligonucleotides (S585-AS595-M33 and S585-AS595-M34) were evaluated. The oligonucleotides were further modified to contain 0, 1, 2, 3, 4, 5, or 6

phosphorothioate linkages at the 5′ end of the antisense strand and were subcutaneously administered to mice at 0.5 mg/kg. Levels of remaining ALDH2 mRNA in mice liver were evaluated by qPCR 4 days post administration. The results showed that different numbers of phosphorothioate linkages had different impact on the potency of the GalNAc-conjugated ALDH2 oligonucleotides (FIG. 12).

[000151] Finally, the GalNAc-conjugated ALDH2 oligonucleotide (S585-AS595) with different modification patterns (M15, M16, M17, M24 and M26) were tested in a duration study. The GalNAc-conjugated ALDH2 oligonucleotides were subcutaneously administered to mice at 3 mg/kg and the levels of ALDH2 mRNA in mice liver were evaluated by qPCR 72 hours post administration. The results showed that the ALDHN2 mRNA suppression activities of the GalNAc-conjugated ALDH2 oligonucleotides tested lasted for at least 35 days (FIG. 13).

Materials and Methods

Transfection

[000152] For the first screen, Lipofectamine RNAiMAX™ was used to complex the oligonucleotides for efficient transfection. Oligonucleotides, RNAiMAX and Opti-MEM incubated together at room temperature for 20 minutes and then 50 µL of this mix was added per well to plates prior to transfection. Media was aspirated from a flask of actively passaging cells and the cells were incubated at 37 °C in the presence of trypsin for 3-5 minutes. After cells no longer adhered to the flask, cell growth media (lacking penicillin and streptomycin) was added to neutralize the trypsin and to suspend the cells. A 10 µL aliquot was removed and counted with a hemocytometer to quantify the cells on a per milliliter basis. For HeLa cells, 25,000 cells were seeded per well in 100 µL of media. A diluted cell suspension was added to the 96-well transfection plates, which already contained the oligonucleotides in Opti-MEM. The transfection plates were then incubated for 24 hours at 37 °C. After 24 hours of incubation, media was aspirated from each well. Cells were lysed using the lysis buffer from the Promega RNA Isolation kit. The lysis buffer was added to each well. The lysed cells were then transferred to the Corbett XtractorGENE (QIAxtractor) for RNA isolation or stored at -80 °C.

[000153] For subsequent screens and experiments, *e.g.*, the secondary screen, Lipofectamine RNAiMAx was used to complex the oligonucleotides for reverse transfection. The complexes were made by mixing RNAiMAX and siRNAs in OptiMEM medium for 15 minutes. The transfection mixture was transferred to multi-well plates and cell suspension was

added to the wells. After 24 hours incubation the cells were washed once with PBS and then lysed using lysis buffer from the Promega SV96 kit. The RNA was purified using the SV96 plates in a vacuum manifold. Four microliters of the purified RNA was then heated at 65 °C for 5 minutes and cooled to 4 °C. The RNA was then used for reverse transcription using the High Capacity Reverse Transcription kit (Life Technologies) in a 10 microliter reaction. The cDNA was then diluted to 50 µL with nuclease free water and used for quantitative PCR with multiplexed 5′-endonuclease assays and SSoFast qPCR mastermix (Bio-Rad laboratories).

cDNA Synthesis

[000154] RNA was isolated from mammalian cells in tissue culture using the Corbett X-tractor Gene™ (QIAxtractor). A modified SuperScript II protocol was used to synthesize cDNA from the isolated RNA. Isolated RNA (approximately 5 ng/µL) was heated to 65 °C for five minutes and incubated with dNPs, random hexamers, oligo dTs, and water. The mixture was cooled for 15 seconds. An "enzyme mix," consisting of water, 5X first strand buffer, DTT, SUPERase•In™ (an RNA inhibitor), and SuperScript II RTase was added to the mixture. The contents were heated to 42 °C for one hour, then to 70 °C for 15 minutes, and then cooled to 4 °C using a thermocycler. The resulting cDNA was then subjected to SYBR®-based qPCR. The qPCR reactions were multiplexed, containing two 5′ endonuclease assays per reaction.

qPCR Assays

[000155] Primer sets were initially screened using SYBR®-based qPCR. Assay specificity was verified by assessing melt curves as well as "minus RT" controls. Dilutions of cDNA template (10-fold serial dilutions from 20 ng and to 0.02 ng per reaction) from HeLa and Hepa1-6 cells are used to test human (Hs) and mouse (Mm) assays, respectively. qPCR assays were set up in 384-well plates, covered with MicroAmp film, and run on the 7900HT from Applied Biosystems. Reagent concentrations and cycling conditions included the following: 2X SYBR mix, 10 µM forward primer, 10 µM reverse primer, DD H_2O, and cDNA template up to a total volume of 10 µL.

Cloning

[000156] PCR amplicons that displayed a single melt-curve were ligated into the pGEM®-T Easy vector kit from Promega according to the manufacturer's instructions. Following the manufacturer's protocol, JM109 High Efficiency cells were transformed with

the newly ligated vectors. The cells were then plated on LB plates containing ampicillin and incubated at 37 °C overnight for colony growth.

PCR Screening and Plasmid Mini-Prep

[000157] PCR was used to identify colonies of *E. coli* that had been transformed with a vector containing the ligated amplicon of interest. Vector-specific primers that flank the insert were used in the PCR reaction. All PCR products were then run on a 1% agarose gel and imaged by a transilluminator following staining. Gels were assessed qualitatively to determine which plasmids appeared to contain a ligated amplicon of the expected size (approximately 300 bp, including the amplicon and the flanking vector sequences specific to the primers used).

[000158] The colonies that were confirmed transformants by PCR screening were then incubated overnight in cultures consisting of 2 mL LB broth with ampicillin at 37 °C with shaking. *E. coli* cells were then lysed, and the plasmids of interest were isolated using Promega's Mini-Prep kit. Plasmid concentration was determined by UV absorbance at 260 nm.

Plasmid Sequencing and Quantification

[000159] Purified plasmids were sequenced using the BigDye® Terminator sequencing kit. The vector-specific primer, T7, was used to give read lengths that span the insert. The following reagents were used in the sequencing reactions: water, 5X sequencing buffer, BigDye terminator mix, T7 primer, and plasmid (100 ng/μL) to a volume of 10 μL. The mixture was held at 96 °C for one minute, then subjected to 15 cycles of 96 °C for 10 seconds, 50 °C for 5 seconds, 60 °C for 1 minute, 15 seconds; 5 cycles of 96 °C for 10 seconds, 50 °C for 5 seconds, 60 °C for 1 minute, 30 seconds; and 5 cycles of 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 2 minutes. Dye termination reactions were then sequenced using Applied Biosystems' capillary electrophoresis sequencers.

[000160] Sequence-verified plasmids were then quantified. They were linearized using a single cutting restriction endonuclease. Linearity was confirmed using agarose gel electrophoresis. All plasmid dilutions were made in TE buffer (pH 7.5) with 100 μg of tRNA per mL buffer to reduce non-specific binding of plasmid to the polypropylene vials.

[000161] The linearized plasmids were then serially diluted from 1,000,000 to 01 copies per μL and subjected to qPCR. Assay efficiency was calculated and the assays were deemed acceptable if the efficiency was in the range of 90-110%.

Multi-Plexing Assays

[000162] For each target, mRNA levels were quantified by two 5' nuclease assays. In general, several assays are screened for each target. The two assays selected displayed a combination of good efficiency, low limit of detection, and broad 5'→3' coverage of the gene of interest (GOI). Both assays against one GOI could be combined in one reaction when different fluorophores were used on the respective probes. Thus, the final step in assay validation was to determine the efficiency of the selected assays when they were combined in the same qPCR or "multi-plexed."

[000163] Linearized plasmids for both assays in 10-fold dilutions were combined and qPCR was performed. The efficiency of each assay was determined as described above. The accepted efficiency rate was 90-110%.

[000164] While validating multi-plexed reactions using linearized plasmid standards, C_q values for the target of interest were also assessed using cDNA as the template. For human or mouse targets, HeLa and Hepa1-6 cDNA were used, respectively. The cDNA, in this case, was derived from RNA isolated on the Corbett (~5 ng/µl in water) from untransfected cells. In this way, the observed C_q values from this sample cDNA were representative of the expected C_q values from a 96-well plate transfection. In cases where C_q values were greater than 30, other cell lines were sought that exhibit higher expression levels of the gene of interest. A library of total RNA isolated from via high-throughput methods on the Corbett from each human and mouse line was generated and used to screen for acceptable levels of target expression.

Description of oligonucleotide nomenclature

[000165] All oligonucleotides described herein are designated either SN_1-ASN_2-MN_3. The following designations apply:

- N_1: sequence identifier number of the sense strand sequence
- N_2: sequence identifier number of the antisense strand sequence
- N_3: reference number of modification pattern, in which each number represents a pattern of modified nucleotides in the oligonucleotide.

For example, S27-AS317-M1 represents an oligonucleotide with a sense sequence that is set forth by SEQ ID NO: 27, an antisense sequence that is set forth by SEQ ID NO: 317, and which is adapted to a modification pattern identified as M1.

Table 4. ALDH2 RNAi Oligonucleotide Sequences

App Name	Sense Sequence/mRNA seq	S SEQ ID NO	Antisense Sequence	AS SEQ ID NO
S1-AS291-M1	GAGGUCUUCUGCAACCAGAUUUUCA	1	UGAAAAUCUGGUUGCAGAAGACCUCGG	291
S2-AS292-M1	AGGUCUUCUGCAACCAGAUUUUCAT	2	AUGAAAAUCUGGUUGCAGAAGACCUCG	292
S3-AS293-M1	GUCUUCUGCAACCAGAUUUUCAUAA	3	UUAUGAAAAUCUGGUUGCAGAAGACCU	293
S4-AS294-M1	CUUCUGCAACCAGAUUUUCAUAAAC	4	GUUUAUGAAAAUCUGGUUGCAGAAGAC	294
S5-AS295-M1	UUCUGCAACCAGAUUUUCAUAAACA	5	UGUUUAUGAAAAUCUGGUUGCAGAAGA	295
S6-AS296-M1	UCUGCAACCAGAUUUUCAUAAACAA	6	UUGUUUAUGAAAAUCUGGUUGCAGAAG	296
S7-AS297-M1	CUGCAACCAGAUUUUCAUAAACAAT	7	AUUGUUUAUGAAAAUCUGGUUGCAGAA	297
S8-AS298-M1	UGCAACCAGAUUUUCAUAAACAATG	8	CAUUGUUUAUGAAAAUCUGGUUGCAGA	298
S9-AS299-M1	GCAACCAGAUUUUCAUAAACAAUGA	9	UCAUUGUUUAUGAAAAUCUGGUUGCAG	299
S10-AS300-M1	CAACCAGAUUUUCAUAAACAAUGAA	10	UUCAUUGUUUAUGAAAAUCUGGUUGCA	300
S11-AS301-M1	AACCAGAUUUUCAUAAACAAUGAAT	11	AUUCAUUGUUUAUGAAAAUCUGGUUGC	301
S12-AS302-M1	ACCAGAUUUUCAUAAACAAUGAATG	12	CAUUCAUUGUUUAUGAAAAUCUGGUUG	302
S13-AS303-M1	CCAGAUUUUCAUAAACAAUGAAUGG	13	CCAUUCAUUGUUUAUGAAAAUCUGGUU	303
S14-AS304-M1	CAGAUUUUCAUAAACAAUGAAUGGC	14	GCCAUUCAUUGUUUAUGAAAAUCUGGU	304
S17-AS307-M1	AGAUUUUCAUAAACAAUGAAUGGCA	17	UGCCAUUCAUUGUUUAUGAAAAUCUGG	307
S18-AS308-M1	GAUUUUCAUAAACAAUGAAUGGCAC	18	GUGCCAUUCAUUGUUUAUGAAAAUCUG	308
S19-AS309-M1	GCCGUCAGCAGGAAAACAUUCCCCA	19	UGGGGAAUGUUUUCCUGCUGACGGCAU	309
S20-AS310-M1	CCGUCAGCAGGAAAACAUUCCCCAC	20	GUGGGGAAUGUUUUCCUGCUGACGGCA	310
S21-AS311-M1	GGCCUUGGAGACCCUGGACAAUGGC	21	GCCAUUGUCCAGGGUCUCCAAGGCCGC	311
S22-AS312-M1	GCCUUGGAGACCCUGGACAAUGGCA	22	UGCCAUUGUCCAGGGUCUCCAAGGCCG	312
S23-AS313-M1	CCUUGGAGACCCUGGACAAUGGCAA	23	UUGCCAUUGUCCAGGGUCUCCAAGGCC	313
S24-AS314-M1	UACCUGGUGGAUUUGGACAUGGUCC	24	GGACCAUGUCCAAAUCCACCAGGUAGG	314
S25-AS315-M1	ACCUGGUGGAUUUGGACAUGGUCCT	25	AGGACCAUGUCCAAAUCCACCAGGUAG	315
S26-AS316-M1	CCUGGUGGAUUUGGACAUGGUCCTC	26	GAGGACCAUGUCCAAAUCCACCAGGUA	316
S27-AS317-M1	CUGGUGGAUUUGGACAUGGUCCUCA	27	UGAGGACCAUGUCCAAAUCCACCAGGU	317
S28-AS318-M1	UGGUGGAUUUGGACAUGGUCCUCAA	28	UUGAGGACCAUGUCCAAAUCCACCAGG	318
S29-AS319-M1	GGUGGAUUUGGACAUGGUCCUCAAA	29	UUUGAGGACCAUGUCCAAAUCCACCAG	319
S30-AS320-M1	GUGGAUUUGGACAUGGUCCUCAAAT	30	AUUUGAGGACCAUGUCCAAAUCCACCA	320
S31-AS321-M1	UGGAUUUGGACAUGGUCCUCAAATG	31	CAUUUGAGGACCAUGUCCAAAUCCACC	321

S32-AS322-M1	GAUUUGGACAUGGUCCUCAAAUGTC	32	GACAUUUGAGGACCAUGUCCAAAUCCA	322
S33-AS323-M1	UUCCCGCUCCUGAUGCAAGCAUGGA	33	UCCAUGCUUGCAUCAGGAGCGGGAAAU	323
S34-AS324-M1	UCCCGCUCCUGAUGCAAGCAUGGAA	34	UUCCAUGCUUGCAUCAGGAGCGGGAAA	324
S35-AS325-M1	CCCGCUCCUGAUGCAAGCAUGGAAG	35	CUUCCAUGCUUGCAUCAGGAGCGGGAA	325
S36-AS326-M1	CCGCUCCUGAUGCAAGCAUGGAAGC	36	GCUUCCAUGCUUGCAUCAGGAGCGGGA	326
S37-AS327-M1	CGCUCCUGAUGCAAGCAUGGAAGCT	37	AGCUUCCAUGCUUGCAUCAGGAGCGGG	327
S38-AS328-M1	GCUCCUGAUGCAAGCAUGGAAGCTG	38	CAGCUUCCAUGCUUGCAUCAGGAGCGG	328
S39-AS329-M1	CUCCUGAUGCAAGCAUGGAAGCUGG	39	CCAGCUUCCAUGCUUGCAUCAGGAGCG	329
S40-AS330-M1	UCCUGAUGCAAGCAUGGAAGCUGGG	40	CCCAGCUUCCAUGCUUGCAUCAGGAGC	330
S41-AS331-M1	AACUGGAAACGUGGUUGUGAUGAAG	41	CUUCAUCACAACCACGUUUCCAGUUGC	331
S42-AS332-M1	ACUGGAAACGUGGUUGUGAUGAAGG	42	CCUUCAUCACAACCACGUUUCCAGUUG	332
S43-AS333-M1	CUGGAAACGUGGUUGUGAUGAAGGT	43	ACCUUCAUCACAACCACGUUUCCAGUU	333
S44-AS334-M1	UGGAAACGUGGUUGUGAUGAAGGTA	44	UACCUUCAUCACAACCACGUUUCCAGU	334
S45-AS335-M1	GGAAACGUGGUUGUGAUGAAGGUAG	45	CUACCUUCAUCACAACCACGUUUCCAG	335
S46-AS336-M1	GAAACGUGGUUGUGAUGAAGGUAGC	46	GCUACCUUCAUCACAACCACGUUUCCA	336
S47-AS337-M1	AACGUGGUUGUGAUGAAGGUAGCTG	47	CAGCUACCUUCAUCACAACCACGUUUC	337
S48-AS338-M1	ACGUGGUUGUGAUGAAGGUAGCUGA	48	UCAGCUACCUUCAUCACAACCACGUUU	338
S49-AS339-M1	CGUGGUUGUGAUGAAGGUAGCUGAG	49	CUCAGCUACCUUCAUCACAACCACGUU	339
S50-AS340-M1	GUUGUGAUGAAGGUAGCUGAGCAGA	50	UCUGCUCAGCUACCUUCAUCACAACCA	340
S51-AS341-M1	GUGAUGAAGGUAGCUGAGCAGACAC	51	GUGUCUGCUCAGCUACCUUCAUCACAA	341
S52-AS342-M1	AGGAUGUGGACAAAGUGGCAUUCAC	52	GUGAAUGCCACUUUGUCCACAUCCUCA	342
S53-AS343-M1	GGGAGCAGCAACCUCAAGAGAGUGA	53	UCACUCUCUUGAGGUUGCUGCUCCCAG	343
S54-AS344-M1	GGAGCAGCAACCUCAAGAGAGUGAC	54	GUCACUCUCUUGAGGUUGCUGCUCCCA	344
S55-AS345-M1	GAGCAGCAACCUCAAGAGAGUGACC	55	GGUCACUCUCUUGAGGUUGCUGCUCCC	345
S56-AS346-M1	AGCAGCAACCUCAAGAGAGUGACCT	56	AGGUCACUCUCUUGAGGUUGCUGCUCC	346
S57-AS347-M1	GCAGCAACCUCAAGAGAGUGACCTT	57	AAGGUCACUCUCUUGAGGUUGCUGCUC	347
S58-AS348-M1	GCCCUGUUCUUCAACCAGGGCCAGT	58	ACUGGCCCUGGUUGAAGAACAGGGCGA	348
S59-AS349-M1	CCCUGUUCUUCAACCAGGGCCAGTG	59	CACUGGCCCUGGUUGAAGAACAGGGCG	349
S60-AS350-M1	CCUGUUCUUCAACCAGGGCCAGUGC	60	GCACUGGCCCUGGUUGAAGAACAGGGC	350
S61-AS351-M1	CUGUUCUUCAACCAGGGCCAGUGCT	61	AGCACUGGCCCUGGUUGAAGAACAGGG	351

S62-AS352-M1	UGUUCUUCAACCAGGGCCAGUGCTG	62	CAGCACUGGCCCUGGUUGAAGAACAGG	352
S63-AS353-M1	GUUCUUCAACCAGGGCCAGUGCUGC	63	GCAGCACUGGCCCUGGUUGAAGAACAG	353
S64-AS354-M1	UUCUUCAACCAGGGCCAGUGCUGCT	64	AGCAGCACUGGCCCUGGUUGAAGAACA	354
S65-AS355-M1	CUUCAACCAGGGCCAGUGCUGCUGT	65	ACAGCAGCACUGGCCCUGGUUGAAGAA	355
S66-AS356-M1	UUCAACCAGGGCCAGUGCUGCUGTG	66	CACAGCAGCACUGGCCCUGGUUGAAGA	356
S67-AS357-M1	CAACCAGGGCCAGUGCUGCUGUGCC	67	GGCACAGCAGCACUGGCCCUGGUUGAA	357
S68-AS358-M1	GGCUCCCGGACCUUCGUGCAGGAGG	68	CCUCCUGCACGAAGGUCCGGGAGCCGG	358
S69-AS359-M1	GCUCCCGGACCUUCGUGCAGGAGGA	69	UCCUCCUGCACGAAGGUCCGGGAGCCG	359
S70-AS360-M1	CUCCCGGACCUUCGUGCAGGAGGAC	70	GUCCUCCUGCACGAAGGUCCGGGAGCC	360
S71-AS361-M1	UCCCGGACCUUCGUGCAGGAGGACA	71	UGUCCUCCUGCACGAAGGUCCGGGAGC	361
S72-AS362-M1	CCCGGACCUUCGUGCAGGAGGACAT	72	AUGUCCUCCUGCACGAAGGUCCGGGAG	362
S73-AS363-M1	CCGGACCUUCGUGCAGGAGGACATC	73	GAUGUCCUCCUGCACGAAGGUCCGGGA	363
S74-AS364-M1	GGAGGACAUCUAUGAUGAGUUUGTG	74	CACAAACUCAUCAUAGAUGUCCUCCUG	364
S75-AS365-M1	CGGGCCAAGUCUCGGGUGGUCGGGA	75	UCCCGACCACCCGAGACUUGGCCCGGG	365
S76-AS366-M1	GGGCCAAGUCUCGGGUGGUCGGGAA	76	UUCCCGACCACCCGAGACUUGGCCCGG	366
S77-AS367-M1	GCAGGUGGAUGAAACUCAGUUUAAG	77	CUUAAACUGAGUUUCAUCCACCUGCGG	367
S78-AS368-M1	CAGGUGGAUGAAACUCAGUUUAAGA	78	UCUUAAACUGAGUUUCAUCCACCUGCG	368
S79-AS369-M1	AGGUGGAUGAAACUCAGUUUAAGAA	79	UUCUUAAACUGAGUUUCAUCCACCUGC	369
S80-AS370-M1	GGUGGAUGAAACUCAGUUUAAGAAG	80	CUUCUUAAACUGAGUUUCAUCCACCUG	370
S81-AS371-M1	GUGGAUGAAACUCAGUUUAAGAAGA	81	UCUUCUUAAACUGAGUUUCAUCCACCU	371
S82-AS372-M1	UGGAUGAAACUCAGUUUAAGAAGAT	82	AUCUUCUUAAACUGAGUUUCAUCCACC	372
S83-AS373-M1	GGAUGAAACUCAGUUUAAGAAGATC	83	GAUCUUCUUAAACUGAGUUUCAUCCAC	373
S84-AS374-M1	GAUGAAACUCAGUUUAAGAAGAUCC	84	GGAUCUUCUUAAACUGAGUUUCAUCCA	374
S85-AS375-M1	AUGAAACUCAGUUUAAGAAGAUCCT	85	AGGAUCUUCUUAAACUGAGUUUCAUCC	375
S86-AS376-M1	UGAAACUCAGUUUAAGAAGAUCCTC	86	GAGGAUCUUCUUAAACUGAGUUUCAUC	376
S87-AS377-M1	GAAACUCAGUUUAAGAAGAUCCUCG	87	CGAGGAUCUUCUUAAACUGAGUUUCAU	377
S88-AS378-M1	AAACUCAGUUUAAGAAGAUCCUCGG	88	CCGAGGAUCUUCUUAAACUGAGUUUCA	378
S89-AS379-M1	AACUCAGUUUAAGAAGAUCCUCGGC	89	GCCGAGGAUCUUCUUAAACUGAGUUUC	379
S90-AS380-M1	ACUCAGUUUAAGAAGAUCCUCGGCT	90	AGCCGAGGAUCUUCUUAAACUGAGUUU	380
S91-AS381-M1	CUCAGUUUAAGAAGAUCCUCGGCTA	91	UAGCCGAGGAUCUUCUUAAACUGAGUU	381

S92-AS382-M1	UCAGUUUAAGAAGAUCCUCGGCUAC	92	GUAGCCGAGGAUCUUCUUAAACUGAGU	382
S93-AS383-M1	CAGUUUAAGAAGAUCCUCGGCUACA	93	UGUAGCCGAGGAUCUUCUUAAACUGAG	383
S94-AS384-M1	AGUUUAAGAAGAUCCUCGGCUACAT	94	AUGUAGCCGAGGAUCUUCUUAAACUGA	384
S95-AS385-M1	GUUUAAGAAGAUCCUCGGCUACATC	95	GAUGUAGCCGAGGAUCUUCUUAAACUG	385
S96-AS386-M1	UUUAAGAAGAUCCUCGGCUACAUCA	96	UGAUGUAGCCGAGGAUCUUCUUAAACU	386
S97-AS387-M1	UUAAGAAGAUCCUCGGCUACAUCAA	97	UUGAUGUAGCCGAGGAUCUUCUUAAAC	387
S98-AS388-M1	UAAGAAGAUCCUCGGCUACAUCAAC	98	GUUGAUGUAGCCGAGGAUCUUCUUAAA	388
S99-AS389-M1	AAGAAGAUCCUCGGCUACAUCAACA	99	UGUUGAUGUAGCCGAGGAUCUUCUUAA	389
S100-AS390-M1	AGAAGAUCCUCGGCUACAUCAACAC	100	GUGUUGAUGUAGCCGAGGAUCUUCUUA	390
S101-AS391-M1	GAAGAUCCUCGGCUACAUCAACACG	101	CGUGUUGAUGUAGCCGAGGAUCUUCUU	391
S102-AS392-M1	AAGAUCCUCGGCUACAUCAACACGG	102	CCGUGUUGAUGUAGCCGAGGAUCUUCU	392
S103-AS393-M1	AGAUCCUCGGCUACAUCAACACGGG	103	CCCGUGUUGAUGUAGCCGAGGAUCUUC	393
S104-AS394-M1	UGCUGCUGACCGUGGUUACUUCATC	104	GAUGAAGUAACCACGGUCAGCAGCAAU	394
S105-AS395-M1	GCUGCUGACCGUGGUUACUUCAUCC	105	GGAUGAAGUAACCACGGUCAGCAGCAA	395
S106-AS396-M1	CUGCUGACCGUGGUUACUUCAUCCA	106	UGGAUGAAGUAACCACGGUCAGCAGCA	396
S107-AS397-M1	GCUGACCGUGGUUACUUCAUCCAGC	107	GCUGGAUGAAGUAACCACGGUCAGCAG	397
S108-AS398-M1	CCAGUGAUGCAGAUCCUGAAGUUCA	108	UGAACUUCAGGAUCUGCAUCACUGGCC	398
S109-AS399-M1	AGUGAUGCAGAUCCUGAAGUUCAAG	109	CUUGAACUUCAGGAUCUGCAUCACUGG	399
S110-AS400-M1	GUGAUGCAGAUCCUGAAGUUCAAGA	110	UCUUGAACUUCAGGAUCUGCAUCACUG	400
S111-AS401-M1	UGAUGCAGAUCCUGAAGUUCAAGAC	111	GUCUUGAACUUCAGGAUCUGCAUCACU	401
S112-AS402-M1	GAUGCAGAUCCUGAAGUUCAAGACC	112	GGUCUUGAACUUCAGGAUCUGCAUCAC	402
S113-AS403-M1	AUGCAGAUCCUGAAGUUCAAGACCA	113	UGGUCUUGAACUUCAGGAUCUGCAUCA	403
S114-AS404-M1	GCAGAUCCUGAAGUUCAAGACCATA	114	UAUGGUCUUGAACUUCAGGAUCUGCAU	404
S115-AS405-M1	CAGAUCCUGAAGUUCAAGACCAUAG	115	CUAUGGUCUUGAACUUCAGGAUCUGCA	405
S116-AS406-M1	AGAUCCUGAAGUUCAAGACCAUAGA	116	UCUAUGGUCUUGAACUUCAGGAUCUGC	406
S117-AS407-M1	GAUCCUGAAGUUCAAGACCAUAGAG	117	CUCUAUGGUCUUGAACUUCAGGAUCUG	407
S118-AS408-M1	UCCUGAAGUUCAAGACCAUAGAGGA	118	UCCUCUAUGGUCUUGAACUUCAGGAUC	408
S119-AS409-M1	AAGUUCAAGACCAUAGAGGAGGUTG	119	CAACCUCCUCUAUGGUCUUGAACUUCA	409
S120-AS410-M1	GCUGUCUUCACAAAGGAUUUGGACA	120	UGUCCAAAUCCUUUGUGAAGACAGCUG	410
S121-AS411-M1	GUCUUCACAAAGGAUUUGGACAAGG	121	CCUUGUCCAAAUCCUUUGUGAAGACAG	411

S122-AS412-M1	GCAGGCAUACACUGAAGUGAAAACT	122	AGUUUUCACUUCAGUGUAUGCCUGCAG	412
S123-AS413-M1	CAGGCAUACACUGAAGUGAAAACTG	123	CAGUUUUCACUUCAGUGUAUGCCUGCA	413
S124-AS414-M1	AGGCAUACACUGAAGUGAAAACUGT	124	ACAGUUUUCACUUCAGUGUAUGCCUGC	414
S125-AS415-M1	GGCAUACACUGAAGUGAAAACUGTC	125	GACAGUUUUCACUUCAGUGUAUGCCUG	415
S126-AS416-M1	GCAUACACUGAAGUGAAAACUGUCA	126	UGACAGUUUUCACUUCAGUGUAUGCCU	416
S127-AS417-M1	AUACACUGAAGUGAAAACUGUCACA	127	UGUGACAGUUUUCACUUCAGUGUAUGC	417
S128-AS418-M1	UACACUGAAGUGAAAACUGUCACAG	128	CUGUGACAGUUUUCACUUCAGUGUAUG	418
S129-AS419-M1	CUGAAGUGAAAACUGUCACAGUCAA	129	UUGACUGUGACAGUUUUCACUUCAGUG	419
S130-AS420-M1	GUCAAAGUGCCUCAGAAGAACUCAT	130	AUGAGUUCUUCUGAGGCACUUUGACUG	420
S131-AS421-M1	CAAAGUGCCUCAGAAGAACUCAUAA	131	UUAUGAGUUCUUCUGAGGCACUUUGAC	421
S132-AS422-M1	AAGUGCCUCAGAAGAACUCAUAAGA	132	UCUUAUGAGUUCUUCUGAGGCACUUUG	422
S133-AS423-M1	AGUGCCUCAGAAGAACUCAUAAGAA	133	UUCUUAUGAGUUCUUCUGAGGCACUUU	423
S134-AS424-M1	GUGCCUCAGAAGAACUCAUAAGAAT	134	AUUCUUAUGAGUUCUUCUGAGGCACUU	424
S135-AS425-M1	UGCCUCAGAAGAACUCAUAAGAATC	135	GAUUCUUAUGAGUUCUUCUGAGGCACU	425
S136-AS426-M1	CCUCAGAAGAACUCAUAAGAAUCAT	136	AUGAUUCUUAUGAGUUCUUCUGAGGCA	426
S137-AS427-M1	CUCAGAAGAACUCAUAAGAAUCATG	137	CAUGAUUCUUAUGAGUUCUUCUGAGGC	427
S138-AS428-M1	UCAGAAGAACUCAUAAGAAUCAUGC	138	GCAUGAUUCUUAUGAGUUCUUCUGAGG	428
S139-AS429-M1	CAGAAGAACUCAUAAGAAUCAUGCA	139	UGCAUGAUUCUUAUGAGUUCUUCUGAG	429
S140-AS430-M1	AGAAGAACUCAUAAGAAUCAUGCAA	140	UUGCAUGAUUCUUAUGAGUUCUUCUGA	430
S141-AS431-M1	GAAGAACUCAUAAGAAUCAUGCAAG	141	CUUGCAUGAUUCUUAUGAGUUCUUCUG	431
S142-AS432-M1	AAGAACUCAUAAGAAUCAUGCAAGC	142	GCUUGCAUGAUUCUUAUGAGUUCUUCU	432
S143-AS433-M1	GAACUCAUAAGAAUCAUGCAAGCTT	143	AAGCUUGCAUGAUUCUUAUGAGUUCUU	433
S144-AS434-M1	AACUCAUAAGAAUCAUGCAAGCUTC	144	GAAGCUUGCAUGAUUCUUAUGAGUUCU	434
S145-AS435-M1	CCCUCAGCCAUUGAUGGAAAGUUCA	145	UGAACUUUCCAUCAAUGGCUGAGGGAG	435
S146-AS436-M1	CCUCAGCCAUUGAUGGAAAGUUCAG	146	CUGAACUUUCCAUCAAUGGCUGAGGGA	436
S147-AS437-M1	UCAGCCAUUGAUGGAAAGUUCAGCA	147	UGCUGAACUUUCCAUCAAUGGCUGAGG	437
S148-AS438-M1	CAGCCAUUGAUGGAAAGUUCAGCAA	148	UUGCUGAACUUUCCAUCAAUGGCUGAG	438
S149-AS439-M1	AGCCAUUGAUGGAAAGUUCAGCAAG	149	CUUGCUGAACUUUCCAUCAAUGGCUGA	439
S150-AS440-M1	GCCAUUGAUGGAAAGUUCAGCAAGA	150	UCUUGCUGAACUUUCCAUCAAUGGCUG	440
S151-AS441-M1	CCAUUGAUGGAAAGUUCAGCAAGAT	151	AUCUUGCUGAACUUUCCAUCAAUGGCU	441

S152-AS442-M1	CAUUGAUGGAAAGUUCAGCAAGATC	152	GAUCUUGCUGAACUUUCCAUCAAUGGC	442
S153-AS443-M1	AUUGAUGGAAAGUUCAGCAAGAUCA	153	UGAUCUUGCUGAACUUUCCAUCAAUGG	443
S154-AS444-M1	UUGAUGGAAAGUUCAGCAAGAUCAG	154	CUGAUCUUGCUGAACUUUCCAUCAAUG	444
S155-AS445-M1	UGAUGGAAAGUUCAGCAAGAUCAGC	155	GCUGAUCUUGCUGAACUUUCCAUCAAU	445
S156-AS446-M1	GAUGGAAAGUUCAGCAAGAUCAGCA	156	UGCUGAUCUUGCUGAACUUUCCAUCAA	446
S157-AS447-M1	AUGGAAAGUUCAGCAAGAUCAGCAA	157	UUGCUGAUCUUGCUGAACUUUCCAUCA	447
S158-AS448-M1	UGGAAAGUUCAGCAAGAUCAGCAAC	158	GUUGCUGAUCUUGCUGAACUUUCCAUC	448
S159-AS449-M1	GGAAAGUUCAGCAAGAUCAGCAACA	159	UGUUGCUGAUCUUGCUGAACUUUCCAU	449
S160-AS450-M1	GAAAGUUCAGCAAGAUCAGCAACAA	160	UUGUUGCUGAUCUUGCUGAACUUUCCA	450
S161-AS451-M1	AAAGUUCAGCAAGAUCAGCAACAAA	161	UUUGUUGCUGAUCUUGCUGAACUUUCC	451
S162-AS452-M1	AAGUUCAGCAAGAUCAGCAACAAAA	162	UUUUGUUGCUGAUCUUGCUGAACUUUC	452
S163-AS453-M1	AUCAGCAACAAAACCAAGAAAAATG	163	CAUUUUUCUUGGUUUUGUUGCUGAUCU	453
S164-AS454-M1	CAGCAACAAAACCAAGAAAAAUGAT	164	AUCAUUUUUCUUGGUUUUGUUGCUGAU	454
S165-AS455-M1	AGCAACAAAACCAAGAAAAAUGATC	165	GAUCAUUUUUCUUGGUUUUGUUGCUGA	455
S166-AS456-M1	ACAAAACCAAGAAAAAUGAUCCUTG	166	CAAGGAUCAUUUUUCUUGGUUUUGUUG	456
S167-AS457-M1	CAAAACCAAGAAAAAUGAUCCUUGC	167	GCAAGGAUCAUUUUUCUUGGUUUUGUU	457
S168-AS458-M1	AGAAAAAUGAUCCUUGCGUGCUGAA	168	UUCAGCACGCAAGGAUCAUUUUUCUUG	458
S169-AS459-M1	AAAAAUGAUCCUUGCGUGCUGAATA	169	UAUUCAGCACGCAAGGAUCAUUUUUCU	459
S170-AS460-M1	AAAAUGAUCCUUGCGUGCUGAAUAT	170	AUAUUCAGCACGCAAGGAUCAUUUUUC	460
S171-AS461-M1	AAAUGAUCCUUGCGUGCUGAAUATC	171	GAUAUUCAGCACGCAAGGAUCAUUUUU	461
S172-AS462-M1	AAUGAUCCUUGCGUGCUGAAUAUCT	172	AGAUAUUCAGCACGCAAGGAUCAUUUU	462
S173-AS463-M1	AUGAUCCUUGCGUGCUGAAUAUCTG	173	CAGAUAUUCAGCACGCAAGGAUCAUUU	463
S174-AS464-M1	UGAUCCUUGCGUGCUGAAUAUCUGA	174	UCAGAUAUUCAGCACGCAAGGAUCAUU	464
S175-AS465-M1	GAUCCUUGCGUGCUGAAUAUCUGAA	175	UUCAGAUAUUCAGCACGCAAGGAUCAU	465
S176-AS466-M1	UCCUUGCGUGCUGAAUAUCUGAAAA	176	UUUUCAGAUAUUCAGCACGCAAGGAUC	466
S177-AS467-M1	CCUUGCGUGCUGAAUAUCUGAAAAG	177	CUUUUCAGAUAUUCAGCACGCAAGGAU	467
S178-AS468-M1	CUUGCGUGCUGAAUAUCUGAAAAGA	178	UCUUUUCAGAUAUUCAGCACGCAAGGA	468
S179-AS469-M1	UUGCGUGCUGAAUAUCUGAAAAGAG	179	CUCUUUUCAGAUAUUCAGCACGCAAGG	469
S180-AS470-M1	UGCGUGCUGAAUAUCUGAAAAGAGA	180	UCUCUUUUCAGAUAUUCAGCACGCAAG	470
S181-AS471-M1	GCGUGCUGAAUAUCUGAAAAGAGAA	181	UUCUCUUUUCAGAUAUUCAGCACGCAA	471

S182-AS472-M1	CGUGCUGAAUAUCUGAAAAGAGAAA	182	UUUCUCUUUUCAGAUAUUCAGCACGCA	472
S183-AS473-M1	GUGCUGAAUAUCUGAAAAGAGAAAT	183	AUUUCUCUUUUCAGAUAUUCAGCACGC	473
S184-AS474-M1	UGCUGAAUAUCUGAAAAGAGAAATT	184	AAUUUCUCUUUUCAGAUAUUCAGCACG	474
S185-AS475-M1	GCUGAAUAUCUGAAAAGAGAAAUTT	185	AAAUUUCUCUUUUCAGAUAUUCAGCAC	475
S186-AS476-M1	CUGAAUAUCUGAAAAGAGAAAUUTT	186	AAAAUUUCUCUUUUCAGAUAUUCAGCA	476
S187-AS477-M1	UGAAUAUCUGAAAAGAGAAAUUUTT	187	AAAAAUUUCUCUUUUCAGAUAUUCAGC	477
S188-AS478-M1	GAAUAUCUGAAAAGAGAAAUUUUTC	188	GAAAAAUUUCUCUUUUCAGAUAUUCAG	478
S189-AS479-M1	AAUAUCUGAAAAGAGAAAUUUUUCC	189	GGAAAAAUUUCUCUUUUCAGAUAUUCA	479
S190-AS480-M1	AUAUCUGAAAAGAGAAAUUUUUCCT	190	AGGAAAAAUUUCUCUUUUCAGAUAUUC	480
S191-AS481-M1	AUCUGAAAAGAGAAAUUUUUCCUAC	191	GUAGGAAAAAUUUCUCUUUUCAGAUAU	481
S192-AS482-M1	GAAAAGAGAAAUUUUUCCUACAAAA	192	UUUUGUAGGAAAAAUUUCUCUUUUCAG	482
S193-AS483-M1	AAAAGAGAAAUUUUUCCUACAAAAT	193	AUUUUGUAGGAAAAAUUUCUCUUUUCA	483
S194-AS484-M1	AGAGAAAUUUUUCCUACAAAAUCTC	194	GAGAUUUUGUAGGAAAAAUUUCUCUUU	484
S195-AS485-M1	GAGAAAUUUUUCCUACAAAAUCUCT	195	AGAGAUUUUGUAGGAAAAAUUUCUCUU	485
S196-AS486-M1	AGAAAUUUUUCCUACAAAAUCUCTT	196	AAGAGAUUUUGUAGGAAAAAUUUCUCU	486
S197-AS487-M1	CUUGGGUCAAGAAAGUUCUAGAATT	197	AAUUCUAGAACUUUCUUGACCCAAGAG	487
S198-AS488-M1	GGGUCAAGAAAGUUCUAGAAUUUGA	198	UCAAAUUCUAGAACUUUCUUGACCCAA	488
S199-AS489-M1	GGUCAAGAAAGUUCUAGAAUUUGAA	199	UUCAAAUUCUAGAACUUUCUUGACCCA	489
S200-AS490-M1	GUCAAGAAAGUUCUAGAAUUUGAAT	200	AUUCAAAUUCUAGAACUUUCUUGACCC	490
S201-AS491-M1	UCAAGAAAGUUCUAGAAUUUGAATT	201	AAUUCAAAUUCUAGAACUUUCUUGACC	491
S202-AS492-M1	CAAGAAAGUUCUAGAAUUUGAAUTG	202	CAAUUCAAAUUCUAGAACUUUCUUGAC	492
S203-AS493-M1	AAGAAAGUUCUAGAAUUUGAAUUGA	203	UCAAUUCAAAUUCUAGAACUUUCUUGA	493
S204-AS494-M1	AGAAAGUUCUAGAAUUUGAAUUGAT	204	AUCAAUUCAAAUUCUAGAACUUUCUUG	494
S205-AS495-M1	GAAAGUUCUAGAAUUUGAAUUGATA	205	UAUCAAUUCAAAUUCUAGAACUUUCUU	495
S206-AS496-M1	AAAGUUCUAGAAUUUGAAUUGAUAA	206	UUAUCAAUUCAAAUUCUAGAACUUUCU	496
S207-AS497-M1	AAGUUCUAGAAUUUGAAUUGAUAAA	207	UUUAUCAAUUCAAAUUCUAGAACUUUC	497
S208-AS498-M1	AGUUCUAGAAUUUGAAUUGAUAAAC	208	GUUUAUCAAUUCAAAUUCUAGAACUUU	498
S209-AS499-M1	GUUCUAGAAUUUGAAUUGAUAAACA	209	UGUUUAUCAAUUCAAAUUCUAGAACUU	499
S210-AS500-M1	UUCUAGAAUUUGAAUUGAUAAACAT	210	AUGUUUAUCAAUUCAAAUUCUAGAACU	500
S211-AS501-M1	UCUAGAAUUUGAAUUGAUAAACATG	211	CAUGUUUAUCAAUUCAAAUUCUAGAAC	501

S212-AS502-M1	CUAGAAUUUGAAUUGAUAAACAUGG	212	CCAUGUUUAUCAAUUCAAAUUCUAGAA	502
S213-AS503-M1	UAGAAUUUGAAUUGAUAAACAUGGT	213	ACCAUGUUUAUCAAUUCAAAUUCUAGA	503
S214-AS504-M1	AGAAUUUGAAUUGAUAAACAUGGTG	214	CACCAUGUUUAUCAAUUCAAAUUCUAG	504
S215-AS505-M1	GAAUUUGAAUUGAUAAACAUGGUGG	215	CCACCAUGUUUAUCAAUUCAAAUUCUA	505
S216-AS506-M1	UAAGAGUAUAUGAGGAACCUUUUAA	216	UUAAAAGGUUCCUCAUAUACUCUUACC	506
S217-AS507-M1	AAGAGUAUAUGAGGAACCUUUUAAA	217	UUUAAAAGGUUCCUCAUAUACUCUUAC	507
S218-AS508-M1	AGAGUAUAUGAGGAACCUUUUAAAC	218	GUUUAAAAGGUUCCUCAUAUACUCUUA	508
S219-AS509-M1	GAGUAUAUGAGGAACCUUUUAAACG	219	CGUUUAAAAGGUUCCUCAUAUACUCUU	509
S220-AS510-M1	AGUAUAUGAGGAACCUUUUAAACGA	220	UCGUUUAAAAGGUUCCUCAUAUACUCU	510
S221-AS511-M1	GUAUAUGAGGAACCUUUUAAACGAC	221	GUCGUUUAAAAGGUUCCUCAUAUACUC	511
S222-AS512-M1	UAUAUGAGGAACCUUUUAAACGACA	222	UGUCGUUUAAAAGGUUCCUCAUAUACU	512
S223-AS513-M1	AUGAGGAACCUUUUAAACGACAACA	223	UGUUGUCGUUUAAAAGGUUCCUCAUAU	513
S224-AS514-M1	GAGGAACCUUUUAAACGACAACAAT	224	AUUGUUGUCGUUUAAAAGGUUCCUCAU	514
S225-AS515-M1	AGGAACCUUUUAAACGACAACAATA	225	UAUUGUUGUCGUUUAAAAGGUUCCUCA	515
S226-AS516-M1	GAACCUUUUAAACGACAACAAUACT	226	AGUAUUGUUGUCGUUUAAAAGGUUCCU	516
S227-AS517-M1	AACCUUUUAAACGACAACAAUACTG	227	CAGUAUUGUUGUCGUUUAAAAGGUUCC	517
S228-AS518-M1	ACCUUUUAAACGACAACAAUACUGC	228	GCAGUAUUGUUGUCGUUUAAAAGGUUC	518
S229-AS519-M1	CCUUUUAAACGACAACAAUACUGCT	229	AGCAGUAUUGUUGUCGUUUAAAAGGUU	519
S230-AS520-M1	CUUUUAAACGACAACAAUACUGCTA	230	UAGCAGUAUUGUUGUCGUUUAAAAGGU	520
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S232-AS522-M1	AAACGACAACAAUACUGCUAGCUTT	232	AAAGCUAGCAGUAUUGUUGUCGUUUAA	522
S233-AS523-M1	AACGACAACAAUACUGCUAGCUUTC	233	GAAAGCUAGCAGUAUUGUUGUCGUUUA	523
S234-AS524-M1	CGACAACAAUACUGCUAGCUUUCAG	234	CUGAAAGCUAGCAGUAUUGUUGUCGUU	524
S235-AS525-M1	GACAACAAUACUGCUAGCUUUCAGG	235	CCUGAAAGCUAGCAGUAUUGUUGUCGU	525
S236-AS526-M1	ACAACAAUACUGCUAGCUUUCAGGA	236	UCCUGAAAGCUAGCAGUAUUGUUGUCG	526
S237-AS527-M1	CAACAAUACUGCUAGCUUUCAGGAT	237	AUCCUGAAAGCUAGCAGUAUUGUUGUC	527
S238-AS528-M1	AACAAUACUGCUAGCUUUCAGGATG	238	CAUCCUGAAAGCUAGCAGUAUUGUUGU	528
S239-AS529-M1	ACAAUACUGCUAGCUUUCAGGAUGA	239	UCAUCCUGAAAGCUAGCAGUAUUGUUG	529
S240-AS530-M1	CAAUACUGCUAGCUUUCAGGAUGAT	240	AUCAUCCUGAAAGCUAGCAGUAUUGUU	530
S241-AS531-M1	AAUACUGCUAGCUUUCAGGAUGATT	241	AAUCAUCCUGAAAGCUAGCAGUAUUGU	531

S242-AS532-M1	AUACUGCUAGCUUUCAGGAUGAUTT	242	AAAUCAUCCUGAAAGCUAGCAGUAUUG	532
S243-AS533-M1	UACUGCUAGCUUUCAGGAUGAUUTT	243	AAAAUCAUCCUGAAAGCUAGCAGUAUU	533
S244-AS534-M1	ACUGCUAGCUUUCAGGAUGAUUUTT	244	AAAAAUCAUCCUGAAAGCUAGCAGUAU	534
S245-AS535-M1	CUGCUAGCUUUCAGGAUGAUUUUTA	245	UAAAAAUCAUCCUGAAAGCUAGCAGUA	535
S246-AS536-M1	UGCUAGCUUUCAGGAUGAUUUUUAA	246	UUAAAAAUCAUCCUGAAAGCUAGCAGU	536
S247-AS537-M1	GCUAGCUUUCAGGAUGAUUUUUAAA	247	UUUAAAAAUCAUCCUGAAAGCUAGCAG	537
S248-AS538-M1	CUAGCUUUCAGGAUGAUUUUUAAAA	248	UUUUAAAAAUCAUCCUGAAAGCUAGCA	538
S249-AS539-M1	AGCUUUCAGGAUGAUUUUUAAAAAA	249	UUUUUUAAAAAUCAUCCUGAAAGCUAG	539
S250-AS540-M1	GCUUUCAGGAUGAUUUUUAAAAAAT	250	AUUUUUUAAAAAUCAUCCUGAAAGCUA	540
S251-AS541-M1	CUUUCAGGAUGAUUUUUAAAAAATA	251	UAUUUUUUAAAAAUCAUCCUGAAAGCU	541
S252-AS542-M1	UUUCAGGAUGAUUUUUAAAAAAUAG	252	CUAUUUUUUAAAAAUCAUCCUGAAAGC	542
S253-AS543-M1	UUCAGGAUGAUUUUUAAAAAAUAGA	253	UCUAUUUUUUAAAAAUCAUCCUGAAAG	543
S254-AS544-M1	UCAGGAUGAUUUUUAAAAAAUAGAT	254	AUCUAUUUUUUAAAAAUCAUCCUGAAA	544
S255-AS545-M1	CAGGAUGAUUUUUAAAAAAUAGATT	255	AAUCUAUUUUUUAAAAAUCAUCCUGAA	545
S256-AS546-M1	AGGAUGAUUUUUAAAAAAUAGAUTC	256	GAAUCUAUUUUUUAAAAAUCAUCCUGA	546
S257-AS547-M1	GGAUGAUUUUUAAAAAAUAGAUUCA	257	UGAAUCUAUUUUUUAAAAAUCAUCCUG	547
S258-AS548-M1	GAUGAUUUUUAAAAAAUAGAUUCAA	258	UUGAAUCUAUUUUUUAAAAAUCAUCCU	548
S259-AS549-M1	AUGAUUUUUAAAAAAUAGAUUCAAA	259	UUUGAAUCUAUUUUUUAAAAAUCAUCC	549
S260-AS550-M1	UGAUUUUUAAAAAAUAGAUUCAAAT	260	AUUUGAAUCUAUUUUUUAAAAAUCAUC	550
S261-AS551-M1	GAUUUUUAAAAAAUAGAUUCAAATG	261	CAUUUGAAUCUAUUUUUUAAAAAUCAU	551
S262-AS552-M1	AUUUUUAAAAAAUAGAUUCAAAUGT	262	ACAUUUGAAUCUAUUUUUUAAAAAUCA	552
S263-AS553-M1	UUUUUAAAAAAUAGAUUCAAAUGTG	263	CACAUUUGAAUCUAUUUUUUAAAAAUC	553
S264-AS554-M1	AAACGCUUCCUAUAACUCGAGUUTA	264	UAAACUCGAGUUAUAGGAAGCGUUUCA	554
S265-AS555-M1	UAUAGGGGAAGAAAAAGCUAUUGTT	265	AACAAUAGCUUUUUCUUCCCCUAUAAA	555
S266-AS556-M1	AUAGGGGAAGAAAAAGCUAUUGUTT	266	AAACAAUAGCUUUUUCUUCCCCUAUAA	556
S267-AS557-M1	GGGGAAGAAAAAGCUAUUGUUUACA	267	UGUAAACAAUAGCUUUUUCUUCCCCUA	557
S268-AS558-M1	GGGAAGAAAAAGCUAUUGUUUACAA	268	UUGUAAACAAUAGCUUUUUCUUCCCCU	558
S269-AS559-M1	GGAAGAAAAAGCUAUUGUUUACAAT	269	AUUGUAAACAAUAGCUUUUUCUUCCCC	559
S270-AS560-M1	GAAGAAAAAGCUAUUGUUUACAATT	270	AAUUGUAAACAAUAGCUUUUUCUUCCC	560
S271-AS561-M1	AAGAAAAAGCUAUUGUUUACAAUTA	271	UAAUUGUAAACAAUAGCUUUUUCUUCC	561

S272-AS562-M1	AGAAAAAGCUAUUGUUUACAAUUAT	272	AUAAUUGUAAACAAUAGCUUUUCUUC	562
S273-AS563-M1	GAAAAAGCUAUUGUUUACAAUUATA	273	UAUAAUUGUAAACAAUAGCUUUUUCUU	563
S274-AS564-M1	AAAAAGCUAUUGUUUACAAUUAUAT	274	AUAUAAUUGUAAACAAUAGCUUUUUCU	564
S275-AS565-M1	AAAAGCUAUUGUUUACAAUUAUATC	275	GAUAUAAUUGUAAACAAUAGCUUUUUC	565
S276-AS566-M1	AAAGCUAUUGUUUACAAUUAUAUCA	276	UGAUAUAAUUGUAAACAAUAGCUUUUU	566
S277-AS567-M1	AAGCUAUUGUUUACAAUUAUAUCAC	277	GUGAUAUAAUUGUAAACAAUAGCUUUU	567
S278-AS568-M1	AGCUAUUGUUUACAAUUAUAUCACC	278	GGUGAUAUAAUUGUAAACAAUAGCUUU	568
S279-AS569-M1	GCUAUUGUUUACAAUUAUAUCACCA	279	UGGUGAUAUAAUUGUAAACAAUAGCUU	569
S280-AS570-M1	CUAUUGUUUACAAUUAUAUCACCAT	280	AUGGUGAUAUAAUUGUAAACAAUAGCU	570
S281-AS571-M1	UAUUGUUUACAAUUAUAUCACCATT	281	AAUGGUGAUAUAAUUGUAAACAAUAGC	571
S282-AS572-M1	AUUGUUUACAAUUAUAUCACCAUTA	282	UAAUGGUGAUAUAAUUGUAAACAAUAG	572
S283-AS573-M1	UUGUUUACAAUUAUAUCACCAUUAA	283	UUAAUGGUGAUAUAAUUGUAAACAAUA	573
S284-AS574-M1	UGUUUACAAUUAUAUCACCAUUAAG	284	CUUAAUGGUGAUAUAAUUGUAAACAAU	574
S285-AS575-M1	GUUUACAAUUAUAUCACCAUUAAGG	285	CCUUAAUGGUGAUAUAAUUGUAAACAA	575
S286-AS576-M1	UACAAUUAUAUCACCAUUAAGGCAA	286	UUGCCUUAAUGGUGAUAUAAUUGUAAA	576
S287-AS577-M1	AUUAUAUCACCAUUAAGGCAACUGC	287	GCAGUUGCCUUAAUGGUGAUAUAAUUG	577
S288-AS578-M1	ACUGCUACACCCUGCUUUGUAUUCT	288	AGAAUACAAAGCAGGGUGUAGCAGUUG	578
S289-AS579-M1	CUGCUACACCCUGCUUUGUAUUCTG	289	CAGAAUACAAAGCAGGGUGUAGCAGUU	579
S290-AS580-M1	UGCUACACCCUGCUUUGUAUUCUGG	290	CCAGAAUACAAAGCAGGGUGUAGCAGU	580
S581-AS591-	UUCAUAAACAAUGAAUGGCAGCAGCCGAAAGGCUGC	581	UGCCAUUCAUUGUUUAUGAAGG	591
S582-AS592-	UCAUAAACAAUGAAUGGCAAGCAGCCGAAAGGCUGC	582	UUGCCAUUCAUUGUUUAUGAGG	592
S583-AS593-M2	GAAACGUGGUUGUGAUGAAGGCAGCCGAAAGGCUGC	583	CUUCAUCACAACCACGUUUCGG	593
S583-AS593-M3	GAAACGUGGUUGUGAUGAAGGCAGCCGAAAGGCUGC	583	CUUCAUCACAACCACGUUUCGG	593
S583-AS593-M4	GAAACGUGGUUGUGAUGAAGGCAGCCGAAAGGCUGC	583	CUUCAUCACAACCACGUUUCGG	593
S583-AS593-M5	GAAACGUGGUUGUGAUGAAGGCAGCCGAAAGGCUGC	583	CUUCAUCACAACCACGUUUCGG	593
S583-AS593-M6	GAAACGUGGUUGUGAUGAAGGCAGCCGAAAGGCUGC	583	CUUCAUCACAACCACGUUUCGG	593
S583-AS593-M7	GAAACGUGGUUGUGAUGAAGGCAGCCGAAAGGCUGC	583	CUUCAUCACAACCACGUUUCGG	593
S584-AS594-M2	GUUGUGAUGAAGGUAGCUGAGCAGCCGAAAGGCUGC	584	UCAGCUACCUUCAUCACAACGG	594
S584-AS594-M3	GUUGUGAUGAAGGUAGCUGAGCAGCCGAAAGGCUGC	584	UCAGCUACCUUCAUCACAACGG	594
S584-AS594-M4	GUUGUGAUGAAGGUAGCUGAGCAGCCGAAAGGCUGC	584	UCAGCUACCUUCAUCACAACGG	594

S584-AS594-M5	GUUGUGAUGAAGGUAGCUGAGCAGCCGAAAGGCUGC	584	UCAGCUACCUUCAUCACAACGG	594
S584-AS594-M6	GUUGUGAUGAAGGUAGCUGAGCAGCCGAAAGGCUGC	584	UCAGCUACCUUCAUCACAACGG	594
S584-AS594-M7	GUUGUGAUGAAGGUAGCUGAGCAGCCGAAAGGCUGC	584	UCAGCUACCUUCAUCACAACGG	594
S585-AS595-M2	GGUGGAUGAAACUCAGUUUAGCAGCCGAAAGGCUGC	585	UAAACUGAGUUUCAUCCACCGG	595
S585-AS595-M3	GGUGGAUGAAACUCAGUUUAGCAGCCGAAAGGCUGC	585	UAAACUGAGUUUCAUCCACCGG	595
S585-AS595-M4	GGUGGAUGAAACUCAGUUUAGCAGCCGAAAGGCUGC	585	UAAACUGAGUUUCAUCCACCGG	595
S585-AS595-M5	GGUGGAUGAAACUCAGUUUAGCAGCCGAAAGGCUGC	585	UAAACUGAGUUUCAUCCACCGG	595
S585-AS595-M6	GGUGGAUGAAACUCAGUUUAGCAGCCGAAAGGCUGC	585	UAAACUGAGUUUCAUCCACCGG	595
S585-AS595-M7	GGUGGAUGAAACUCAGUUUAGCAGCCGAAAGGCUGC	585	UAAACUGAGUUUCAUCCACCGG	595
S585-AS595-M8	GGUGGAUGAAACUCAGUUUAGCAGCCGAAAGGCUGC	585	UAAACUGAGUUUCAUCCACCGG	595
S585-AS595-M9	GGUGGAUGAAACUCAGUUUAGCAGCCGAAAGGCUGC	585	UAAACUGAGUUUCAUCCACCGG	595
S585-AS595-M10	GGUGGAUGAAACUCAGUUUAGCAGCCGAAAGGCUGC	585	UAAACUGAGUUUCAUCCACCGG	595
S585-AS595-M11	GGUGGAUGAAACUCAGUUUAGCAGCCGAAAGGCUGC	585	UAAACUGAGUUUCAUCCACCGG	595
S586-AS596-M2	CAGUUUAAGAAGAUCCUCGGGCAGCCGAAAGGCUGC	586	CCGAGGAUCUUCUUAAACUGGG	596
S586-AS596-M3	CAGUUUAAGAAGAUCCUCGGGCAGCCGAAAGGCUGC	586	CCGAGGAUCUUCUUAAACUGGG	596
S586-AS596-M4	CAGUUUAAGAAGAUCCUCGGGCAGCCGAAAGGCUGC	586	CCGAGGAUCUUCUUAAACUGGG	596
S586-AS596-M5	CAGUUUAAGAAGAUCCUCGGGCAGCCGAAAGGCUGC	586	CCGAGGAUCUUCUUAAACUGGG	596
S586-AS596-M6	CAGUUUAAGAAGAUCCUCGGGCAGCCGAAAGGCUGC	586	CCGAGGAUCUUCUUAAACUGGG	596
S586-AS596-M7	CAGUUUAAGAAGAUCCUCGGGCAGCCGAAAGGCUGC	586	CCGAGGAUCUUCUUAAACUGGG	596
S586-AS596-M12	CAGUUUAAGAAGAUCCUCGGGCAGCCGAAAGGCUGC	586	CCGAGGAUCUUCUUAAACUGGG	596
S587-AS597-M2	UUUAAGAAGAUCCUCGGCUAGCAGCCGAAAGGCUGC	587	UAGCCGAGGAUCUUCUUAAAGG	597
S587-AS597-M3	UUUAAGAAGAUCCUCGGCUAGCAGCCGAAAGGCUGC	587	UAGCCGAGGAUCUUCUUAAAGG	597
S587-AS597-M4	UUUAAGAAGAUCCUCGGCUAGCAGCCGAAAGGCUGC	587	UAGCCGAGGAUCUUCUUAAAGG	597
S587-AS597-M5	UUUAAGAAGAUCCUCGGCUAGCAGCCGAAAGGCUGC	587	UAGCCGAGGAUCUUCUUAAAGG	597
S587-AS597-M6	UUUAAGAAGAUCCUCGGCUAGCAGCCGAAAGGCUGC	587	UAGCCGAGGAUCUUCUUAAAGG	597
S587-AS597-M7	UUUAAGAAGAUCCUCGGCUAGCAGCCGAAAGGCUGC	587	UAGCCGAGGAUCUUCUUAAAGG	597
S587-AS597-M13	UUUAAGAAGAUCCUCGGCUAGCAGCCGAAAGGCUGC	587	UAGCCGAGGAUCUUCUUAAAGG	597
S588-AS598-M2	GUUCUAGAAUUUGAAUUGAUGCAGCCGAAAGGCUGC	588	AUCAAUUCAAAUUCUAGAACGG	598

S588-AS598-M3	GUUCUAGAAUUUGAAUUG AUGCAGCCGAAAGGCUGC	588	AUCAAUUCAAAUUCUAGAA CGG	598
S588-AS598-M4	GUUCUAGAAUUUGAAUUG AUGCAGCCGAAAGGCUGC	588	AUCAAUUCAAAUUCUAGAA CGG	598
S588-AS598-M5	GUUCUAGAAUUUGAAUUG AUGCAGCCGAAAGGCUGC	588	AUCAAUUCAAAUUCUAGAA CGG	598
S588-AS598-M6	GUUCUAGAAUUUGAAUUG AUGCAGCCGAAAGGCUGC	588	AUCAAUUCAAAUUCUAGAA CGG	598
S588-AS598-M7	GUUCUAGAAUUUGAAUUG AUGCAGCCGAAAGGCUGC	588	AUCAAUUCAAAUUCUAGAA CGG	598
S589-AS599-M2	CCUUUUAAACGACAACAAU AGCAGCCGAAAGGCUGC	589	UAUUGUUGUCGUUUAAAAG GGG	599
S589-AS599-M3	CCUUUUAAACGACAACAAU AGCAGCCGAAAGGCUGC	589	UAUUGUUGUCGUUUAAAAG GGG	599
S589-AS599-M4	CCUUUUAAACGACAACAAU AGCAGCCGAAAGGCUGC	589	UAUUGUUGUCGUUUAAAAG GGG	599
S589-AS599-M5	CCUUUUAAACGACAACAAU AGCAGCCGAAAGGCUGC	589	UAUUGUUGUCGUUUAAAAG GGG	599
S589-AS599-M6	CCUUUUAAACGACAACAAU AGCAGCCGAAAGGCUGC	589	UAUUGUUGUCGUUUAAAAG GGG	599
S589-AS599-M7	CCUUUUAAACGACAACAAU AGCAGCCGAAAGGCUGC	589	UAUUGUUGUCGUUUAAAAG GGG	599
S590-AS600-M2	AUGAUUUUUAAAAAAUAG AUGCAGCCGAAAGGCUGC	590	AUCUAUUUUUUAAAAAUCA UGG	600
S590-AS600-M3	AUGAUUUUUAAAAAAUAG AUGCAGCCGAAAGGCUGC	590	AUCUAUUUUUUAAAAAUCA UGG	600
S590-AS600-M4	AUGAUUUUUAAAAAAUAG AUGCAGCCGAAAGGCUGC	590	AUCUAUUUUUUAAAAAUCA UGG	600
S590-AS600-M5	AUGAUUUUUAAAAAAUAG AUGCAGCCGAAAGGCUGC	590	AUCUAUUUUUUAAAAAUCA UGG	600
S590-AS600-M6	AUGAUUUUUAAAAAAUAG AUGCAGCCGAAAGGCUGC	590	AUCUAUUUUUUAAAAAUCA UGG	600
S590-AS600-M7	AUGAUUUUUAAAAAAUAG AUGCAGCCGAAAGGCUGC	590	AUCUAUUUUUUAAAAAUCA UGG	600

[000166] The disclosure illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

[000167] In addition, where features or aspects of the invention are described in terms

of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

[000168] 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 compared with the specified sequence while retaining essentially same or similar complementary properties as the specified sequence.

[000169] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (*i.e.*, meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[000170] Embodiments of this invention are described herein. Variations of those embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description.

[000171] The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly

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

CLAIMS

What is claimed is:

1. An oligonucleotide for reducing expression of ALDH2, the oligonucleotide comprising an antisense strand comprising a sequence as set forth in any one of SEQ ID NOs: 591-600.

2. The oligonucleotide of claim 1, further comprising a sense strand that comprises a sequence as set forth in any one of SEQ ID NOs: 581-590.

3. The oligonucleotide of claim 1 or 2, wherein the antisense strand consists of a sequence as set forth in any one of SEQ ID NOs: 591-600.

4. The oligonucleotide of claim 2 or 3, wherein the sense strand consists of a sequence as set forth in any one of SEQ ID NOs: 581-590.

5. An oligonucleotide for reducing expression of ALDH2, the oligonucleotide comprising an antisense strand of 15 to 30 nucleotides in length, wherein the antisense strand has a region of complementarity to a target sequence of ALDH2 as set forth in any one of SEQ ID NOs: 601-607, wherein the region of complementarity is at least 15 contiguous nucleotides in length.

6. The oligonucleotide of claim 5, wherein the region of complementarity is fully complementary to the target sequence of ALDH2.

7. The oligonucleotide of any one of claims 1 to 6, wherein the antisense strand is 19 to 27 nucleotides in length.

8. The oligonucleotide of any one of claims 1 to 7, wherein the antisense strand is 21 to 27 nucleotides in length.

9. The oligonucleotide of any one of claims 1 to 8, further comprising a sense strand of 15 to 40 nucleotides in length, wherein the sense strand forms a duplex region with the antisense strand.

10. The oligonucleotide of claim 9, wherein the sense strand is 19 to 40 nucleotides in length.

11. The oligonucleotide of claim 9 or 10, wherein the duplex region is at least 19 nucleotides in length.

12. The oligonucleotide of any one of claims 9 to 11, wherein the duplex region is at least 21 nucleotides in length.

13. The oligonucleotide of any one of claims 5 to 12, wherein the region of complementarity to ALDH2 is at least 19 contiguous nucleotides in length.

14. The oligonucleotide of any one of claims 5 to 13, wherein the region of complementarity to ALDH2 is at least 21 contiguous nucleotides in length.

15. The oligonucleotide of any one of claims 9 to 14, wherein the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 581-590.

16. The oligonucleotide of any one of claims 5 to 15, wherein the antisense strand comprises a sequence as set forth in any one of SEQ ID NOs: 591-600.

17. The oligonucleotide of any one of claims 9 to 16, wherein the sense strand consists of a sequence as set forth in any one of SEQ ID NOs: 581-590.

18. The oligonucleotide of any one of claims 5 to 17, wherein the antisense strand consists of a sequence as set forth in any one of SEQ ID NOs: 591-600.

19. The oligonucleotide of any one of claims 9 to 18, wherein the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L forms a loop between S_1 and S_2 of 3 to 5 nucleotides in length.

20. An oligonucleotide for reducing expression of ALDH2, the oligonucleotide comprising an antisense strand and a sense strand,

wherein the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to ALDH2,

wherein the sense strand comprises at its 3′-end a stem-loop set forth as: S_1-L-S_2, wherein S_1 is complementary to S_2, and wherein L forms a loop between S_1 and S_2 of 3 to 5 nucleotides in length,

and wherein the antisense strand and the sense strand form a duplex structure of at least 19 nucleotides in length but are not covalently linked.

21. The oligonucleotide of claim 20, wherein the region of complementarity is fully complementary to at least 19 contiguous nucleotides of ALDH2 mRNA.

22. The oligonucleotide of any one of claims 19 to 21, wherein L is a tetraloop.

23. The oligonucleotide of any one of claims 19 to 22, wherein L is 4 nucleotides in length.

24. The oligonucleotide of any one of claims 19 to 23, wherein L comprises a sequence set forth as GAAA.

25. The oligonucleotide of any one of claims 9 to 18, wherein the antisense strand is 27 nucleotides in length and the sense strand is 25 nucleotides in length.

26. The oligonucleotide of claim 25, wherein the antisense strand and sense strand form a duplex region of 25 nucleotides in length.

27. The oligonucleotide of any one of claims 20 to 24, further comprising a 3′-overhang sequence on the antisense strand of two nucleotides in length.

28. The oligonucleotide of any one of claims 9 to 18, wherein the oligonucleotide comprises an antisense strand and a sense strand that are each in a range of 21 to 23 nucleotides in length.

29. The oligonucleotide of claim 28, wherein the oligonucleotide comprises a duplex structure in a range of 19 to 21 nucleotides in length.

30. The oligonucleotide of claim 28 or 29, wherein the oligonucleotide comprises a 3′-overhang sequence of one or more nucleotides in length, wherein the 3′-overhang sequence is present on the antisense strand, the sense strand, or the antisense strand and sense strand.

31. The oligonucleotide of claim 28 or 29, wherein the oligonucleotide comprises a 3′-overhang sequence of two nucleotides in length, wherein the 3′-overhang sequence is present on the antisense strand, and wherein 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.

32. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises at least one modified nucleotide.

33. The oligonucleotide of claim 32, wherein the modified nucleotide comprises a 2′-modification.

34. The oligonucleotide of claim 33, wherein the 2′-modification is a modification selected from: 2′-aminoethyl, 2′-fluoro, 2′-O-methyl, 2′-O-methoxyethyl, and 2′-deoxy-2′-fluoro-β-d-arabinonucleic acid.

35. The oligonucleotide of any one of claims 32 to 34, wherein all of the nucleotides of the oligonucleotide are modified.

36. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises at least one modified internucleotide linkage.

37. The oligonucleotide of claim 36, wherein the at least one modified internucleotide linkage is a phosphorothioate linkage.

38. The oligonucleotide of any one of the preceding claims, wherein the 4′-carbon of the sugar of the 5′-nucleotide of the antisense strand comprises a phosphate analog.

39. The oligonucleotide of claim 38, wherein the phosphate analog is oxymethylphosphonate, vinylphosphonate, or malonylphosphonate.

40. The oligonucleotide of any one of the preceding claims, wherein at least one nucleotide of the oligonucleotide is conjugated to one or more targeting ligands.

41. The oligonucleotide of claim 40, wherein each targeting ligand comprises a carbohydrate, amino sugar, cholesterol, polypeptide or lipid.

42. The oligonucleotide of claim 41, wherein each targeting ligand comprises a N-acetylgalactosamine (GalNAc) moiety.

43. The oligonucleotide of claim 42, wherein the GalNac moiety is a monovalent GalNAc moiety, a bivalent GalNAc moiety, a trivalent GalNAc moiety, or a tetravalent GalNAc moiety.

44. The oligonucleotide of any one of claims 19 to 24, wherein up to 4 nucleotides of L of the stem-loop are each conjugated to a monovalent GalNAc moiety.

45. The oligonucleotide of claim 40, wherein the targeting ligand comprises an aptamer.

46. A composition comprising an oligonucleotide of any one of the preceding claims and an excipient.

47. A method of delivering an oligonucleotide to a subject, the method comprising administering the composition of claim 46 to the subject.

48. A method of decreasing ethanol tolerance in a subject, the method comprising administering the composition of claim 46 to the subject.

49. A method of inhibiting ethanol intake by a subject, the method comprising administering the composition of claim 46 to the subject.

50. A method of decreasing the desire of a subject to consume ethanol, the method comprising administering the composition of claim 46 to the subject.

51. The method of any one of claims 47 to 50, wherein the subject suffers from alcoholism.

52. An oligonucleotide for reducing expression of ALDH2, the oligonucleotide comprising a sense strand of 15 to 50 nucleotides in length and an antisense strand of 15 to 30 nucleotides in length, wherein the sense strand forms a duplex region with the antisense strand, wherein the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 581-590 and wherein the antisense strand comprises a complementary sequence selected from SEQ ID NOs: 591-600.

53. An oligonucleotide for reducing expression of ALDH2, the oligonucleotide comprising a pair of sense and antisense strands selected from a row of the table set forth in Table 4.

FIG. 1

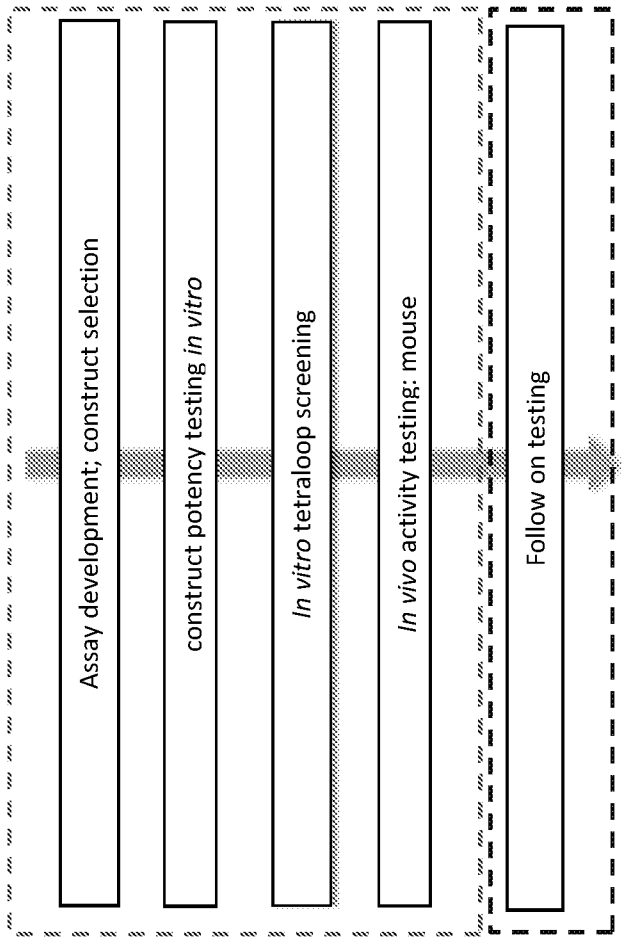

Assay development; construct selection
construct potency testing *in vitro*
In vitro tetraloop screening
In vivo activity testing: mouse
Follow on testing

FIG. 2

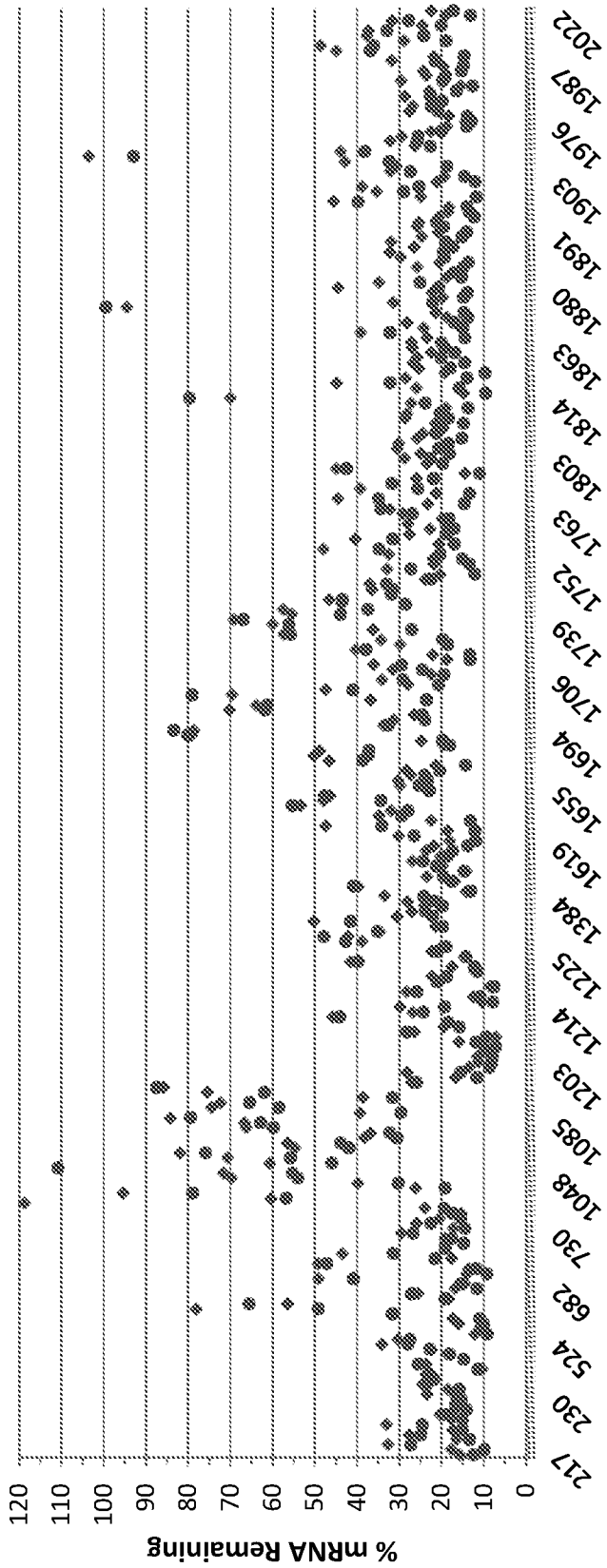

Human ALDH2 Knockdown in HepG2 Cells Normalized to Human HPRT 517-591 (FAM) and Human SFRS9 594-690 (HEX) vs M107-M48 | NC1 control, M107-M48 | NC5 control, M107-M48|NC7 Control
% mRNA Remaining
120
110
100
90
80
70
60
50
40
30
20
10
0
217
230
524
682
730
1048
1085
1203
1224
1225
1384
1619
1655
1694
1706
1739
1752
1763
1803
1814
1863
1880
1891
1903
1976
1987
2022
siRNA (Hs Location)
Hs ALDH2 196-305 (FAM)
Hs ALDH2 1455-1559 (HEX)

FIG. 3A

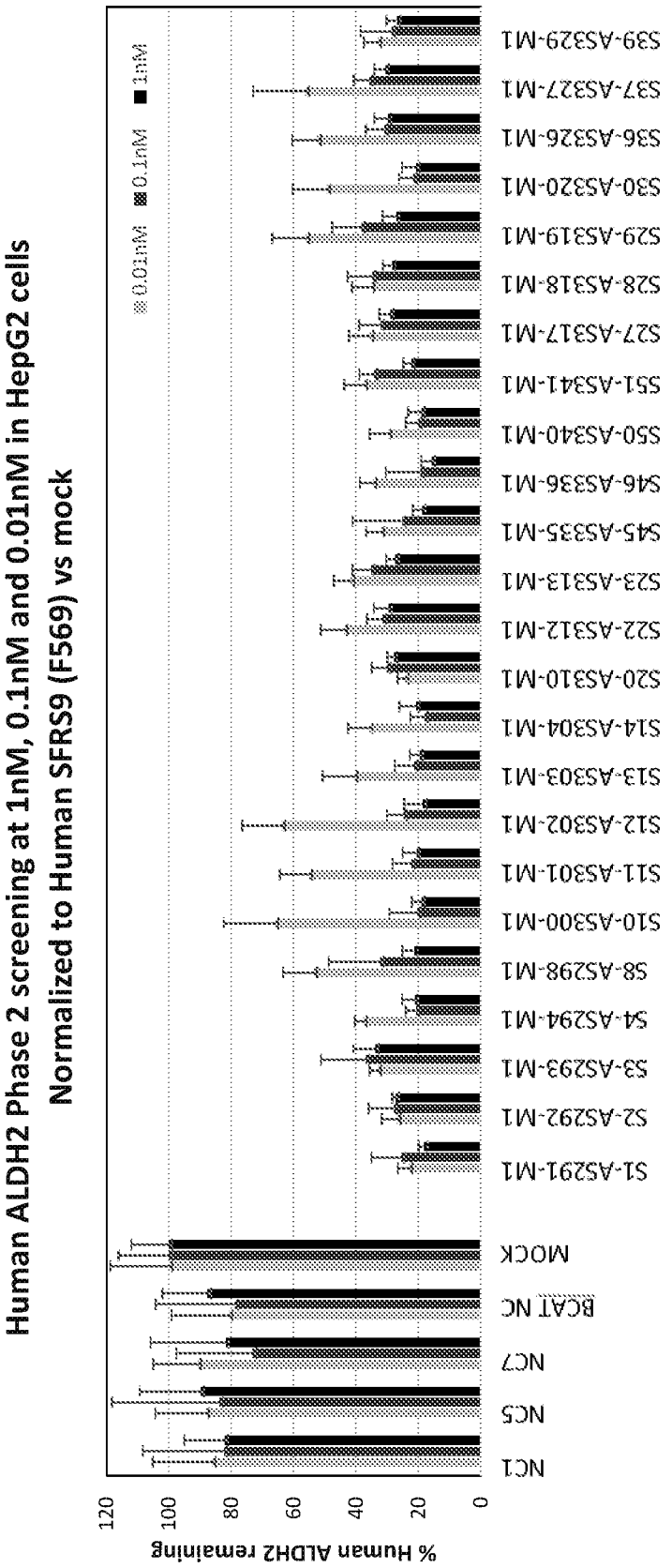

Human ALDH2 Phase 2 screening at 1nM, 0.1nM and 0.01nM in HepG2 cells
Normalized to Human SFRS9 (F569) vs mock
0.01nM
0.1nM
1nM
% Human ALDH2 remaining
120
100
80
60
40
20
0
NC1
NC5
NC7
BCAT NC
MOCK
S1-AS291-M1
S2-AS292-M1
S3-AS293-M1
S4-AS294-M1
S8-AS298-M1
S10-AS300-M1
S11-AS301-M1
S12-AS302-M1
S13-AS303-M1
S14-AS304-M1
S20-AS310-M1
S22-AS312-M1
S23-AS313-M1
S45-AS335-M1
S46-AS336-M1
S50-AS340-M1
S51-AS341-M1
S27-AS317-M1
S28-AS318-M1
S29-AS319-M1
S30-AS320-M1
S36-AS326-M1
S37-AS327-M1
S39-AS329-M1

FIG. 3B

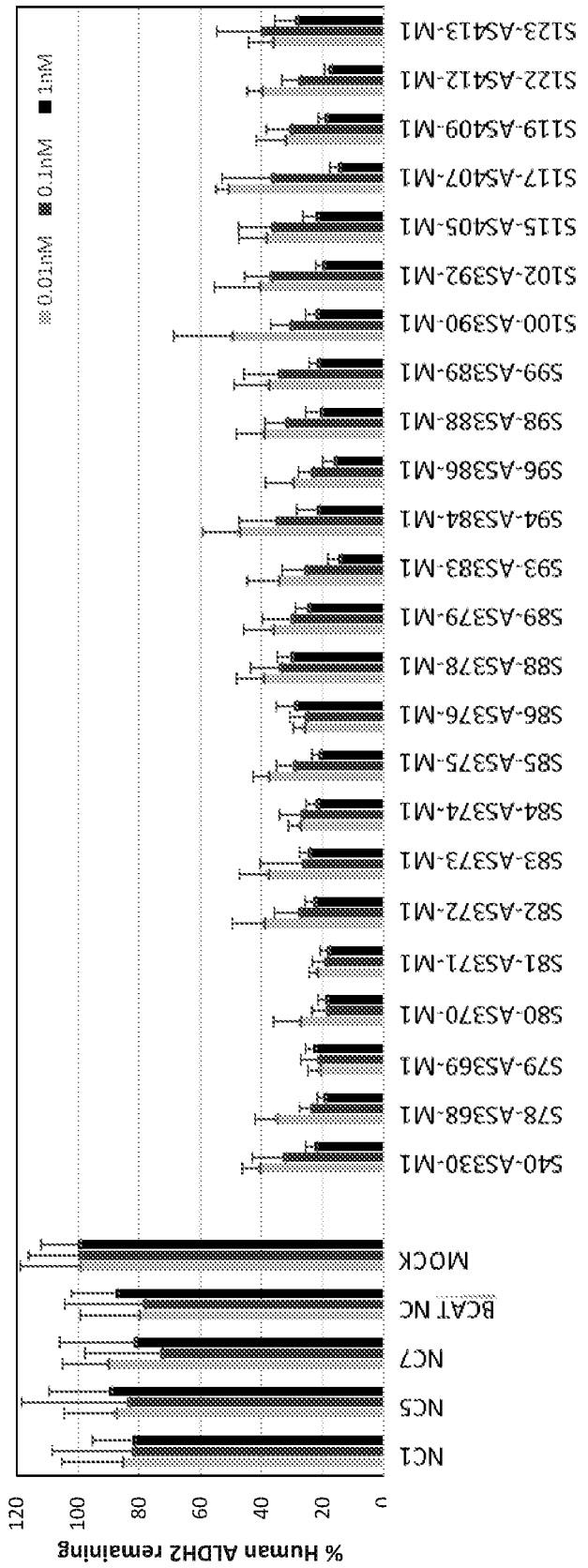

Human ALDH2 Phase 2 screening at 1nM, 0.1nM and 0.01nM in HepG2 cells
Normalized to Human SFRS9 (F569) vs mock
0.01nM
0.1nM
1nM
% Human ALDH2 remaining
120
100
80
60
40
20
0
NC1
NC5
NC7
BCAT NC
MOCK
S40-AS330-M1
S78-AS368-M1
S79-AS369-M1
S80-AS370-M1
S81-AS371-M1
S82-AS372-M1
S83-AS373-M1
S84-AS374-M1
S85-AS375-M1
S86-AS376-M1
S88-AS378-M1
S89-AS379-M1
S93-AS383-M1
S94-AS384-M1
S96-AS386-M1
S98-AS388-M1
S99-AS389-M1
S100-AS390-M1
S102-AS392-M1
S115-AS405-M1
S117-AS407-M1
S119-AS409-M1
S122-AS412-M1
S123-AS413-M1

FIG. 3C

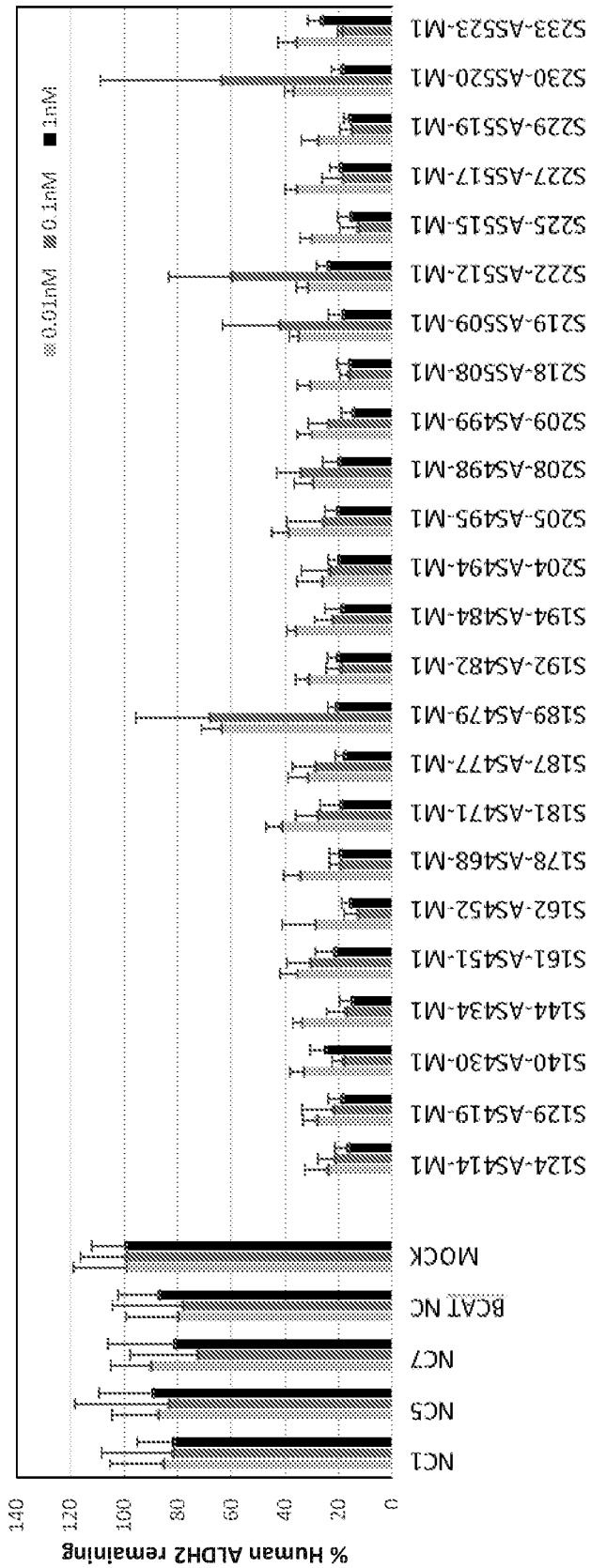

Human ALDH2 Phase 2 screening at 1nM, 0.1nM and 0.01nM in HepG2 cells
Normalized to Human SFRS9 (F569) vs mock
0.01nM
0.1nM
1nM
% Human ALDH2 remaining
0
20
40
60
80
100
120
140
NC1
NC5
NC7
BCAT NC
MOCK
S124-AS414-M1
S129-AS419-M1
S140-AS430-M1
S144-AS434-M1
S161-AS451-M1
S162-AS452-M1
S178-AS468-M1
S181-AS471-M1
S187-AS477-M1
S189-AS479-M1
S192-AS482-M1
S194-AS484-M1
S204-AS494-M1
S205-AS495-M1
S208-AS498-M1
S209-AS499-M1
S218-AS508-M1
S219-AS509-M1
S222-AS512-M1
S225-AS515-M1
S227-AS517-M1
S229-AS519-M1
S230-AS520-M1
S233-AS523-M1

FIG. 3D

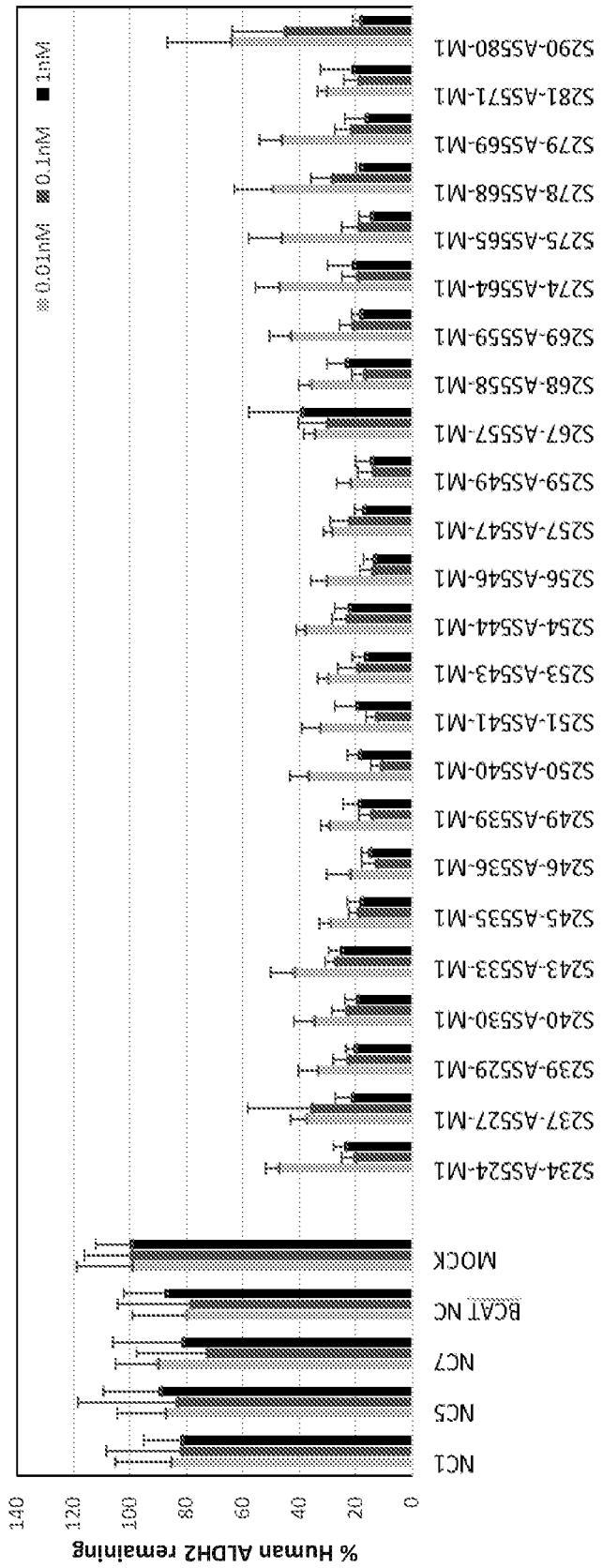

Human ALDH2 Phase 2 screening at 1nM, 0.1nM and 0.01nM in HepG2 cells
Normalized to Human SFRS9 (F569) vs mock
0.01nM
0.1nM
1nM
% Human ALDH2 remaining
0
20
40
60
80
100
120
140
NC1
NC5
NC7
BCAT NC
MOCK
S234-AS524-M1
S237-AS527-M1
S239-AS529-M1
S240-AS530-M1
S243-AS533-M1
S245-AS535-M1
S246-AS536-M1
S249-AS539-M1
S250-AS540-M1
S251-AS541-M1
S253-AS543-M1
S254-AS544-M1
S256-AS546-M1
S257-AS547-M1
S259-AS549-M1
S267-AS557-M1
S268-AS558-M1
S269-AS559-M1
S274-AS564-M1
S275-AS565-M1
S278-AS568-M1
S279-AS569-M1
S281-AS571-M1
S290-AS580-M1

FIG. 4

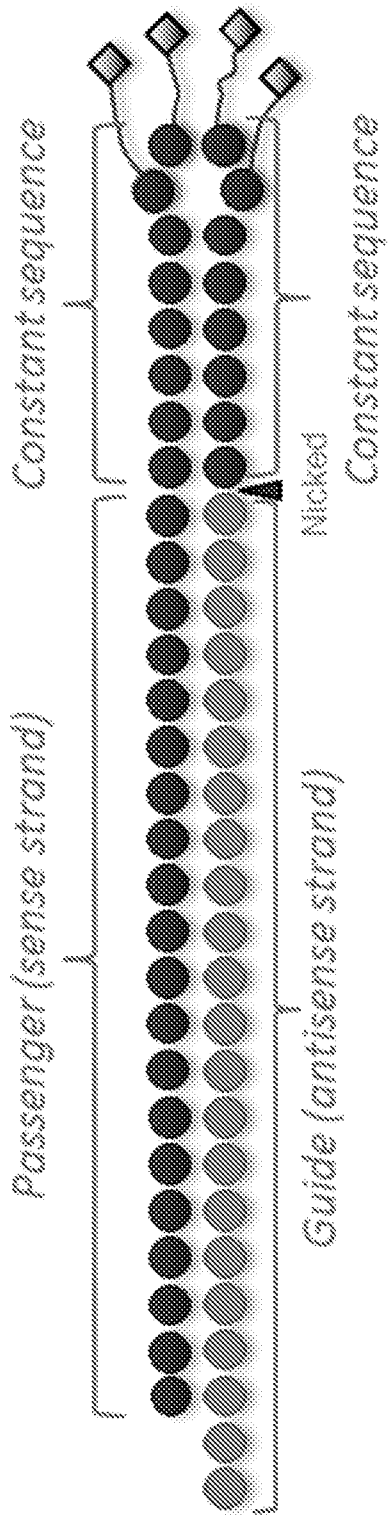

Constant sequence
Nicked
Constant sequence
Passenger (sense strand)
Guide (antisense strand)

FIG. 5A

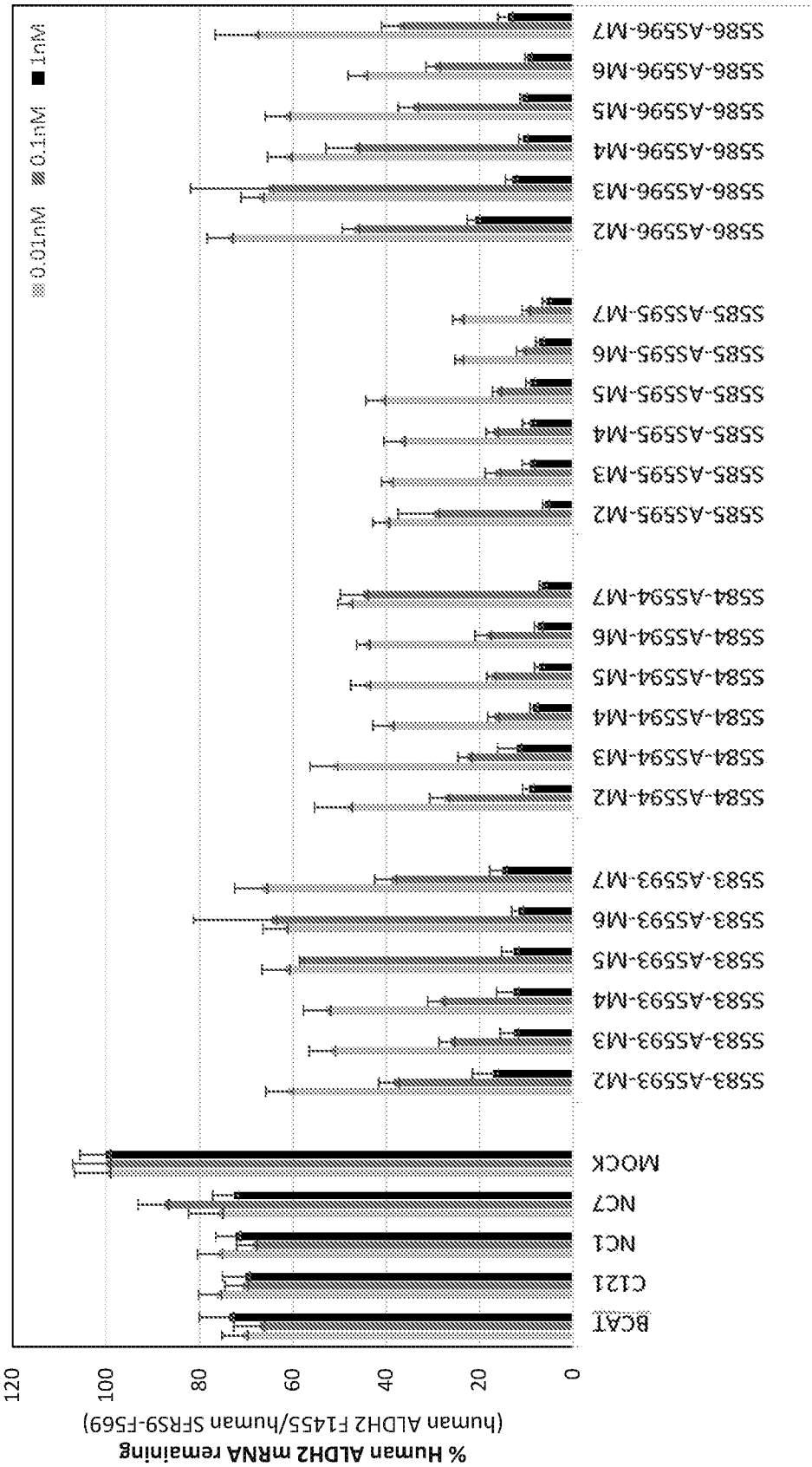

Human ALDH2 Tetraloop screening in HepG2 cells
Normalized to Human SFRS9 vs Mock
0.01nM
0.1nM
1nM
% Human ALDH2 mRNA remaining
(human ALDH2 F1455/human SFRS9-F569)
120
100
80
60
40
20
0
BCAT
C121
NC1
NC7
MOCK
S583-AS593-M2
S583-AS593-M3
S583-AS593-M4
S583-AS593-M5
S583-AS593-M6
S583-AS593-M7
S584-AS594-M2
S584-AS594-M3
S584-AS594-M4
S584-AS594-M5
S584-AS594-M6
S584-AS594-M7
S585-AS595-M2
S585-AS595-M3
S585-AS595-M4
S585-AS595-M5
S585-AS595-M6
S585-AS595-M7
S586-AS596-M2
S586-AS596-M3
S586-AS596-M4
S586-AS596-M5
S586-AS596-M6
S586-AS596-M7

FIG. 5B

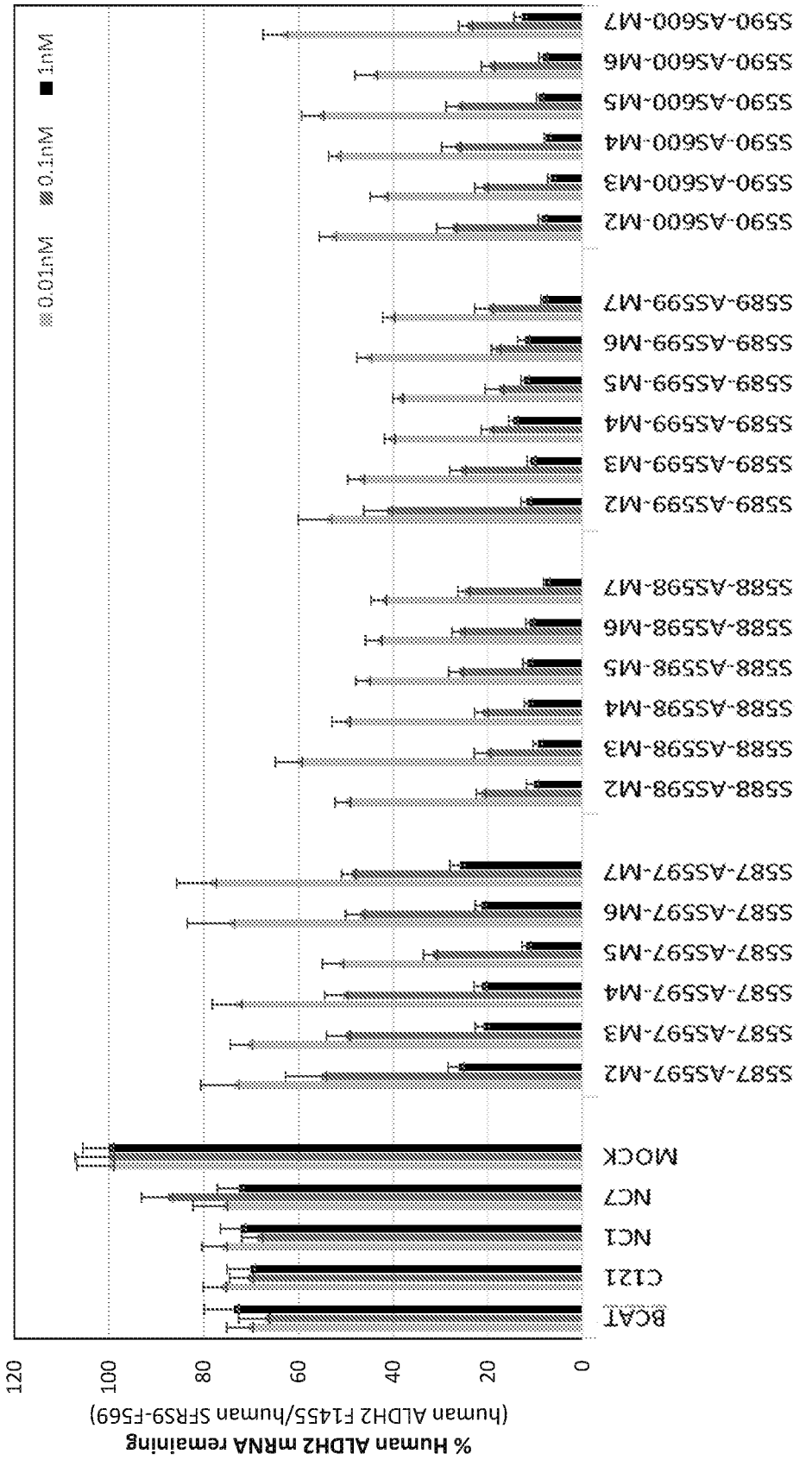

Human ALDH2 Tetraloop screening in HepG2 cells
Normalized to Human SFRS9 vs Mock
0.01nM
0.1nM
1nM
% Human ALDH2 mRNA remaining
(human ALDH2 F1455/human SFRS9-F569)
120
100
80
60
40
20
0
BCAT
C121
NC1
NC7
MOCK
S587-AS597-M2
S587-AS597-M3
S587-AS597-M4
S587-AS597-M5
S587-AS597-M6
S587-AS597-M7
S588-AS598-M2
S588-AS598-M3
S588-AS598-M4
S588-AS598-M5
S588-AS598-M6
S588-AS598-M7
S589-AS599-M2
S589-AS599-M3
S589-AS599-M4
S589-AS599-M5
S589-AS599-M6
S589-AS599-M7
S590-AS600-M2
S590-AS600-M3
S590-AS600-M4
S590-AS600-M5
S590-AS600-M6
S590-AS600-M7

FIG. 6

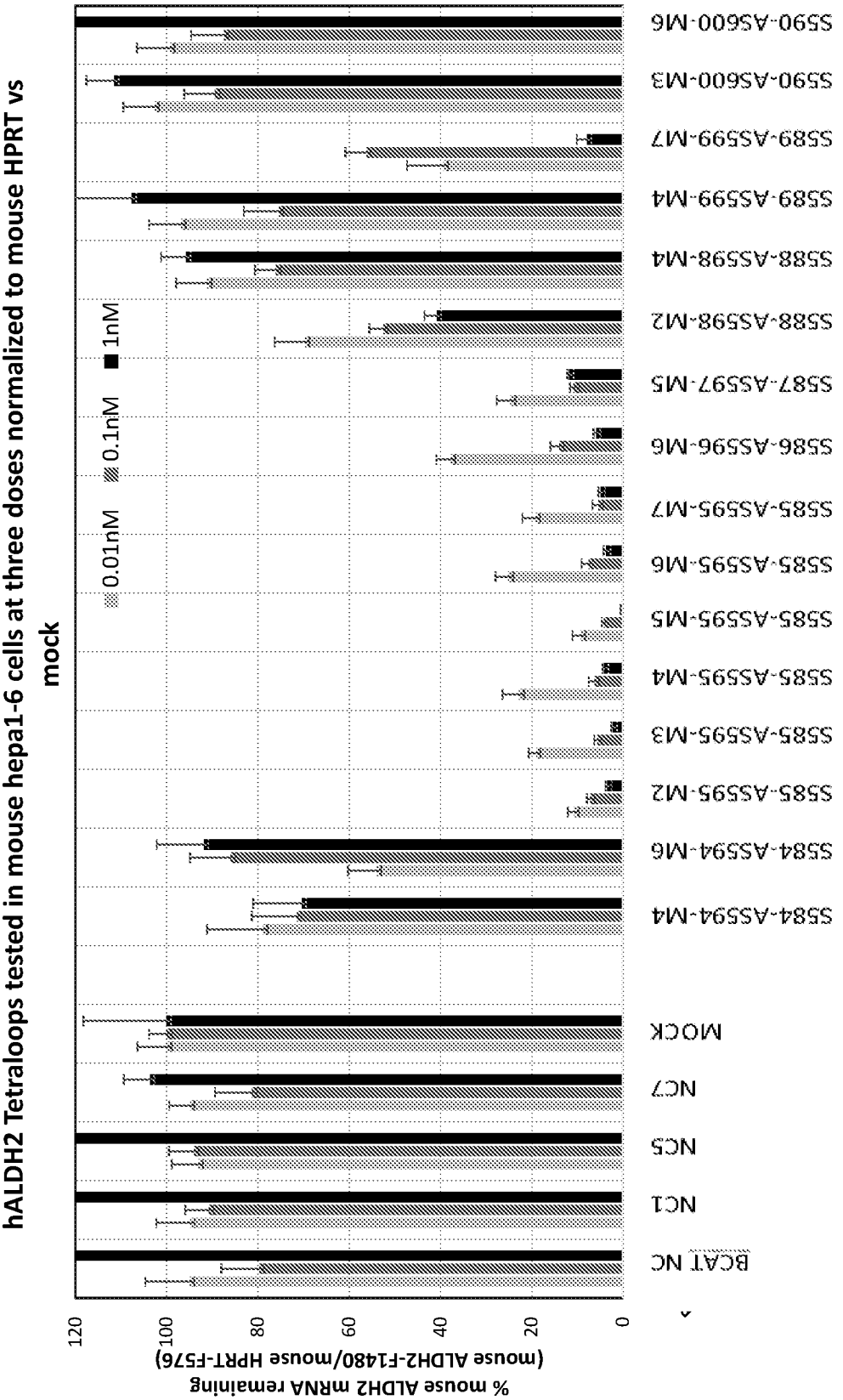

hALDH2 Tetraloops tested in mouse hepa1-6 cells at three doses normalized to mouse HPRT vs mock
% mouse ALDH2 mRNA remaining (mouse ALDH2-F1480/mouse HPRT-F576)
0
20
40
60
80
100
120
0.01nM
0.1nM
1nM
BCAT NC
NC1
NC5
NC7
MOCK
S584-AS594-M4
S584-AS594-M6
S585-AS595-M2
S585-AS595-M3
S585-AS595-M4
S585-AS595-M5
S585-AS595-M6
S585-AS595-M7
S586-AS596-M6
S587-AS597-M5
S588-AS598-M2
S588-AS598-M4
S589-AS599-M4
S589-AS599-M7
S590-AS600-M3
S590-AS600-M6

FIG. 7

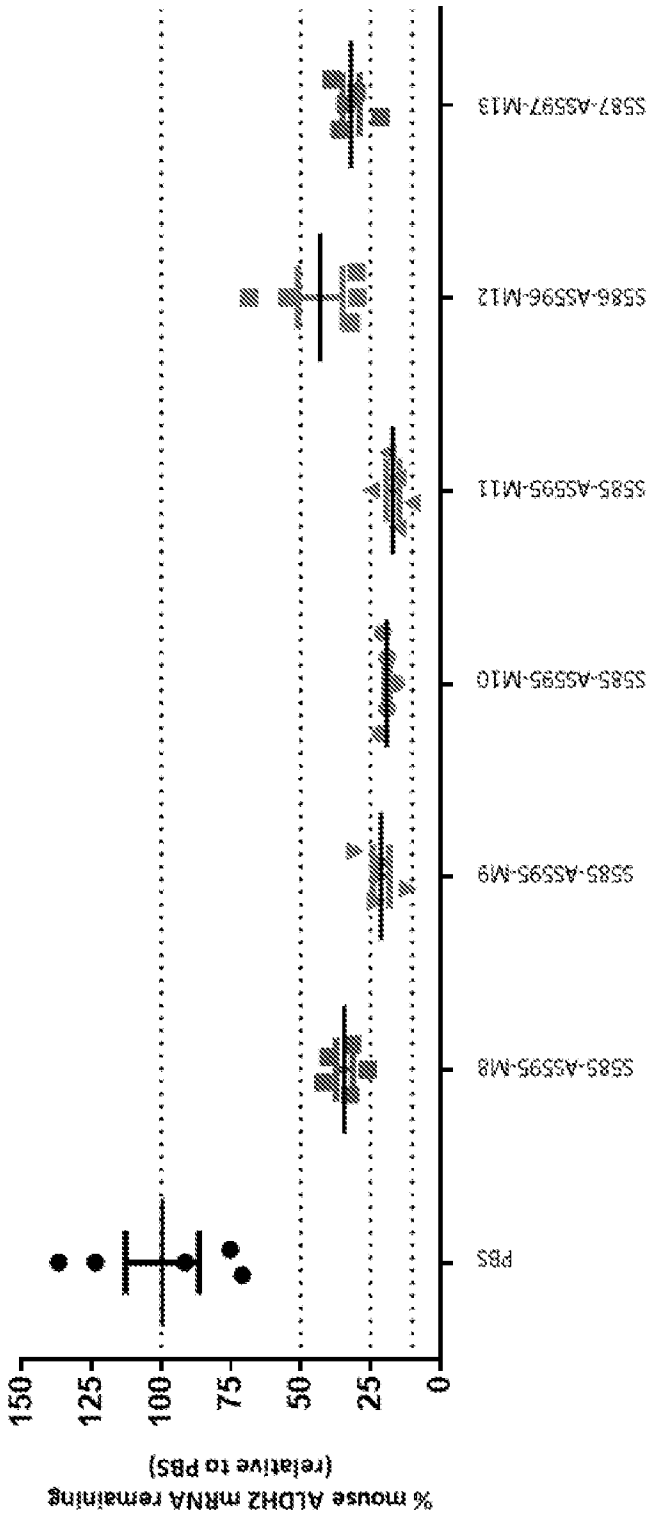

Day 4, dosed at 3mpk in CD-1 mice
% mouse ALDH2 mRNA remaining
(relative to PBS)
150
125
100
75
50
25
0
PBS
S585-A5595-M8
S585-A5595-M9
S585-A5595-M10
S585-A5595-M11
S586-A5596-M12
S587-A5597-M13

FIG. 8

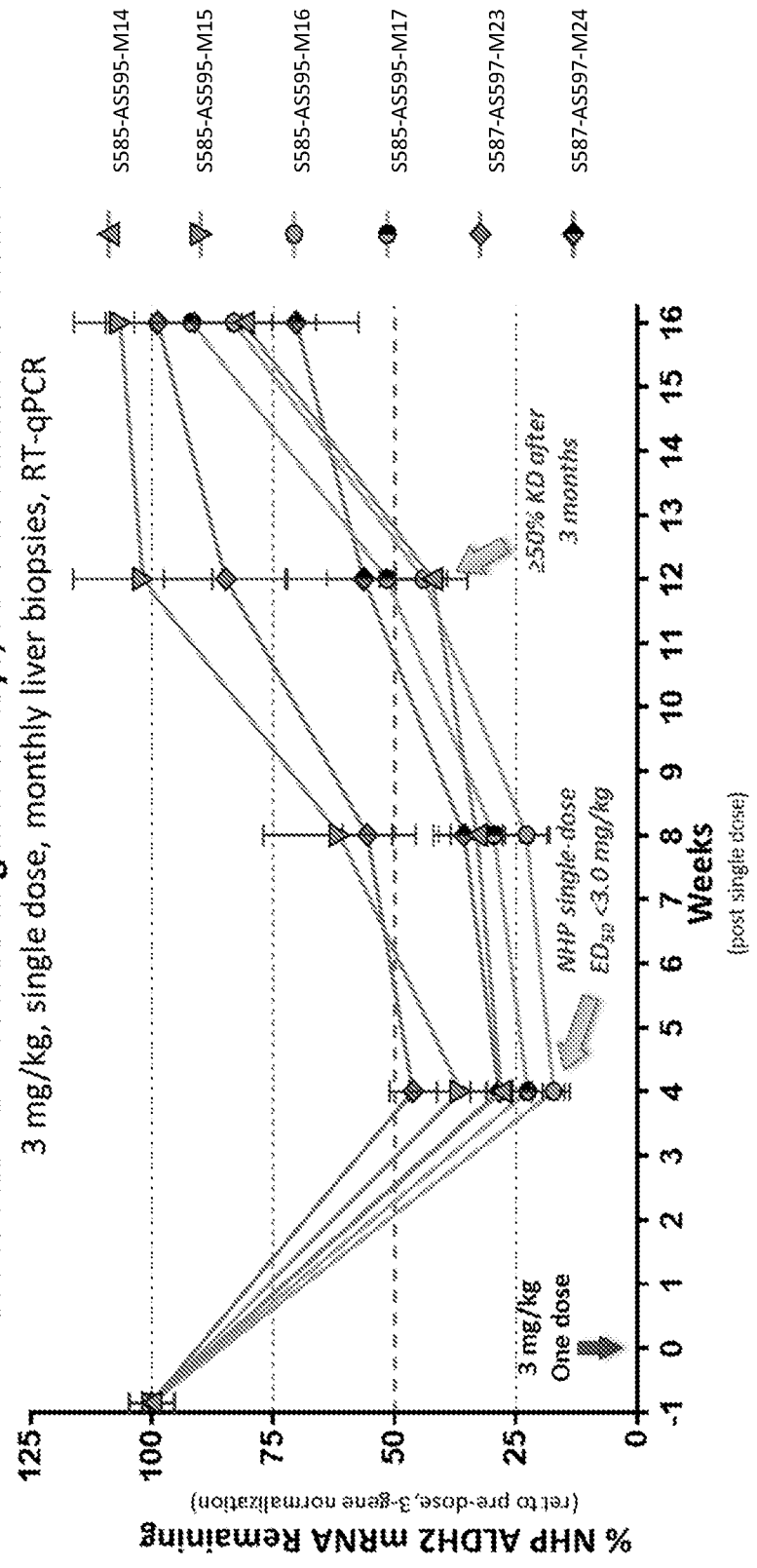

GalXC-ALDH2 Hit Screening in Monkeys, PD and Duration of Action
3 mg/kg, single dose, monthly liver biopsies, RT-qPCR
S585-AS595-M14
S585-AS595-M15
S585-AS595-M16
S585-AS595-M17
S587-AS597-M23
S587-AS597-M24
≥50% KD after 3 months
NHP single-dose ED50 <3.0 mg/kg
3 mg/kg One dose
% NHP ALDH2 mRNA Remaining
(rel to pre-dose, 3-gene normalization)
Weeks
(post single dose)
125
100
75
50
25
0
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

FIG. 9

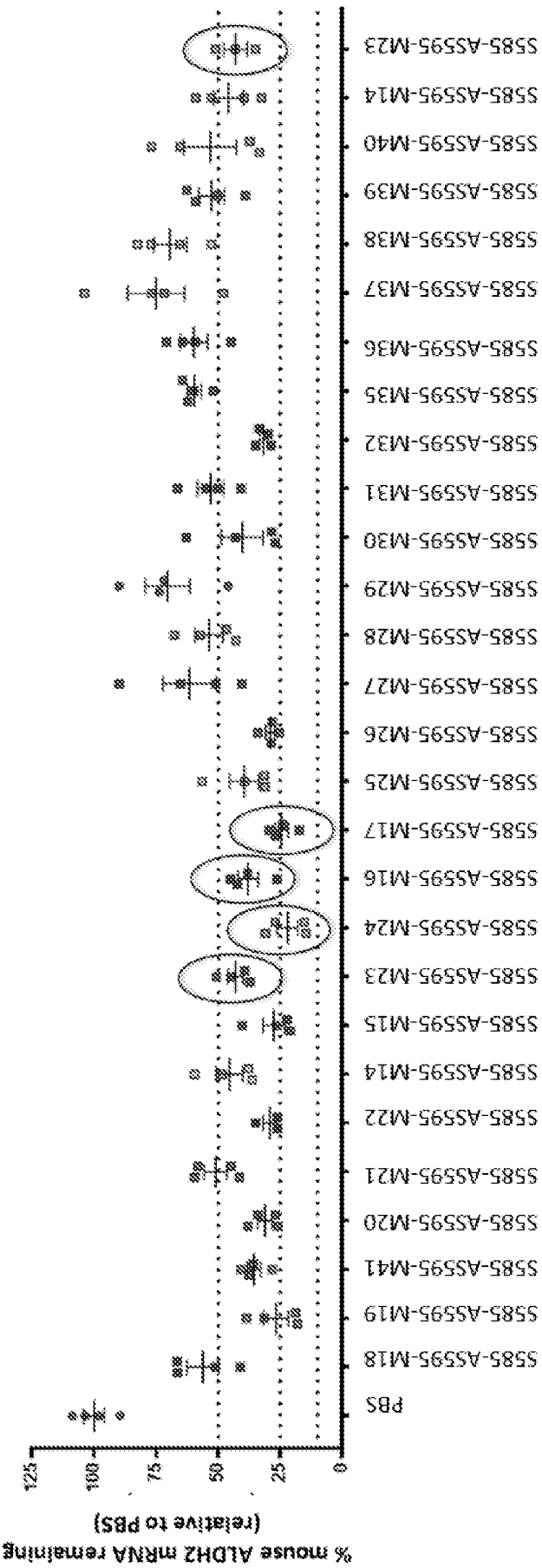

% mouse ALDH2 mRNA remaining
(relative to PBS)
125
100
75
50
25
0
PBS
S585-AS595-M18
S585-AS595-M19
S585-AS595-M41
S585-AS595-M20
S585-AS595-M21
S585-AS595-M22
S585-AS595-M14
S585-AS595-M15
S585-AS595-M23
S585-AS595-M24
S585-AS595-M16
S585-AS595-M17
S585-AS595-M25
S585-AS595-M26
S585-AS595-M27
S585-AS595-M28
S585-AS595-M29
S585-AS595-M30
S585-AS595-M31
S585-AS595-M32
S585-AS595-M35
S585-AS595-M36
S585-AS595-M37
S585-AS595-M38
S585-AS595-M39
S585-AS595-M40
S585-AS595-M14
S585-AS595-M23

FIG. 10

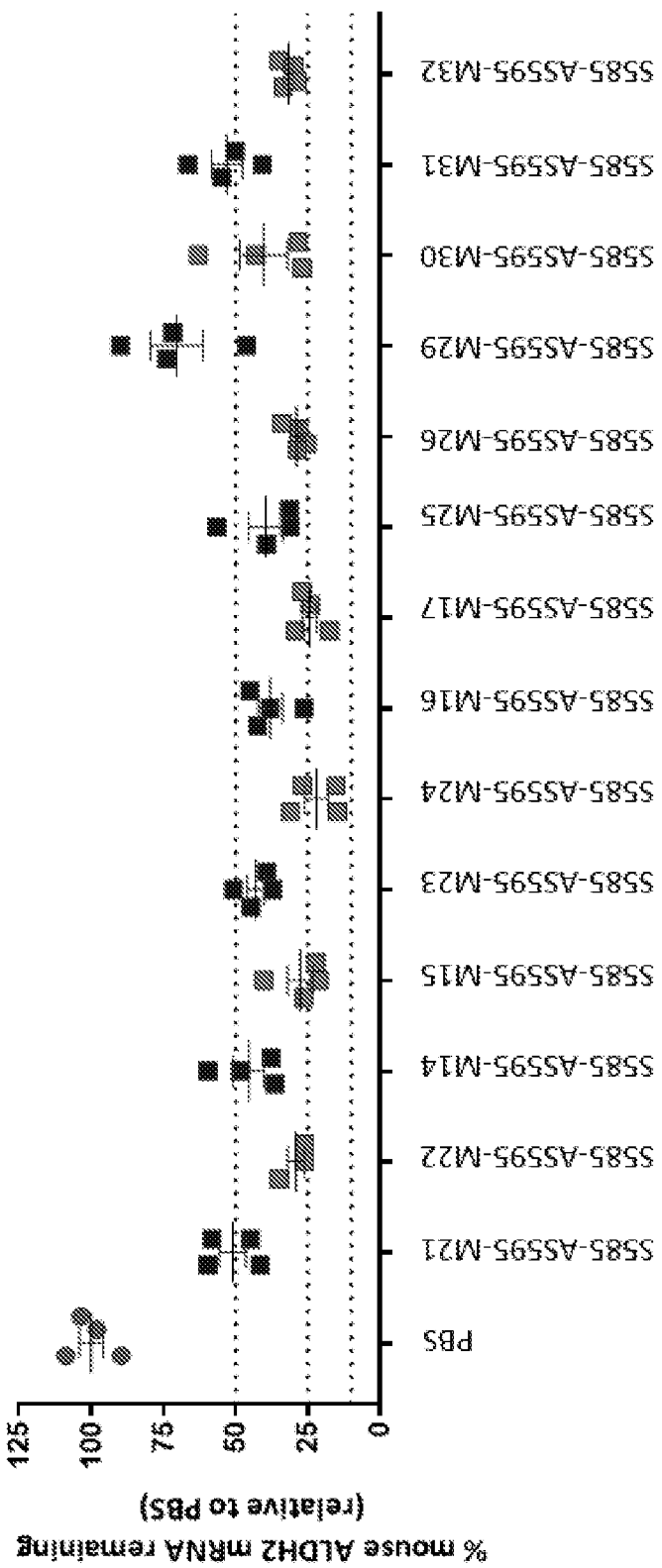

Dosed at 0.5mpk; Day 4 knockdown data
% mouse ALDH2 mRNA remaining
(relative to PBS)
125
100
75
50
25
0
PBS
S585-AS595-M21
S585-AS595-M22
S585-AS595-M14
S585-AS595-M15
S585-AS595-M23
S585-AS595-M24
S585-AS595-M16
S585-AS595-M17
S585-AS595-M25
S585-AS595-M26
S585-AS595-M29
S585-AS595-M30
S585-AS595-M31
S585-AS595-M32

FIG. 11

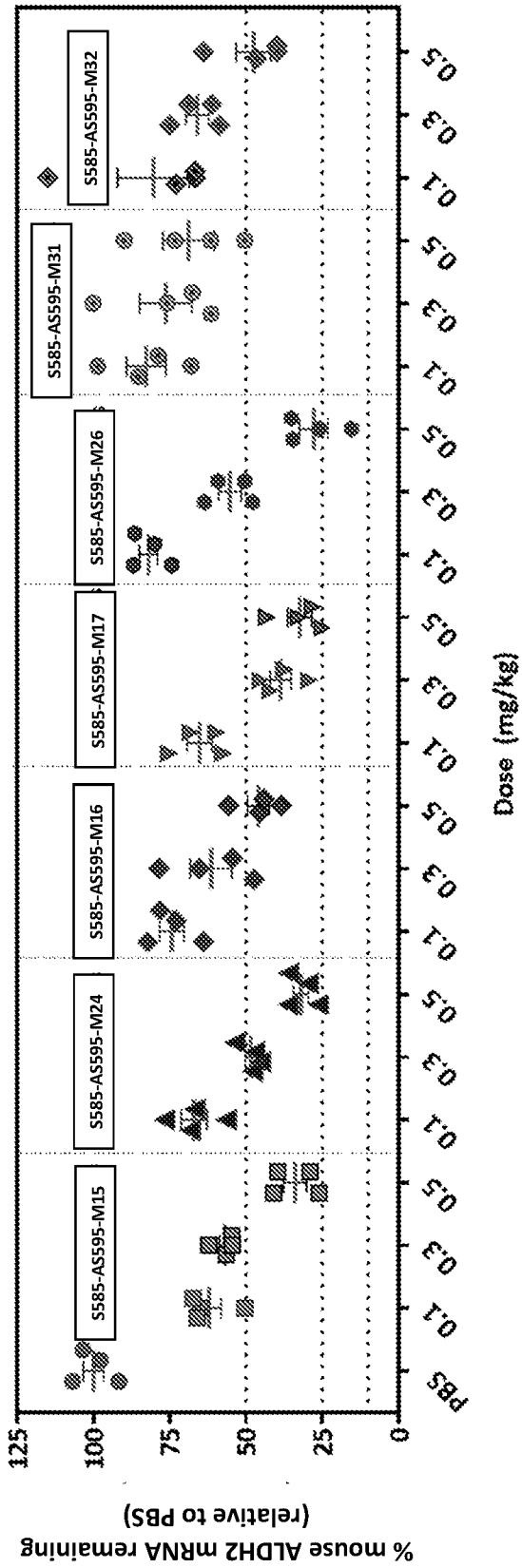

ALDH2 dose response in CD-1 mice; Day 3 knockdown data; 3' assay
% mouse ALDH2 mRNA remaining (relative to PBS)
125
100
75
50
25
0
S585-AS595-M15
S585-AS595-M24
S585-AS595-M16
S585-AS595-M17
S585-AS595-M26
S585-AS595-M31
S585-AS595-M32
PBS
0.1
0.3
0.5
1
3
5
10
Dose (mg/kg)

FIG. 12

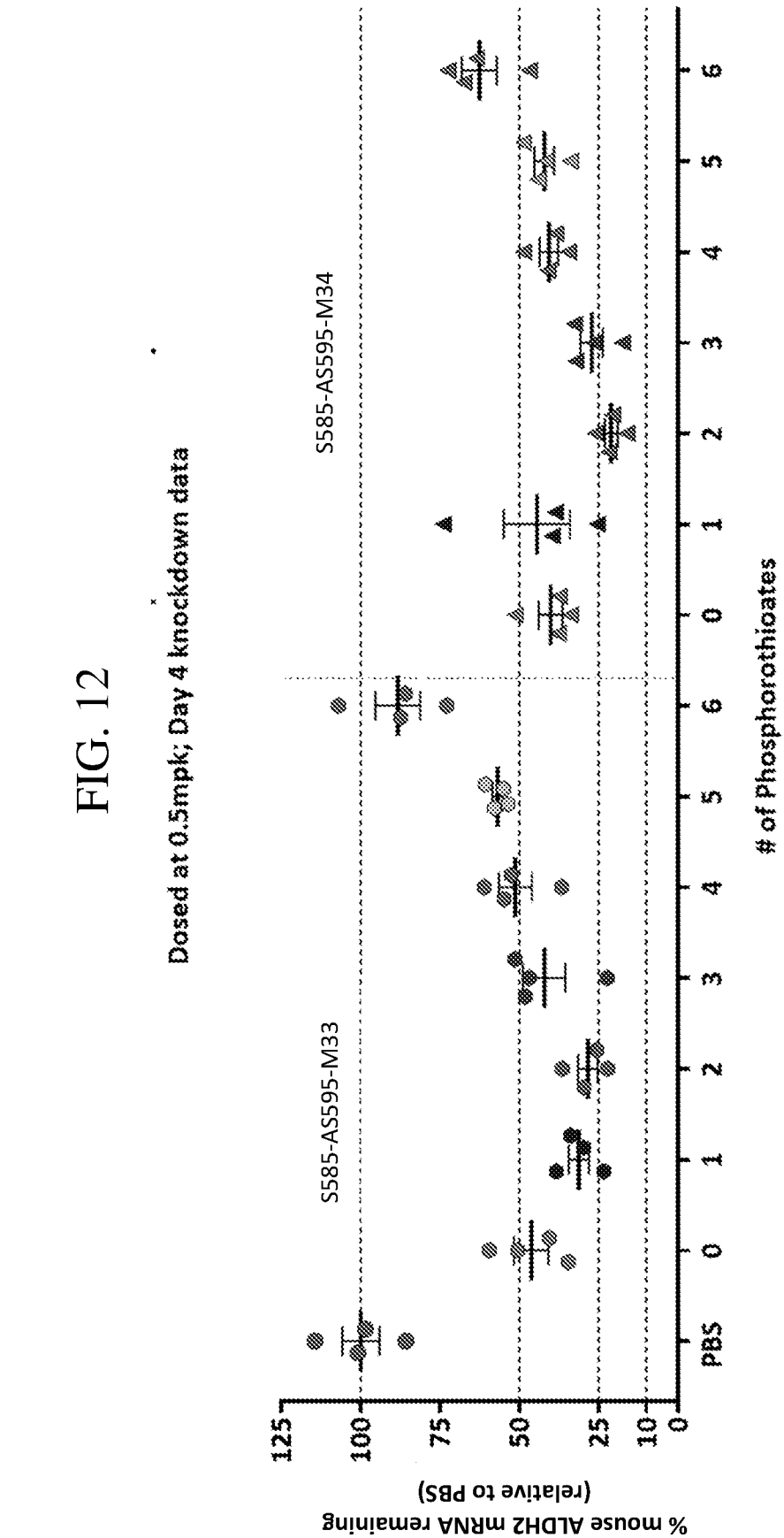
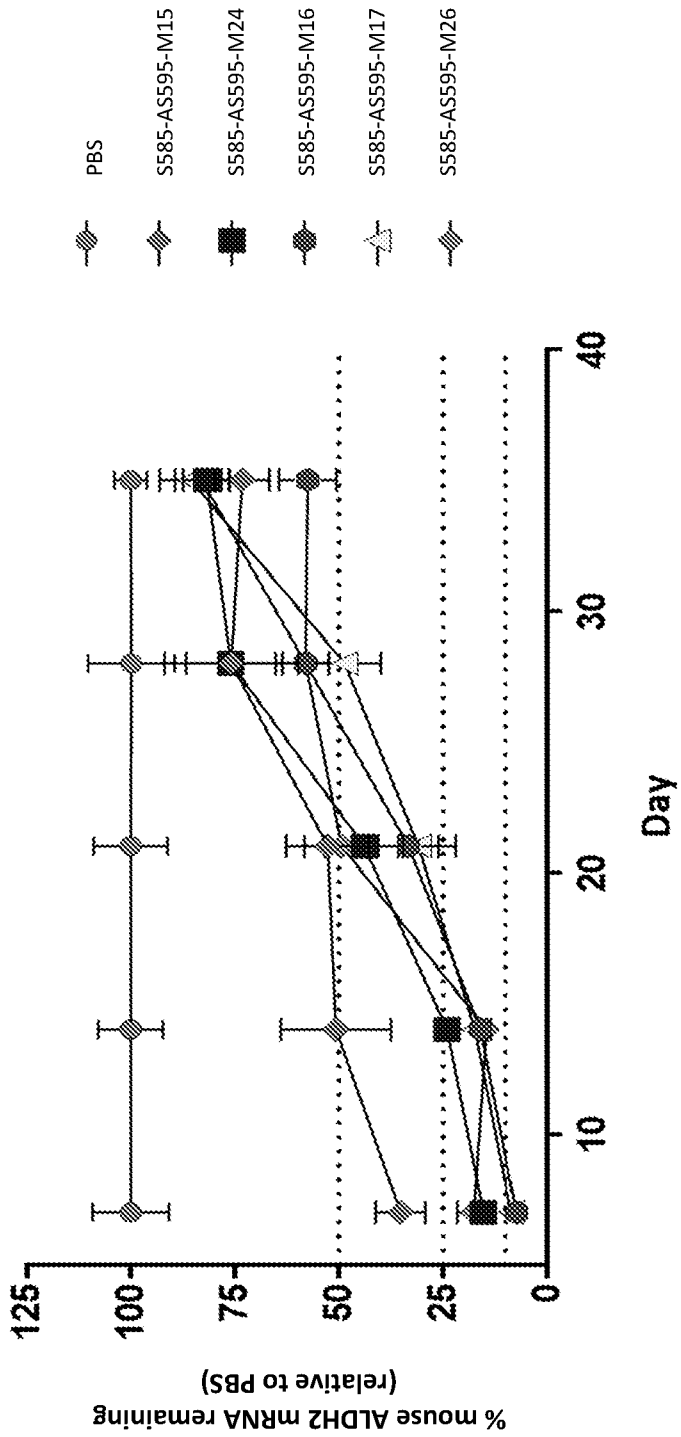

Dosed at 0.5mpk; Day 4 knockdown data
S585-AS595-M33
S585-AS595-M34
% mouse ALDH2 mRNA remaining
(relative to PBS)
125
100
75
50
25
10
0
PBS
0
1
2
3
4
5
6
# of Phosphorothioates

FIG. 13


ALDH2 Duration study
% mouse ALDH2 mRNA remaining
(relative to PBS)
125
100
75
50
25
0
10
20
30
40
Day
PBS
S585-AS595-M15
S585-AS595-M24
S585-AS595-M16
S585-AS595-M17
S585-AS595-M26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/13672

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/113; A61K 31/713, 31/712, 31/7115 (2019.01)

CPC - C12N 15/113, 15/1137; A61K 31/713, 31/712, 31/7115, 31/7125; C07H 21/02

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2014/0248338 A1 (PROTIVA BIOTHERAPEUTICS, INC.) 04 September 2014; paragraphs [0009], [0012], [0035], [0118], [0120], [0133], [0171], [0173], [0177], [0179], [0186]; claims 1, 11	20, 21 -------- 5, 6
Y	WO 01/16346 A1 (WISCONSIN ALUMNI RESEARCH FOUNDATION) 08 March 2001; page 9, lines 91-5; SEQ ID NO: 3	5, 6
A	US 2003/0032788 A1 (GARVER E, et al.) 13 February 2003; paragraphs [0036], [0037], [0297]	1, 2, 3/1-2, 52, 53
A	WO 2015/169973 A2 (PROTAGEN AG) 12 November 2015; SEQ ID NO: 39	1, 2, 3/1-2, 52, 53

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 May, 2019 (06.05.2019)	23 MAY 2019
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/13672

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4, 7-19, 22-51
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Continued Within the Next Supplemental Box-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 20, 21, 52, 53; SEQ ID NOs: 591, 581, 601

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US19/13672

-***-Continued from Box III: Lack of Unity of Invention-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-3, 5, 6, 20-22, 52, 53 and SEQ ID NOs: 591, 581 and 601 are directed toward sense and antisense oligonucleotides for reducing expression of ALDH2, wherein the antisense strands are complementary to sense strands to form a duplex in a stem-loop structure.

The oligonucleotides will be searched to the extent that they encompass a pair of RNAi oligonucleotides encompassing SEQ ID NOs: 591 (first exemplary antisense oligonucleotide), 581 (first exemplary sense oligonucleotide) and a target encompassing SEQ ID NO: 601 (first exemplary ALDH target sequence). Applicant is invited to elect additional oligonucleotide(s), and/or target(s) thereof with specified SEQ ID NO: for each, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), to be searched. Additional oligonucleotide sequence(s) and/or associated target sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1-3 (each in-part), 5 (in-part), 6 (in-part), 20-22 and 52-53 (each in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 591 (antisense oligonucleotide), SEQ ID NO: 581 (sense oligonucleotide) and SEQ ID NO: 601 (ALDH2 target sequence). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a pair of olignonucleotides encompassing SEQ ID NO: 592 (antisense oligonucleotide), SEQ ID NO: 582 (sense oligonucleotide), and SEQ ID NO: 602 (ALDH2 target sequence).

No technical features are shared between the oligonucletide sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of: an oligonucleotide for reducing expression of ALDH2, the oligonucleotide comprising an antisense strand comprising a defined sequence; an oligonucleotide for reducing expression of ALDH2, the oligonucleotide comprising an antisense strand of 15 to 30 nucleotides in length, wherein the antisense strand has a region of complementarity to a defined target sequence of ALDH2, wherein the region of complementarity is at least 15 contiguous nucleotides in length; an oligonucleotide for reducing expression of ALDH2, the oligonucleotide comprising an antisense strand and a sense strand, wherein the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to ALDH2, wherein the sense strand comprises at its 3'-end a stem-loop set forth as: S1-L-S2, wherein S1 is complementary to S2, and wherein L forms a loop between S1 and S2 of 3 to 5 nucleotides in length, and wherein the antisense strand and the sense strand form a duplex structure of at least 19 nucleotides in length but are not covalently linked; and an oligonucleotide for reducing expression of ALDH2, the oligonucleotide comprising a sense strand of 15 to 50 nucleotides in length and an antisense strand of 15 to 30 nucleotides in length, wherein the sense strand forms a duplex region with the antisense strand, wherein the sense strand comprises a defined sequence and wherein the antisense strand comprises a defined complementary sequence; however, these shared technical features are previously disclosed by US 2014/0248338 A1 to Protiva Biotherapeutics, Inc. (hereinafter 'Protiva').

Protiva discloses an oligonucleotide (antisense, siRNA oligonucleotides, etc.; paragraphs [0118], [0120]) for reducing expression of ALDH2 (inhibiting ALDH2 expression; paragraph [0009]; claim 11), the oligonucleotide comprising an antisense strand (paragraphs [0012], [0014]) comprising a defined sequence (paragraph [0017]); an oligonucleotide (antisense, siRNA oligonucleotides, etc.; paragraphs [0118], [0120]) for reducing expression of ALDH2 (inhibiting ALDH2 expression; paragraph [0009]; claim 11), the oligonucleotide comprising an antisense strand of 15 to 30 nucleotides in length (15 to 60 nucleotides in length; paragraph [0012]), wherein the antisense strand has a region of complementarity (paragraph [0034]) to a defined target sequence of ALDH2 (paragraphs [0084], [0120]), wherein the region of complementarity is at least 15 contiguous nucleotides in length (up to 16-contiguous homologous base pairs; paragraph [0130]); an oligonucleotide (antisense, siRNA oligonucleotides, etc.; paragraphs [0118], [0120]) for reducing expression of ALDH2 (inhibiting ALDH2 expression; paragraph [0009]; claim 11), the oligonucleotide comprising an antisense strand and a sense strand (paragraph [0012]; claim 1), wherein the antisense strand is 21 to 27 nucleotides in length (15 to 60 nucleotides in length; paragraph [0012]) and has a region of complementarity (paragraph [0034]) to ALDH2 (paragraphs [0084], [0120]), wherein the sense strand comprises at its 3'-end a stem-loop set forth as: S1-L-S2 (stem-loop structure; paragraph [0133]), wherein S1 is complementary to S2, and wherein L forms a loop between S1 and S2 regions in the loop(self-complementary stem-loop structure; paragraphs [0035], [0133]) of 3 to 5 nucleotides in length (loop of 1-25 nucleotides; paragraph [0186]), and wherein the antisense strand and the sense strand form a duplex structure of at least 19 nucleotides in length (duplex of 15-60 nucleotides; paragraph [0035]) but are not covalently linked (base-paired linkages; paragraph [0133]); and an oligonucleotide (antisense, siRNA oligonucleotides, etc.; paragraphs [0118], [0120]) for reducing expression of ALDH2 (inhibiting ALDH2 expression; paragraph [0009]; claim 11), the oligonucleotide comprising a sense strand of 15 to 50 nucleotides in length (15 to 60 nucleotides in length; paragraph [0012]) and an antisense strand of 15 to 30 nucleotides in length (15 to 60 nucleotides in length; paragraph [0012]), wherein the sense strand forms a duplex region with the antisense strand (duplex of 15-60 nucleotides; paragraph [0035]), wherein the sense strand comprises a defined sequence (paragraph [0017]) and wherein the antisense strand comprises a defined complementary sequence (paragraph [0017]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Protiva reference, unity of invention is lacking.