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COMPOUNDS FOR THE INHIBITION OF miRNA

FIELD OF THE INVENTION

[001] The invention relates generally to drug, bio-affecting and body treating compositions, generally to organic compounds, particularly to nucleic acid expression inhibitors, and more particularly to anti-miRNA oligonucleotides.

BACKGROUND OF THE INVENTION

[002] MicroRNAs (or miRNAs) are small, naturally-occurring, double-stranded RNA molecules that participate in gene regulation. They are non-coding, but target the mRNA of protein-coding genes for cleavage or repression of translation. miRNAs appear in many organisms, including plants, invertebrates such as nematodes, and vertebrates, from fish such as zebrafish to humans. They are a family of molecules, differing from each other in sequence. Many hundreds have been identified in humans, and there may be as many as 1000 or more. Up to 60% or more of human genes may be regulated, at least in part, by miRNAs. Friedman et al. 2009 Genome Res. 19: 92-105.

[003] miRNAs are sequence-specific, though this specificity is imperfect. The key recognition site is the seed region sequence (positions 2-8 of the antisense strand). Sequence identity at other positions is less critical. Therefore, a single miRNA can regulate several genes with slightly different sequences. In addition, a single gene can be regulated by several miRNAs.

[004] Of the genes regulated at least in part by miRNAs, many are involved in diseases. For example, miRNAs are known to be overexpressed in many cancers. Several miRNAs are located at the sites of translocation breakpoints or deletions linked to human leukemias. Calin et al. 2004 Proc. Natl. Acad. Sci. USA 101: 2999-3004. Altered miRNA expression has been reported in leukemia, lung cancer, and colon cancer. Calin et al. 2002 Proc. Natl. Acad. Sci. USA 99: 15524-9; Calin et al. 2004 Proc. Natl. Acad. Sci. USA 101: 11755-60; and Michael et al. 2003 Mol. Cancer Res. 1: 889-91.

[005] Expression or over-expression of several miRNAs is directly implicated in different proliferative diseases. The miRNA miR-21 blocks expression of critical apoptosis-related genes in human glioblastoma tissues; knockdown of this miRNA leads to increased apoptotic cell death. Chan et al. 2005 Cancer Res. 65: 6029-6033. Over-expression of miR-21, which is also frequently highly expressed in haematological malignancies, results in a pre-B malignant lymphoid-like phenotype; miR-21 inactivation leads to apoptosis and tumor regression. Medina et al. 2010 Nat. 467: 86-90. Over-expression of the oncogenic miR-17-92 cluster leads to a lymphoproliferative disorder, and higher level expression of the cluster in MYC-driven B cell lymphomas dramatically increased tumorigenicity. He et al. 2005

Nature 435: 828-833; Xiao et al. 2008 Nat. Immun. 9: 405-414. Ectopic expression of miR-155 in bone marrow induced polyclonal pre-B cell proliferation progressing to B cell leukaemia or myeloproliferation in mice. Costinean et al. 2006 Proc. Natl. Acad. Sci. USA 103: 7024-7029; and O'Connell et al. 2008 J. Exp. Med. 205: 585-594.

[006] Thus, a need exists for novel methods and compositions for inhibiting miRNAs, including those involved in diseases.

SUMMARY OF THE INVENTION

[007] In a first embodiment, the miRNA inhibitor comprises a polymer, e.g., a molecule comprising multiple mers (subunits), wherein a mer can be a nucleotide (nt), modified nucleotide or nucleotide analogue. In a second embodiment, the polymer is about 6 to about 21 mers long.

[008] In a third embodiment, the invention provides a miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises a region of complementarity complementary to the Dicer-excised region (or a portion thereof) of a pri-miRNA hairpin or pre-miRNA hairpin, wherein (a) the region of complementarity is at the 3' end of the miRNA inhibitor and the mer at the 3' end of the region of complementarity anneals to any of the first to fifth nt counting from the 5' end of the Dicer-excised region; or (b) the region of complementarity is at the 5' end of the miRNA inhibitor, and the mer at the 5' end of the region of complementarity anneals to any of the third to seventh nt counting from the 3' end of the Dicer-excised region.

[009] In a fourth embodiment, the invention provides an miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises a region of complementarity complementary to the Dicer-excised region (or a portion thereof) of a pri-miRNA hairpin or pre-miRNA hairpin, wherein the region of complementarity is at the 3' end of the miRNA inhibitor and the mer at the 3' end of the region of complementarity anneals to any of the third to seventh nt counting from the 3' end of the Dicer-excised region.

[0010] In a fifth embodiment, the miRNA inhibitor anneals to a pri-miRNA and/or pre-miRNA in a sequence-dependent manner and comprises a sequence which can straddle a Dicer or Drosha cleavage site and/or anneal to the Dicer-excised region of the pri-miRNA and/or pre-miRNA and reduce production of the miRNA from the pri-miRNA and/or pre-miRNA.

[0011] In a sixth embodiment, the entire miRNA inhibitor is complementary to the Dicer-excised region (or a portion thereof); thus, the miRNA inhibitor consists of a region of complementarity.

[0012] In a seventh embodiment, the region of complementarity has 1 to 3 mismatches to the Dicer-excised region or Drosha-excised region.

[0013] In an eighth embodiment, the miRNA inhibitor is at least about 6 mers long. In a ninth embodiment, the miRNA inhibitor is no more than about 21 mers long. In a tenth

embodiment, the miRNA inhibitor is about 6 to about 21. In an eleventh embodiment, the miRNA inhibitor is 6 to 21 mers long.

[0014] In a twelfth embodiment, the miRNA inhibitor comprises a first strand (thus, the miRNA inhibitor is single-stranded). In a thirteenth embodiment, the miRNA inhibitor comprises a first and a second strand (thus, the miRNA inhibitor is double-stranded).

[0015] In a fourteenth embodiment, the miRNA inhibitor comprises the full sequence of, or at least about 15 contiguous positions of any miRNA inhibitor disclosed herein. In a fifteenth embodiment, the sequence of the miRNA inhibitor is the sequence of any miRNA inhibitor disclosed herein, and the miRNA inhibitor is modified or unmodified, and comprises any combination of nucleotides, modified nucleotides and nucleotide analogues.

[0016] In sixteenth embodiment, the invention provides a method of reducing the formation of a miRNA from a pri-miRNA and/or pre-miRNA, the method comprising the step of contacting the pri-miRNA and/or pre-miRNA with a miRNA inhibitor as described herein.

[0017] In a seventeenth embodiment, the invention provides a method of treating, preventing or ameliorating a disease or condition related to expression or over-expression a miRNA (e.g., a miRNA-related disease), wherein the method comprises the step of administering a therapeutically effective amount of a miRNA inhibitor of the invention. In an eighteenth embodiment, the miRNA inhibitor is administered along with a pharmaceutically acceptable carrier.

[0018] In a nineteenth embodiment, the miRNA inhibitor inhibits miR-145, miR-208a, miR-137 or miR-122. In a twentieth embodiment, the invention provides methods of using these miRNA inhibitors to inhibit the miRNA, and/or to treat, prevent or ameliorate a disease related to expression or over-expression of the miRNA. In a twenty-first embodiment, the invention provides for the use of the miR-145 inhibitor in the treatment or amelioration of various diseases related to expression or over-expression of miR-145, including Marfan syndrome, Loeys-Dietz syndrome, Takayasu's arteritis, giant cell arteritis, liver fibrosis, kidney fibrosis, heart fibrosis, respiratory disease, pulmonary arterial hypertension (PAH), idiopathic pulmonary fibrosis (IPF), Asthma, Chronic Obstructive Pulmonary Disease (COPD), chronic/scarless wound healing, and primary open angle glaucoma (POAG). In a twenty-second embodiment, the invention provides for the use of the miR-208a inhibitor in the treatment or amelioration of various diseases related to expression or over-expression of miR-208a, including Non-alcoholic fatty liver disease (NAFLD), Non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, heart disease, heart failure, acute coronary syndrome (ACS), hypertrophic heart dysfunction, acute myocardial infarction, obesity, fatty liver, metabolic syndrome, and Type II diabetes mellitus (T2DM). In a twenty-third embodiment, the invention provides for the use of the miRNA inhibitor in the treatment or amelioration of various diseases related to expression or over-expression of miRNA-137, or miRNA-137 variants

with a single nucleotide polymorphism, including schizophrenia. In a twenty-fourth embodiment, the invention provides for the use of the miRNA-122 inhibitor in the treatment or amelioration of various diseases related to expression or over-expression of miRNA-122, including viral infections, including HCV infection, and dysregulated homeostasis, including high cholesterol and fatty liver disease.

[0019] In a twenty-fifth embodiment, the invention provides a miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises a region of complementarity complementary to the Dicer-excised region (or a portion thereof) of a pri-miRNA hairpin or pre-miRNA hairpin, wherein (a) the region of complementarity is at the 3' end of the miRNA inhibitor and the mer at the 3' end of the region of complementarity anneals to any of the first to fifth nt counting from the 5' end of the Dicer-excised region; or (b) the region of complementarity is at the 5' end of the miRNA inhibitor, and the mer at the 5' end of the region of complementarity anneals to any of the third to seventh nt counting from the 3' end of the Dicer-excised region. In a twenty-sixth embodiment, the invention provides a miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises a region of complementarity complementary to the Dicer-excised region (or a portion thereof) of a pri-miRNA hairpin or pre-miRNA hairpin, wherein the region of complementarity is at the 3' end of the miRNA inhibitor and the mer at the 3' end of the region of complementarity anneals to any of the third to seventh nt counting from the 3' end of the Dicer-excised region. In a twenty-seventh embodiment, the entire miRNA inhibitor is complementary to the Dicer-excised region (or a portion thereof); thus, the miRNA inhibitor consists of a region of complementarity. In a twenty-eighth embodiment, the region of complementarity has 1 to 3 mismatches to the Dicer-excised region.

[0020] In a twenty-ninth embodiment, the miRNA inhibitor can anneal to a pri-miRNA and/or pre-miRNA in a sequence-dependent manner and comprises a sequence that can straddle a cleavage site and/or anneal to the Dicer-excised region of the pri-miRNA and/or pre-miRNA and reduce production of the miRNA from the pri-miRNA and/or pre-miRNA.

[0021] In a first aspect, and without being bound by any particular theory or proposed biochemical mechanism, at least some of the miRNA inhibitors of the invention can mediate invasion of the stem loop of a miRNA, leading to repression of Dicer- or Drosha-mediated cleavage and thus decrease production of the mature miRNA.

[0022] In a thirtieth embodiment, the miRNA inhibitor inhibits miR-145, miR-208a, miR-137 or miR-122. In a thirty-first embodiment, the invention provides methods of using these miRNA inhibitors to inhibit the miRNA, and/or to treat, prevent or ameliorate a disease related to expression or over-expression of the miRNA.

[0023] The details of one or more aspects of the invention are set forth in the accompanying drawings and the description below. Elements of the various aspects (e.g., sequences, modifications, substitutions, spacers, modified internucleoside linkers, endcaps,

combinations of miRNA inhibitors, delivery vehicles, combination therapy involving a miRNA inhibitor and another agent, etc.) disclosed herein or known in the art that are not mutually exclusive can be combined with each other, provided the inhibitor or inhibitors are still capable of inhibiting miRNA. For example, any miRNA inhibitor sequence disclosed herein can be combined with any set of modifications or endcaps disclosed herein. Similarly, any combination of modifications, 5' end modification, and/or 3' end modification can be used with any miRNA inhibitor sequence disclosed herein. Any miRNA inhibitor disclosed herein (with any combination of modifications or 5' or 3' end modification or without either modifications or 5' or 3' end modifications) can be combined with any other miRNA inhibitor or other treatment composition or method disclosed herein.

[0024] Other features, objects, and advantages of the invention will be apparent from this description, the drawings, and from the claims.

BRIEF DESCRIPTION OF THE FIGURES

[0025] FIG. 1 shows an anti-miR oligonucleotide (AMO) walk. (A) Lowest energy predicted secondary structure of human miR-145 stem loop hairpin using MC-FOLD algorithm (Parisien & Major 2008 Nature 452, 51-55). miR-145-5p (red), the terminal loop (blue), and miR-145-3p (yellow) sequences are highlighted. (B) Linearized hsa-miR-145 stem loop hairpin. A miR-145 AMO hairpin 'walk' containing all possible 21mer oligonucleotides was generated to interrogate the optimal sequences for stem loop hairpin 'invasion' and repression of Dicer-mediated processing.

[0026] FIG. 2 shows miR-145 stem loop hairpin binding and in vitro Dicer processing screens. (A) In vitro binding of AMOs (analytes) was assessed by surface plasmon resonance assay (SPR) and plotted as association (report point) versus the mapped position (5' end of AMO) on the miR-145 stem loop hairpin (ligand). (B) Gel-based in vitro Dicer enzymatic assay assessing processing of the pre-miR-145 hairpin (substrate) $\pm$ pre-incubation with AMO inhibitors. Processing is reported as densitometry of the miR-145 duplex (product) relative to total intensity in the lane and normalized to product production in the absence of AMO inhibitors. (C) Correlation plot of miR-145 processing versus binding. (D, E) Mapping of 05AF (2'MOE chemistry) (D) and 15MB (2'F/2'MOE mixmer chemistry) (E) as representative examples of the two major 'hot spots' for binding and biogenesis inhibition. Alignments are consistent with AMO nucleation in the single-stranded terminal loop from the 5' and 3' ends, respectively and disruption of the miR-145 hairpin structure at the distal Dicer cut site.

[0027] FIG. 3 shows confirmation of miR-145 stem loop hairpin invasion using biochemical and cell-based assays. (A) Full kinetic analysis by SPR of 15MB and LXH874 (a peptide nucleic acid (PNA) targeting the same nucleotide sequence as 15MB) binding to miR-145

stem loop hairpin ligand. (B) Gel-based in vitro Dicer processing assay highlighting binding in the absence of Dicer (Lane 1 vs. Lane2; gel shift) and processing inhibition in the presence of Dicer (Lane 3 vs. Lane 4; duplex production) for 15MB and the pre-miR-145 stem-loop hairpin substrate. (C) Comparison of 15MB and LXH874 with respect to binding (gel-shift) and processing inhibition (product production) in the presence of Dicer. (D) Inhibition of TGF β 1-dependent 'phenotypic switching' in primary human pulmonary arterial smooth muscle cells (PASMCs) using an α -smooth muscle actin immunofluorescence assay. 20HB and 15MB are highlighted as representative examples of inactive and active AMOs, respectively.

[0028] FIG. 4 shows miR-208a stem loop hairpin binding and in vitro Dicer processing screens. (A) In vitro binding of AMOs (analytes) was assessed by SPR and plotted as association (report point) versus the mapped position (5' end of AMO) on the miR-208a stem loop hairpin (ligand). (B) Gel-based in vitro Dicer enzymatic assay assessing processing of the pre-miR-208a hairpin (substrate) $\pm$ pre-incubation with AMO inhibitors. Processing is reported as densitometry of the miR-208a duplex (product) relative to total intensity in the lane and normalized to product production in the absence of AMO inhibitors. (C) Correlation plot of miR-208a processing versus binding. (D, E) Mapping of 57MF (2'MOE chemistry) (D) and 68ME (2'MOE chemistry) (E) as representative examples of the two major 'hot spots' for binding and biogenesis inhibition. Alignments are consistent with AMO nucleation in the single-stranded terminal loop from the 5' and 3' ends, respectively and disruption of the miR-208a hairpin structure at the distal Dicer cut site.

[0029] FIG. 5 shows confirmation of miR-208a stem loop hairpin invasion using biochemical assays. (A) Full kinetic analysis by SPR of 57MF and 68ME to miR-208a stem loop hairpin ligand. (B) Comparison of 57MF and 68ME with respect to binding (gel-shift) and processing inhibition (product production) in the presence of Dicer.

[0030] FIG. 6 shows the AMO inhibition of pre-miR-137 processing by Dicer. (A) Plot of the amount of remaining miR-137 hairpin after incubation with Dicer and AMO molecules. The amount of pre-miRNA hairpin remaining is compared to the amount of pre-miRNA incubated without Dicer enzyme. A representative gel showing the pre-miRNA protection in the presence of AMOs is beneath the plotted data. (B) Structure of pri-miR-137 hairpin with mature miR-137 sequence in red, the loop sequence in blue, and the passenger strand in yellow. (C) Mapping of AMOs to the pri-miR-137 hairpin to show binding regions.

[0031] FIG. 7 shows the AMO inhibition of pre-miR-122 processing by Dicer. (A) Plot of the amount of mature miR-122 produced by Dicer in the presence of different AMOs. The production of mature miR-122 is compared to the processing in the absence of any inhibitor. (B) Structure of pri-miR-122 hairpin with mature miR-122 sequence in red, the loop

sequence in blue, and the passenger strand in yellow. (C) Mapping of AMOs to the pri-miR-122 hairpin to show binding regions.

DETAILED DESCRIPTION OF THE INVENTION

[0032] A mature miRNA is formed from a pre-miRNA, which is in turn formed from a pri-miRNA. This multi-step process requires cleavage by Drosha and Dicer, and involves various other biological components. Cleavage by Drosha and Dicer excise RNA adjacent to, but not a part of, the mature miRNA. The region excised by Dicer is termed herein the “Dicer-excised region” (and the like), and is a region that is not part of the mature miRNA. The region excised by Drosha is termed herein the “Drosha-excised region” (and the like), and is also a region that is not part of the mature miRNA.

[0033] While miRNA inhibitors known in the art generally bind to a mature miRNA, thus preventing the miRNA's activity after it has been formed, the miRNA inhibitors of the invention prevent or reduce formation of the miRNA inhibitor from the pri-miRNA and/or pre-miRNA. In a thirty-second embodiment, the miRNA inhibitors of the invention can invade the stem loop hairpin structure of a pri-miRNA and/or pre-miRNA, binding to the loop or other part of the Dicer-excised region or Drosha-excised region and/or straddling and preventing or reducing cleavage at a Dicer cleavage site and/or a Drosha cleavage site. In a thirty-third embodiment, the miRNA inhibitors of the invention bind to the Dicer-excised region of the pre-miRNA or pri-miRNA. This prevents cleavage and formation of the mature miRNA.

[0034] In a thirty-fourth embodiment, the miRNA inhibitor of the invention is a polymer, a molecule comprising multiple mers or subunits, wherein each mer is a nucleotide, modified nucleotide, or nucleotide analogue.

[0035] In a thirty-fifth embodiment, the miRNA inhibitor of the invention comprises a sequence of 15 contiguous positions of or comprises the sequence of any miRNA inhibitor disclosed herein. In a thirty-sixth embodiment, the sequence of the miRNA inhibitor of the invention has the sequence of any miRNA inhibitor disclosed herein.

[0036] In a thirty-seventh embodiment, the invention provides a method of reducing the formation of a miRNA from a pri-miRNA and/or pre-miRNA, the method comprising the step of contacting the pri-miRNA and/or pre-miRNA with a miRNA inhibitor of the invention as described herein.

[0037] In a thirty-eighth embodiment, the invention provides specific miRNA inhibitors that inhibit miR-145, miR-208a, miR-137 or miR-122. In a thirty-ninth embodiment, the invention provides methods of using these miR-145, miR-208a, miR-137 or miR-122 miRNA inhibitors in treating or ameliorating diseases related to expression or over-expression of these miRNAs.

DEFINITIONS

[0038] The terms “microRNA” or “miRNA” or “miR” or the like mean small RNA molecules that naturally participate in gene regulation; these are naturally produced in the cells of many organisms, including plants, and invertebrates and vertebrates, including humans. A miRNA comprises an antisense strand and (in some contexts) a sense strand. A miRNA antisense strand is generally 18-25 nt long and binds to fully or partially complementary target mRNAs, thereby targeting them for degradation or translational inhibition. The antisense strand can be fully or partially complementary to the sense strand. There may be as many as four mismatches or more between an antisense and a corresponding sense strand.

[0039] In some scientific references cited herein, the term “miRNA” refers to the combination of the antisense strand and the complementary sense strand. In other scientific references, the term “miRNA” refers only to the antisense strand. In still other references, the terminology “miRNA / miRNA*” is used to refer to the combination of a miRNA antisense and sense strand, wherein the term “miRNA” refers to the antisense strand and the term “miRNA*” refers to the sense strand. More recent research has shown that the sense strand is sometimes active (e.g., incorporated into the miRISC). Thus, the terms “5p” and “3p” are sometimes used to refer to strands that derive from the 5’ side or the 3’ side of the pre-miRNA hairpin, respectively. Sometimes, the antisense strand is termed the “guide strand”, and the sense strand is accordingly termed the “passenger strand”. As used herein, unless otherwise stated, the term “miRNA” will usually indicate the double-stranded molecule, including both anti-sense and sense strand.

[0040] The term “pri-miRNA” means an intermediate in miRNA biogenesis. miRNA biogenesis is complex. The genes encoding miRNAs are much longer than the processed mature miRNA. Many miRNAs are known to reside in introns of their host genes and share their regulatory elements and primary transcript, and have a similar expression profile. Some miRNA genes are transcribed from their own promoters. A microRNA is transcribed by RNA polymerase II as a large RNA precursor that is called a “pri-miRNA” and comprises a 5’ cap and a poly-A tail. The pri-miRNA is processed in the nucleus by the microprocessor complex, comprising the RNase III enzyme Drosha, and the double-stranded-RNA-binding protein, Pasha/DGCR8. The result is the “pre-miRNA”, which is approximately 70 nucleotides in length and folded into an imperfect stem-loop structure. The pre-miRNA is then exported into the cytoplasm by the karyopherin exportin 5 (exp5) and Ran-GTP complex. Ran (ras-related nuclear protein) is a small GTP binding protein that belongs to the RAS superfamily and is essential for the translocation of RNA and proteins through the nuclear pore complex. The Ran GTPase binds Exp5 and forms a nuclear heterotrimer with pre-miRNAs. Once in the cytoplasm, the pre-miRNA undergoes an additional processing step by the RNase III

enzyme Dicer, generating the miRNA. Thus, "pri-miRNA" or "pri-miRNA hairpin" is a naturally occurring RNA structure that is processed by Drosha to yield a pre-miRNA hairpin.

[0041] The terms "pre-miRNA" or "pre-miRNA hairpin" mean a naturally occurring RNA hairpin that is processed by Dicer to yield the mature miRNA. The "loop" (or "loop sequence") is the RNA nucleotide sequence that is between the Dicer cleavage positions in a pre-miRNA hairpin, e.g., the sequence extending from the 5'-cleavage Dicer cleavage site to the 3'-Dicer cleavage site of a pre-miRNA. Additional information about miRNAs and their biogenesis is available in the scientific literature, for example: Lee et al. 2004 EMBO J. 23: 4051-4060; Han et al. 2004 Genes Dev. 18: 3016-3027; Denli et al. 2004 Nature 432: 231-235; Yi et al. 2003 Genes Dev. 17: 3011-3016; Moore et al. 1993 Nature 365: 661-663; Lund et al. 2004 science 30: 95-98; Bernstein et al. 2001 Nature 409: 363-366; and Hammond et al. 2005 FEBS Lett. 579: 5822-5829.

[0042] The terms "miRNA inhibitor", "inhibitor of miRNA" and the like, as used herein, mean any composition or method capable of reducing the level, activity, stability and/or production of a miRNA. miRNA inhibitors include, inter alia, anti-miRNA oligonucleotides (AMOs) and other types of inhibitors. Several authors have described methods of inhibiting miRNAs. General inhibition of miRNA production can be mediated by factors inhibiting components which produce miRNAs, such as factors which generally inhibit Dicer, where the inhibitor effect is global, rather than specific to a particular miRNA. Specific inhibitors targeting individual miRNAs have been described, including AMOs.

[0043] The terms "anti-miRNA oligonucleotide", "anti-miRNA molecule", "anti-miRNA oligo", or "AMO" and the like, as used herein, mean a single or double-stranded molecule comprising nucleotides, modified nucleotides and/or nucleotide analogs, which recognizes (anneals to) a miRNA (or pre-miRNA or pri-miRNA) in a sequence-specific manner, and reduces the level, activity, stability and/or production of a miRNA. This sequence specificity, however, can be imperfect, as one or more mismatches may exist between the miRNA and the AMO. An AMO can comprise an antisense strand, or an antisense strand and a sense strand. Many AMOs are reviewed in, for example: Lennox et al. 2011 Gene Ther. 18: 1111-1120.

[0044] The term "polynucleotide", as used herein, means a polymer of nucleotides. By "nucleotide" or is meant a molecule consisting of a phosphate, a sugar and a base. A nucleotide can form a subunit of a polynucleotide such as a strand of RNA or DNA, or a subunit of a polymer or other molecule comprising one or more nucleotides and one or more of another type of molecule such as a modified nucleotide or nucleotide analogue. In the case of a ribonucleotide, the sugar is a ribose; a ribonucleotide is a subunit of RNA. In the case of a deoxyribonucleotide, the sugar is deoxyribose; a deoxyribonucleotide is a subunit

of DNA. A polynucleotide can comprise RNA, DNA, or both; one or more nucleotide can be modified or unmodified.

[0045] The terms “nucleotide,” “polynucleotide,” “ribonucleotide,” “RNA,” “DNA,” as used herein, encompass unmodified or modified variants of these molecules (e.g., a modified nucleotide or modified polynucleotide); a polynucleotide can comprise any number of one or more nucleotide, modified nucleotide and/or nucleotide analogues.

[0046] The terms “modified nucleotide” or “modified polynucleotide” and the like, as used herein, mean any nucleotide or polynucleotide with one or more modifications or substitutions of the phosphate, base, or sugar.

[0047] The terms “nucleotide analogue” or “nucleotide analog” or “nt analog” or “nucleotide substitute” or the like, as used herein, mean a polymer, or individual component (“mer”) of such a polymer, which is not a nucleotide, but which is capable of mediating sequence-specific annealing to a nucleotide.

[0048] The terms “minor groove binders”, “minor groove binding molecules”, “minor groove binding agent”, “MGB” and the like, as used herein, mean any molecule which is capable of binding non-covalently to the minor groove of a nucleic acid double helix in either a sequence-specific or sequence-independent manner. The outer surface of a double-helical nucleic acid such as DNA or RNA has two channels, namely the major and minor grooves. Both of these grooves contain chemical information by way of arrangements of hydrogen-bond donors and acceptors, electrostatic charges, dipoles, hydrophobic regions and so on. The major groove contains approximately twice the information content of the minor groove in terms of the number of potential hydrogen-bonding contacts. The major groove is therefore the preferred recognition site for cellular proteins such as control proteins, promoters and repressors. However, the minor groove is normally (with a few exceptions) relatively unoccupied. The vulnerability of the minor groove makes it a particularly useful target for compounds that bind to nucleic acids.

[0049] The term “pharmaceutical composition” comprises a pharmaceutically effective amount of one or more miRNA inhibitor, a pharmaceutically acceptable carrier, and, optionally, an additional disease treatment which works synergistically with the miRNA inhibitor.

[0050] The terms “pharmacologically effective amount,” “therapeutically effective amount” or simply “effective amount” mean the amount of a miRNA inhibitor effective to produce the intended pharmacological, therapeutic or preventive result. For example, if a given clinical treatment is considered effective where there is at least a 10% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to effect at least a 10% reduction in that parameter.

[0051] The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, lipid nanoparticles, saline, buffered saline, dextrose, water, glycerol, ethanol, or any other carrier known in the art, and combinations thereof. Any appropriate pharmaceutical carrier known in the art can be used in conjunction with the miRNA inhibitors disclosed herein.

[0052] The term "terminal loop" means the single-stranded portion of the stem loop hairpin structure of a pri-miRNA and/or pre-miRNA, wherein the sequence immediately 5' to the terminal loop and the sequence immediately 3' to the terminal loop are complementary to each other and form a double-stranded region; the terminal loop is not the stem.

[0053] The term "stem" means the substantially double-stranded portion of the stem loop hairpin structure of a pri-miRNA and/or pre-miRNA, which double-stranded portion is not the terminal loop. The term "substantially double-stranded" means that the stem is largely or mostly double-stranded, though it may comprise single-stranded regions.

[0054] The "Dicer-excised region (or portion or segment)" of a pre-miRNA or pri-miRNA is the region, portion, segment, etc., deleted after cleavage by Dicer. This region, portion, segment, etc., is not part of the mature miRNA, but is adjacent to it.

[0055] The term "anneals" means that a position (mer) of the miRNA inhibitor hydrogen bonds (e.g., base pairs) with the complementary base at a corresponding position of the target pre-miRNA or pri-miRNA. In general, G bonds to C, A to U. Thus, if for example, position 37 of the example pre-miRNA is U, then the mer of a miRNA inhibitor which anneals to position 37 would be A [or a modified or substitute base which hydrogen bonds to U, e.g., hypoxanthine (I)]. G can hydrogen bond with (anneal to) U, or I to A, forming a wobble base pair.

[0056] The term "SNALP" refers to a stable nucleic acid-lipid particle. A SNALP represents a vesicle of lipids coating a reduced aqueous interior comprising a nucleic acid. SNALPs are described, e.g., in U.S. Patent Application Publication Nos. 20060240093, 20070135372, and in International Application No. WO 2009082817.

[0057] By "straddles" is meant that the sequence (or its complement comprises at least one nucleotide on either side of a cleavage site.

[0058] The term "delay of progression" as used herein means administration of the combination to patients being in a pre-stage or in an early phase, of the first manifestation or a relapse of the disease to be treated, in which patients, e.g., a pre-form of the corresponding disease is diagnosed or which patients are in a condition, e.g., during a medical treatment or a condition resulting from an accident, under which a corresponding disease will likely develop.

[0059] "Jointly therapeutically active" or "joint therapeutic effect" means that the compounds may be given separately (in a chronically staggered manner, especially a

sequence-specific manner) in such time intervals that they preferably, in the warm-blooded animal, especially human, to be treated, still show a (preferably synergistic) interaction (joint therapeutic effect). Whether this is the case, can inter alia be determined by following the blood levels, showing that both compounds are present in the blood of the human to be treated at least during certain time intervals.

miRNA in human diseases or conditions

[0060] miRNAs are a family of molecules differing from each other in sequence. Many hundreds of them have been identified in humans, and there may be as many as 1000 or more. Up to 60% or more of human genes may be regulated, at least in part, by miRNAs. Friedman et al. 2009 Genome Res. 19: 92-105.

[0061] miRNAs have many important or essential functions in various biochemical processes. These include differentiation, apoptosis proliferation, stress response, and cell fate determination. Hornstein et al. 2010 Cell Cycle 9: 4041-42. In *Caenorhabditis elegans*, miRNAs regulate temporal transitions between developmental stages. Feinbaum et al. 1992 Dev. Biol. 210: 87-95; Olsen et al. 1999 Dev. Biol. 216: 671-80. In *Drosophila*, the miRNA bantam both prevents apoptosis and stimulates cell proliferation by suppressing the proapoptotic gene hid. Bennecke et al. 2003 Cell 113: 25-36. In mammals, functions for specific miRNAs have been described in the processes of hematopoietic and adipocyte differentiation. Chen et al. 2004 Science 303: 83-6; and Esau et al. 2004 J. Biol. Chem. 279: 52361-5. miRNAs are also involved in insulin secretion. Poy et al. 2004 Nature 432: 226-30. miRNAs are precisely regulated and characteristic patterns of miRNA expression appear during brain development and neuronal differentiation. Krichevsky et al. 2003 RNA 9: 1274-81; Miska et al. 2004 Genome Biol. 5: R68; and Sempere et al. 2004 Genome Biol. 5: R13.

[0062] Many miRNAs are also involved in disease states. Dysregulation of miRNAs have been implicated in inflammatory and autoimmune disease, neurological disorders, myocardial disease and several types of cancer. See, Haramati et al. 2010 Proc. Natl. Acad. Sci. USA 107: 13111-13116; Thum et al. 2008 Nature 456: 980-984; and Garzon et al. 2010 Nat. Rev. Drug Disc. 9: 775-789. miRNAs are known to be overexpressed in many cancers. Some are important factors in the development or maintenance of the neoplastic state.

[0063] For example, miRNA miR-21 is elevated in human glioblastoma tumor tissues, early-passage glioblastoma cultures and in six established glioblastoma cell lines. This miR contributes to the malignant phenotype by blocking expression of critical apoptosis-related genes. Knockdown of miR-21 triggers activation of caspases and leads to increased apoptotic cell death. Chan et al. 2005 Cancer Res. 65: 6029-6033. Additional information about miRNAs can be found in the literature, e.g., Bartel. 2004 Cell 116: 281-297; Yi et al. 2003 Genes Dev. 17: 3011-3016; Lee et al. 2003 Nature 425: 415-419; Lee et al. 2002

EMBO J. 21: 4663-4670; Bartel et al. 2009 Cell 136: 215-233; Brodersen et al. 2009 Nat. Rev. Mol. Cell. Biol. 10: 141-148; Carthew et al. 2009 Cell 136: 642-655; Winter et al. 2009 Nat. Cell Biol. 11: 228-234; Ghildiyal et al. 2009 Nat. Rev. Genet. 10: 94-108; Kim et al. 2009 Nat. Rev. Mol. Cell. Biol. 10: 126-139; and Krol et al. 2010 Nat. Rev. Genet. 11: 597-610.

[0064] A variety of miRNAs are known. These include several which are described in more detail below, and to which AMOs are shown herein. These include: miR-145, miR-208a, miR-137 and miR-122.

miR-145

[0065] miR-145 (also known as miRNA145 and MIRN145) is a miRNA involved in several diseases, including respiratory diseases, including pulmonary arterial hypertension (PAH). 2009 Nature 46, 705-710. miR-145 plasma expression is increased in PAH patients compared to matched controls. Inhibition of miR-145 will block pathophysiological vascular remodeling (SMA+ cell hyperplasia) in PAH. miR-145 is also involved in idiopathic pulmonary fibrosis (IPF). miR-145 expression increases in TGFβ1-treated lung fibroblasts and IPF patient lung samples compared to normal lung. miR-145 over-expression in lung fibroblasts represses KLF4 and increases SMA expression; miR-145 inhibition reduces TGFβ1-induced SMA expression. miR-145 $-/-$ mice are protected from bleomycin-induced pulmonary fibrosis. See, for example, 2013 FASEB J. 27: 2382-92. miR-145 is also involved in Asthma/Chronic Obstructive Pulmonary Disease (COPD). TGFβ, IFN-β and IFN-γ increase miR-145/SMA expression in human airway SMCs and/or fibroblasts. miR-145 increases in house dust mite (HDM) acute asthma mouse model. Intranasal delivery of 2'OMe-AMO prevents development of allergic airway disease in HDM model; blocks TH2 cytokines, mucous hypersecretion and airway hyper-responsiveness; the effects are equivalent to DEX. See, for example, 2011 J. Allergy Clin. Immunol. 128: 16-176. miR-145 is also believed to be involved in chronic/scarless wound healing and/or liver fibrosis and primary open angle glaucoma (POAG). miR-145 is also associated with Marfan syndrome, Loeys-Dietz syndrome, Takayasu's arteritis, giant cell arteritis, liver fibrosis, kidney fibrosis, and heart fibrosis. miR-145 is thus associated with various diseases, including Marfan syndrome, Loeys-Dietz syndrome, Takayasu's arteritis, giant cell arteritis, liver fibrosis, kidney fibrosis, heart fibrosis, respiratory disease, pulmonary arterial hypertension (PAH), idiopathic pulmonary fibrosis (IPF), Asthma, Chronic Obstructive Pulmonary Disease (COPD), chronic/scarless wound healing, and primary open angle glaucoma (POAG). A miR-145 inhibitor can thus be used to treat any of these diseases, or any other disease known in the art related to expression or over-expression of miR-145.

[0066] Anti-miR-145 oligos can be designed and tested for the ability to decrease production of a mature miR-145 from the corresponding pre-miR-145. In a fortieth embodiment, the

putative anti-miR-145 oligos are designed from simple walking through the pre-miR-145 sequence.

[0067] The efficacy of putative AMOs to miR-145 can be tested by various methods known in the art. The efficacy of putative AMOs to miR-145 can be tested, for example, in a SPR screen (see Example 1, for example). In addition, anti-miR-145 oligos can be tested using 1° human pulmonary arterial smooth muscle cells (PASMCs) (See, for example, the Immunofluorescence assay, in Example 1). The efficacy of putative AMOs to miR-145 can also be tested in vitro using a Dicer processing assay (see also, Example 1). In this assay, the pre-miR-145 (e.g., the substrate) is mixed with Dicer and/or the putative AMO; the various products are examined electrophoretically, and the presence is determined of: the complex of the pre-miR-145 with the putative AMO, the pre-miR-145 substrate; the mature miR-145; the AMO; and other products. A decrease in the production of the mature miR from the pre-miR indicates that the AMO is effective. Similar assays can be used to test the activity of other putative AMOs on their corresponding pre-miR.

[0068] AMOs to miR-145 can thus be used in methods of treating any of the indications related to miR-145 described herein.

miR-208a

[0069] miR-208a (also known as miR208a, MIRNA208A, MIRN208a, and the like) is a miRNA specifically expressed in the heart and other organs, and is involved in various diseases. miR-208a can regulate the expression of myosin heavy chain (MHC) gene, is closely related to the differentiation of cardiac embryonic stem cells and involved in the myocardial fibrosis and hypertrophy via regulating subunits alpha and beta of MHC. Evidence in the art shows that the expression of miR-208a and miR-208b in ST-segment elevation acute myocardial infarction (STEMI) patients increased significantly when compared with healthy controls and chest pain patients without coronary lesions. Bi et al. 2015 Int. J. Clin. Exp. Med. 8: 4275-4280; Cortez et al. 2013 J. Thorac. Oncol. 8: 1156-1162. miR-208a is associated with heart disease, including heart failure. High miR-208a expression is also associated with acute coronary syndrome (ACS). Bi et al. 2015 Int. J. Clin. Exp. Med. 8: 4275-4280. miR-208a is also a key factor in hypertrophic heart dysfunction. Doka et al. 2015 J. Hypertension. miR-208a was also undetectable in non-AMI (acute myocardial infarction) patients, but detectable in all or almost all patients with AMI within 4 hours. Wang et al. 2010 Eur. Heart J. 31: 659-666. See also, WO2008016924 and US20050182005. miR-208a is also implicated in obesity, and administration of Nebivolol (Neb), a β_1 adrenergic receptor blocker, suppressed miR-208a and is thought to underlie Neb-induced body weight loss in leptin-resistant rats. Mahmood et al. 2015 FASEB J. 29 (supp. 1): 716.15. Inhibiting miR-208a can be useful in de-bulking fatty liver. miR-208a-

related indications also include metabolic syndrome and Type II diabetes mellitus (T2DM). miR-208a is also associated with Non-alcoholic fatty liver disease (NAFLD), Non-alcoholic steatohepatitis (NASH), liver fibrosis, and cirrhosis. miR-208a expression or over-expression is thus associated with several diseases, including Non-alcoholic fatty liver disease (NAFLD), Non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, heart disease, heart failure, acute coronary syndrome (ACS), hypertrophic heart dysfunction, acute myocardial infarction, obesity, fatty liver, metabolic syndrome, and Type II diabetes mellitus (T2DM). A miR-208a inhibitor can thus be used to treat any of these diseases or any other disease known in the art related to expression or over-expression of miR-208a.

miR-137

[0070] miR-137 (also known as miR137 and MIRN137) is highly expressed in the brain, and is involved in various diseases, including schizophrenia. Non-coding variants of miR-137 increase schizophrenia risk with genome-wide significance. A study of induced human neurons harboring the minor alleles of four disease-associated single nucleotide polymorphisms (SNPs) in miR-137 showed increased miR-137 levels compared to those in major allele-carrying cells. miR-137 gain-of-function caused downregulation of the presynaptic target genes complexin-1 (Cplx1), Nsf and synaptotagmin-1 (Syt1). Siebert et al. 2015 Nature Neurosci. 18: 1008-1016. Sometimes the variants are in the microRNA sequence itself. Sometimes they are localized to flanking regulatory regions that may or may not result in differential regulation of expression. Also, SNPs in mRNAs can either create or destroy miRNA target sites. Any of these scenarios can be a source of increased disease risk.

[0071] Abnormal expression of miR-137 affects vesicle release at presynaptic terminals and in turn alters hippocampal functioning. Han et al. 2015 Nature Neurosci. 18: 931-933. See also, Yates et al. 2015 Nature Rev. 16, 373. miRNA-137, and miRNA-137 variants with a single nucleotide polymorphism, are thus associated with schizophrenia. A miR-137 inhibitor can be used to treat this disease or any other disease known in the art related to expression or over-expression of miR-137. However, down-regulated miR-137 has been observed in various cancers such as colorectal cancer, gastric cancer, oral cancer, squamous cell carcinoma of the head and neck and breast cancer, as reviewed in Lee et al. Oncotarget, May 20 2015 and references cited therein.

miR-122

[0072] miR-122 (also known as miR122 and MIRN122) is largely expressed in the liver, and is associated with various diseases, including various liver diseases. miR-122 is a completely conserved liver-specific miRNA in vertebrates, and is essential for the

maintenance of liver homeostasis. This 22-nt miRNA regulates diverse functions, such as cholesterol, glucose and iron homeostasis, lipid metabolism and infection of hepatitis C virus (HCV) and the parasitic protozoa, *Leishmania donovani*. miR-122 expression is reduced in non-alcoholic steatohepatitis (NASH) patients, and in a subset of hepatocellular carcinoma (HCC) patients including Hepatitis B virus (HB) positive patients with highly invasive and metastatic cancer. Some studies, however, have shown some uses for miR-122 inhibitors (e.g., antigomirs). Silencing miR-122 resulted in a decrease in serum cholesterol levels compared to controls that correlated with down regulation of expression of many genes involved in cholesterol biosynthesis and transport. Similar observations were reported by silencing miR-122 in normal mice and mice that developed fatty liver upon feeding a high-fat diet. An anti-miR-122 which was a LNA showed a significant lowering of plasma cholesterol. Thus, anti-miR-122 molecules may be useful in treating high cholesterol, fatty liver disease and related diseases. Thakral et al. 2015 Curr. Gene Ther. 15:142-150 and references cited therein. miR-122 has a key role in some viral infections, facilitating replication of infectious viruses in hepatic cells. Jopling et al. 2005 Science 309: 1577-81. Santaris Pharma has developed miravirsin, an anti-miR-122 drug that showed promising results in chronic HCV type 1 infected patients. Janssen et al. 2013 N. Eng. J. Med. 368:1685-94. Thus, anti-miR-122 molecules may be useful in treating viral infections in the liver, including Hepatitis C virus. Lanford et al. 2010 Science 327: 198-201. miRNA-122 expression or over-expression is thus associated with viral infections, including HCV infection, and dysregulated homeostasis, including high cholesterol and fatty liver disease. A miR-122 inhibitor can thus be used to treat any of these diseases or any other disease known in the art related to expression or over-expression of miR-122.

LENGTH AND REVERSE COMPLEMENTARITY OF AMOs

[0073] Many AMOs have been described in the art. Of the myriad AMOs described in the art, almost all comprise a strand which is the same length as and completely reverse complementary to the miRNA antisense strand. A minority of AMOs in the art are shorter than the miRNA. In 2011, Lennox et al. noted that for earlier AMOs, "it is essential that the AMO binds with high affinity to the miRNA 'seed region,' which spans bases 2-8 from the 5' end of the miRNA [antisense strand]". Lennox et al. 2011 Gene Ther. 18: 1111-1120 (Abstract). Generally, an AMO known in the art can be shortened on its 5' end (the complement to the 3' end of the miRNA antisense strand), which is the end furthest from the seed region. The seed region (positions 2-8 of the miRNA antisense strand) is the most critical region for sequence recognition. Efficacious tiny AMOs have been constructed which are only 8 nt long and only bind to the seed region. As these are constructed from LNA, these are also known as "tiny LNAs". Obad et al. 2011 Nat. Genet. 43: 371-378.

[0074] In contrast to these AMOs shown in the art, an AMO of the invention need not recognize or anneal to the seed region of the AMO. Instead, an anti-miRNA oligo can, in fact, not anneal to the miRNA at all, but rather to a sequence adjacent to the miRNA, such as a Dicer-excised region. Alternatively, an anti-miRNA oligo can anneal to a portion of a miRNA and also a portion of an adjacent sequence, such as a Dicer-excised region or Drosha-excised region. Many efficacious AMOs are disclosed herein, wherein the AMO does not anneal to the seed region of the miRNA.

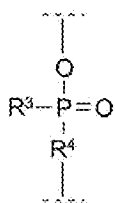
[0075] A miRNA inhibitor of the invention can be, for example, about 6 to about 21 mers long; about 6 to 21; 6 to about 21; 6 to 21; or 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 mers long; or longer than any of these lengths.

[0076] miRNA inhibitors of the invention, such as oligos that sequence-specifically anneal to miRNAs, can be constructed from mers (subunits), wherein the mers are nucleotides (RNA or DNA), modified nucleotides and/or nucleotide analogues. Multiple types of mers (e.g., DNA and LNA, modified RNA and non-modified RNA, etc.) can be combined in the same AMO. Some of these molecules may be designated as mixmers.

NUCLEOTIDES, MODIFIED NUCLEOTIDES AND NUCLEOTIDE ANALOGS FOR USE IN AMOs

[0077] Early AMOs were constructed from DNA, but these often degraded rapidly. Later approaches replaced nucleotides with modified nucleotides or nucleotide analogs. Boutla et al. 2003 Nucl. Acids Res. 31: 4973-4980; and Weiler et al. 2006 Gene Ther. 13: 496-502.

[0078] Various modifications confer greater stability against nucleases; increase binding affinity to the target miRNA; aid in cellular uptake and/or reduce the triggering of an immune response. Non-limiting examples of modifications at the phosphate include replacement of one or more phosphate with any of: phosphorothioate, phosphorodithioate, phosphoramidate, boranophosphonate, an amide linker, C₁₋₆ alkyl, and a compound of formula (I):



(I), where R³ is selected from O⁻, S⁻, NH₂, BH₃, CH₃, C₁₋₆ alkyl, C₆₋₁₀ aryl, C₁₋₆ alkoxy and C₆₋₁₀ aryl-oxy, wherein C₁₋₆ alkyl and C₆₋₁₀ aryl are unsubstituted or optionally independently substituted with 1 to 3 groups independently selected from halo, hydroxyl and NH₂; and R⁴ is selected from O, S, NH, or CH₂.

[0079] In a forty-first embodiment, one or more phosphate of the modified nucleotide or polynucleotide is replaced by phosphorothioate (PS). Both endo- and exonucleases degrade nucleic acids by cleaving the phosphate bonds between nucleotides. The phosphorothioate

modification substitutes a sulfur atom for a non-bridging oxygen in the phosphate bond, which reduces the ability of nucleases to degrade this bond. Several AMOs have been constructed which comprise a phosphorothioate. AMOs comprising a PS have been described in: Krutzfeldt et al. 2005 Nature 438: 685-689; Krutzfeldt et al. 2007 Nucl. Acids Res. 35: 2885-2892; Esau et al. 2004 J. Biol. Chem. 279: 52361-52365; Davis et al. 2006 Nucl. Acids Res. 34: 2294-2304; Esau et al. 2008 Methods 44: 55-60; and Lennox et al. 2011 Gene Ther. 18: 1111-1120.

[0080] Non-limiting examples of modified nucleotides which can be used to generate the miRNA inhibitor include 2-aminopurine, (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

[0081] Non-limiting examples of the sugar include, for example, a modification at the 2' carbon. In a forty-second embodiment, at least one modified nucleotide is selected from among 2' alkoxyribonucleotide, 2' alkoxyalkoxy ribonucleotide, or 2'-fluoro ribonucleotide. In a forty-third embodiment, said at least one modified nucleotide is selected from 2'-OMe, 2'-MOE and 2'-H. In a second aspect, the nucleotide subunit is chemically modified at the 2' position of the sugar. In a third aspect, the 2' chemical modification is selected from a halo, a C1-10 alkyl, a C1-10 alkoxy, and the like. In a fourth aspect, the 2' chemical modification is a C1-10 alkoxy selected from -OCH₃ (i.e., "OMe"), -OCH₂CH₃ (i.e., "OEt") or -CH₂OCH₂CH₃ (i.e., methoxyethyl or "MOE"); or is a halo selected from F.

[0082] As a non-limiting example, a 2'-OMe can improve binding affinity; the melting temperature of a 2'-OMe:RNA duplex is about 1.6 degrees C higher than that of a corresponding DNA:RNA duplex of the same sequence.

[0083] AMOs comprising a 2'-MOE, 2'-OMe, or 2'F have described in, inter alia: Meister et al. 2004 RNA 10: 544-550; Hutvagner et al. 2004 PLoS 2: E98; Davis et al. 2006 Nucl. Acids Res. 34: 2294-2304; and Davis et al. 2009 Nucl. Acids Res. 37: 70-77.

[0084] An additional modification, naphthyl-based chemical modifying group, has been successfully used in AMOs, particularly near each end of the antisense strand. Lennox et al. 2008 Oligo. 16: 26-42; and Melkman-Zehavi et al. 2011 EMBO J. 30: 835-845. An AMO can thus comprise any of: a nucleotide, a modified nucleotide, and/or a nucleotide analogue.

[0085] In a forty-fourth embodiment, the nucleotide analogue useful in this invention is any nucleotide analogue known in the art, including, but not limited to, peptide nucleic acid (PNA), PNA carrying an alternating α/β amino acid backbone consisting of (2'R,4'R)-nucleobase-substituted proline and (1S,2S)-2-aminocyclopentanecarboxylic acid (acpcPNA), locked nucleic acid (LNA), morpholino nucleotide, threose nucleic acid (TNA), glycol nucleic acid (GNA), arabinose nucleic acid (ANA), 2'-fluoroarabinose nucleic acid (FANA), cyclohexene nucleic acid (CeNA), anhydrohexitol nucleic acid (HNA), and/or unlocked nucleic acid (UNA), or xeno nucleic acid (XNA), or xylo nucleic acid (XNA).

[0086] Some nucleotide equivalents have advantages over nucleotides in some cases. PNAs, for example, have been used to create inhibitors to specific miRNAs; these have exceptional biological and chemical stability. Because the intra-molecular distances and the configuration of the bases are similar to those found in naturally occurring DNA molecules, hybridization occurs specifically between the PNA and its complementary RNA sequences. Compared with DNA-RNA duplexes, PNA-RNA duplexes exhibit increased thermal stability. Oh et al. 2009 Mol. Cells 28: 341-345.

[0087] Replacing a nucleotide with LNA stabilizes the molecule against nucleases and offers significant increase in binding affinity, adding about 1.6 degrees C per LNA unit compared to RNA.

[0088] An AMO comprising both nucleotides and nucleotide analogs is sometimes designated a "mixmer". AMOs comprising a nucleotide analog such as LNA, PNA or morpholino, or a mixture of nucleotides and nucleotide analogues have been described in, for example: Lennox et al. 2010 Pharm. Res. 27: 1788-1799; Chan et al. 2005 Cancer Res. 65: 6029-6033; Flynt et al. 2007 Nat. Genet. 39: 259-263; Obad et al. 2011 Nat. Genet. 43: 371-378; Fabani et al. 2008 RNA 14: 336-346; Orom et al. 2006 Gene 372: 137-141; Oh et al. 2009 Mol. Cells 28: 341-345; Orom et al. 2006 Gene 372: 137-141; and Kloosterman et al. PLoS 2007 5: e203. Elmen et al. 2008 Nature 452: 896 and Lanford et al. 2010 Science 327: 198-201 describe an AMO to miRNA-122 for use in treating Hepatitis C virus, comprising a LNA and DNA, with a phosphorothioate backbone, and methylcytosine LNA. Thus, an AMO can comprise nucleotides, modified nucleotides and/or nucleotide analogues, or a mixture thereof.

[0089] When a given clinical treatment is considered effective where there is at least a 10% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the

amount necessary to effect at least a 10% reduction in that parameter. In this fifth aspect, a therapeutically effective amount of a miRNA inhibitor targeting a miRNA can reduce miRNA levels by at least 10%. In a sixth aspect, a given clinical treatment is considered effective where there is at least a 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95% reduction in a measurable parameter associated with a disease or disorder, and the therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to effect at least a 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95% reduction, respectively, in that parameter.

MODIFICATIONS AT THE 5' AND/OR 3' END OF AN AMO

[0090] miRNA inhibitors can also be modified at the 5' and/or 3' end. Such modifications may increase performance, and/or may decrease degradation or increase stability. Any modification at the 5' and/or 3' end of a miRNA inhibitor can be used with any miRNA comprising any combination of nucleotides, modified nucleotides, and/or nucleotide analogues.

MODIFICATIONS AT THE 5' END

[0091] miRNA inhibitors can also be modified on the 5' end. As non-limiting examples, any of the following can be conjugated to the AMO at the 5' end:

- cell-penetrating peptides (CPP) such as R6 Pen, Tat, 47Tat57, Cationic, C-myc tag, H region, PTD-4, Transportan, SSBPI(I) and Tat-modified. Oh et al. 2009 Mol. Cells 28: 341-345; in the work of Oh et al., of the tested CPPs, Tat modified and R6 Pen were most effective;
- Biotin; Hutvagner et al. 2004 PLoS 2: E98;
- CysLys; Fabani et al. 2008 RNA 14: 336-346;
- Hairpin; Vermeulen et al. 2007 RNA 13: 723-730;
- RNA loop binders, which bind to the loop of a pre-miRNA or pri-miRNA; these include, without limitation, dimers of deoxystreptomycin, Thomas et al. 2005 J. Am. Chem. Soc. 127: 12434-12435; and molecules that bind to TAR, U1A, and GNRA, Thomas et al. 2008 Chem. Rev. 108: 1171;
- Neomycin; or
- Kanamycin.

[0092] Various additional modifications at the 5' end of a miRNA inhibitor are known in the art and can be used on miRNA inhibitors of the invention.

MODIFICATIONS AT THE 3' END

[0093] miRNA inhibitors can also be modified on the 3' end. As non-limiting examples, any of the following can be conjugated to the AMO at the 3' end:

- 3' end cap, as described in, for example, U.S. Pat. Nos. 8,097,716; 8,084,600; 8,404,831; 8,404,832; 8,344,128; and PCT/US14/58705 (WO2015051366); these include the C3, C6, biphenyl, and X058 3' end caps;
- C3 with amino linker. Cheng et al. 2005 Nucl. Acids. Res. 33: 1290-1297;
- Aliphatic amino modifier. Meister et al. 2004 RNA 10: 544-550;
- Cholesterol, which assists in delivery in vivo. Krutzfeldt et al. 2005 Nature 438: 685-689; and Krutzfeldt et al. 2007 Nucl. Acids Res. 35: 2885-2892;
- LysLysLys. Fabani et al. 2008 RNA 14: 336-346;
- Hairpin. Vermeulen et al. 2007 RNA 13: 723-730;
- Lipids: or
- Minor groove binders (described in greater detail below).

[0094] Various additional modifications at the 3' end of a miRNA inhibitor are known in the art and can be used on miRNA inhibitors of the invention.

[0095] Various modifications of the 5' end or the 3' end of a miRNA inhibitor known in the art can be used in any combination on a miRNA inhibitor of the invention.

[0096] MINOR GROOVE BINDERS

[0097] Any minor groove binder known in the art can be used in combination with a miRNA inhibitor described herein (e.g., the miRNA inhibitor can have a 3' end modification that is a minor groove binder).

[0098] Many minor groove binders have a molecular weight of approximately 150 to approximately 2000 Daltons. A minor groove binder generally binds in a non-intercalating manner into the minor groove of double stranded (or higher order aggregation) DNA, RNA or hybrids thereof, preferably, with an association constant greater than approximately 10^3 M^{-1} . Minor groove binding compounds have widely varying chemical structures, but many minor groove binders have a crescent shape three-dimensional structure.

[0099] Minor groove binders are known in the art. And of these can be conjugated to an anti-miRNA molecule of the invention. As a non-limiting example, the minor groove binder can be present at the 3' or 5' end of a molecule comprising an anti-miRNA molecule.

[00100] Minor groove binders useful for the invention include any of those known in the art. These include, as non-limiting examples:

- Naturally-occurring compounds and antibiotics, such as netropsin, distamycin, and lexitropsin, mithramycin, chromomycin A3, olivomycin, anthramycin, sibiromycin;

- Derivatives or analogues of naturally-occurring compounds and antibiotics, such as derivatives or analogues of netropsin, distamycin, lexitropsin, mithramycin, chromomycin A3, olivomycin, anthramycin, sibiromycin [Sondhi et al 1997 Curr. Med. Chem. 4: 313; Reddy et al. 1999 Pharm. Therap. 84: 1; Wemmer 2001 Biopolymers 52: 197; Dervan 2001 Bioorg. Med. Chem. 9: 2215; J. Am. Chem. Soc. 114(15), 5911 (1992); Biochemistry 31, 8349 (1992); Bioconjugate Chem. 5, 475 (1994); Biochem. Biophys. Res. Commun. 222, 764 (1996); J. Med. Chem. 43, 3257 (2000); Tetrahedron 56, 5225 (2000); Molecular Pharmacology 54, 280 (1998), Bioorg. Med. Chem. Lett. 6(18), 2169 (1996), J. Med. Chem. 45, 805 (2002), Bioorg. Med. Chem. Lett. 12, 2007 (2002), international patent applications WO 97/28123, WO 98/21202, WO 01/74898 and WO 02/00650; U.S. Pat. Nos. 4,912,199, 5,273,991, 5,637,621, 5,698,674 and 5,753,629; U.S. Pat. No. 6,090,947; Tet. Lett. 37(43), 7801-7804 (1996); and Bioorg. Med. Chem. Lett. 11, 769 (2001)];
- Minor groove binding polyamides containing N-methylimidazole and N-methylpyrrole amino acids [Wemmer et al. 1997 Curr. Opin. Struct. Biol. 7: 355-361];
- A multimer (e.g., a dimer, trimer, tetramer, pentamer, hexamer or longer) of 1,2-dihydro-(3H)-pyrrolo[3,2-e]indole-7-carboxylate (CDPI);
- A multimer (e.g., a dimer, trimer, tetramer, pentamer, hexamer or longer) of N-methylpyrrole-4-carbox-2-amide (MPC);
- Minor groove binding compounds comprising an acrylamide-type linker between the 2-position of a pyrrole group and a terminal basic group are described in J. Am. Chem. Soc. 122, 1602-1608 (2000), *ibid.* 123, 5158-5159 (2001), *ibid.* 125, 3471-3485 (2003), *ibid.* 126, 3406-3407 (2004), Chem. Eur. J. 8, 4781-4790 (2002), Chem. & Biol. 10, 751-758 (2003) and Bioconjugate Chem. 17, 715-720 (2006);
- Minor groove binding compounds comprising an acrylamide-type linker between the 3-position of a pyrrole group and a terminal aromatic group, for example, as described in J. Med. Chem. 47, 2133-2156 (2004);
- Bisquaternary ammonium heterocyclic compounds, diarylamidines, pentamidine, stilbamidine and berenil, CC-1065 and related pyrroloindole and indole polypeptides, Hoechst 33258, 4'-6-diamidino-2-phenylindole (DAPI); Synthetic minor groove binding molecule, USPN 8,012,967;
- Negatively charged minor groove binding molecules, USPN 7,851,606;
- Compounds designed to bind to DNA regions containing GC base pairs [Anti-Cancer Drug Design 5, 3 (1990); Proc. Natl. Acad. Sci. USA 89, 7586 (1992); Biochemistry 32, 4237 (1993); Science 266, 647 (1994); Anti-Cancer Drug Design 10, 155 (1995); Bioorg. Med. Chem. 8, 985 (2000); and Mol. Biol. 34, 357 (2000)];

- Oligomers of heterocyclic groups, in which the group linking the heterocycles may be methyleneamino, amido, thioamido, iminydyl or ethenylene [U.S. Pat. No. 6,143,901];
- oligopeptides comprising naturally-occurring or synthetic amino acids are minor groove binder compounds. Exemplary minor groove binders are described in U.S. Pat. Nos. 6,084,102 and 7,700,765; or
- Additional synthetic minor groove binders, described for example, in U.S. Pat. Nos. 8,465,921, 8,674,094, 6,303,312 and 6,221,589; Dervan et al. 2003 Curr. Opin. Struct. Biol. 13: 284-299; Wemmer et al. 1997 Curr. Opin. Struct. Biol. 7: 355-361.

[00101] Any minor groove binder known in the art can be used in combination with a miRNA inhibitor described herein (e.g., the miRNA inhibitor can have a 3' end modification that is a minor groove binder).

miRNA INHIBITORS WHICH INVADE THE pri-miRNA AND/OR pre-miRNA HAIRPIN, BIND TO THE LOOP AND/OR STRADDLE A CLEAVAGE SITE

[00102] Inhibitors of miRNA known in the art generally act by annealing to the mature miRNA, especially the seed region, and blocking the recognition of the target by the miRNA. The invention provides miRNA inhibitors which anneal to a sequence outside the miRNA (e.g., in a Dicer-excised region or Drosha-excised region), and - in a forty-fifth embodiment - also anneal to at least a portion of the mature miRNA sequence. Without being bound by any particular theory or suggested biochemical mechanism, these miRNA inhibitors function by preventing or reducing the formation of the mature miRNA from a pre-miRNA or pri-miRNA, e.g., by preventing or reducing cleavage by Dicer and/or Drosha.

[00103] As noted above, mature miRNA molecules comprise two at-least-partially complementary strands, each strand about 18-25 nt long, often with a 3' overhang (single-stranded) region on either side. These molecules are cleaved from a longer molecule, a pre-miRNA, which forms a stem loop hairpin structure. Cleavage is performed by two enzymes, Dicer and Drosha. The portions deleted from the molecule to form a mature miRNA are designated the Dicer-excised region and Drosha-excised region.

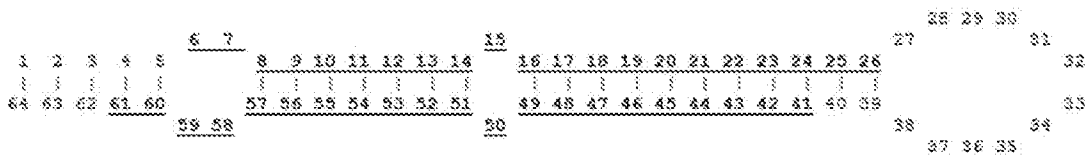
[00104] In a forty-sixth embodiment, the miRNA inhibitor comprises a sequence such that it invades the hairpin and/or the terminal loop (e.g., a part of the Dicer-excised region) of a pri-miRNA and/or pre-miRNA. Annealing to one strand, the terminal loop, Dicer-excised region or Drosha-excised region, and/or the other strand disturbs the structure of the pri-miRNA and/or pre-miRNA, thus preventing processing (e.g., cleavage) of the pri-miRNA and/or pre-miRNA to produce a miRNA. In a forty-seventh embodiment, prevention of cleavage is performed by steric hindrance. In a forty-eighth embodiment, the miRNA inhibitor comprises a sequence (or its complement) which straddles at least one cleavage site of the

pri-miRNA and/or pre-miRNA and/or anneals to the terminal loop of the pri-miRNA and/or pre-miRNA.

[00105] In a forty-ninth embodiment, the invention provides a miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises a region of complementarity complementary to the Dicer-excised region (or a portion thereof) of a pri-miRNA hairpin or pre-miRNA hairpin, wherein (a) the region of complementarity is at the 3' end of the miRNA inhibitor and the mer at the 3' end of the region of complementarity anneals to any of the first to fifth nt counting from the 5' end of the Dicer-excised region; or (b) the region of complementarity is at the 5' end of the miRNA inhibitor, and the mer at the 5' end of the region of complementarity anneals to any of the third to seventh nt counting from the 3' end of the Dicer-excised region. In a fiftieth embodiment, the entire miRNA inhibitor is complementary to the Dicer-excised region (or a portion thereof); thus, the miRNA inhibitor consists of a region of complementarity. In a fifty-first embodiment, the region of complementarity has 1 or 2 or 3 mismatches to the Dicer-excised region.

Example of a generic pri-miRNA.

[00106] A generic example pri-miRNA is shown below, with numbers standing in for nucleotides:



[00107] Position 1 is the 5' end; position 64 is the 3' end. This represents a portion of the molecule; the complete molecule would extend 5' to nt 1, and 3' to nt 64. The miRNA has an antisense strand (or 5p strand) of nt 6 to 26 (bold, underlined), and a sense strand (or 3p strand) of 41 to 61 (also bold, underlined). The loop (e.g., terminal loop) is represented by nucleotides 27 to 38. The stem shown is represented by nt 1 to 26 and 39 to 64. A hairpin comprises a stem and a loop. In this example, the staggered Dicer cleavage site is close to the loop; Dicer cleaves between nt 26 and 27, and between nt 40 and 41; Drosha cleaves between nt 5 and 6, and between nt 61 and 62.

[00108] In this example, the Dicer-excised region is nt 27 to 40 (inclusive). In some miRNAs, the Dicer-excised region can comprise a single large terminal loop (e.g., a loop at the end) and an additional sequence, as in this example. Other example Dicer-excised regions are nt 39 to 53 (inclusive) of miR-145 (see Fig. 1A), and nt 37 to 50 (inclusive) of miR-122 (Fig. 7B). In other miRNAs, the Dicer-excised region can comprise a small terminal

loop and/or one or more loops which are not terminal loops, along with additional sequences; examples of such a Dicer-excised region is nt 47 to 58 (inclusive) of miRNA-137 (Fig. 6B), and nt 31 to 43 (inclusive) of miR-208a (Fig. 4D).

[00109] In this example, the Drosha-excised region is positions 1 to 5 (and positions 5' of 1, but not shown here) and positions 62 to 64 (and positions 3' of 64, but also not shown here).

[00110] As a non-limiting example, the nt 27-38 in the pre-miRNA diagram above indicate the terminal loop. Nucleotides 6-7, 15, 50, and 58-59, while forming single-stranded regions, would not represent a terminal loop. In the structure shown in FIG. 1. A., C (nt 14), UC (17-18), C (28), U (67), and UU (72-73) are not terminal loops, but UAGAUGCUAAGAU (39-51) (SEQ ID NO: 324) is a terminal loop. In the structure shown in FIG. 4. D., G (nt 5), G (7), GG (23-24), C (31), GA (48-49), A (52), A (55), and G (67) are not terminal loops, but UGC (35-37) is a terminal loop.

[00111] As a non-limiting example, the nt 1-26 and 39-64 form the stem of a pri-miRNA in the diagram above. In the structure shown in FIG. 1. A., the nt 1-38 and 52-88 form the stem of a pre-miRNA. In the structure shown in FIG. 4. D., nt 1-34 and 38-71 form the stem of a pri-miRNA.

[00112] In a fifty-second embodiment, the invention provides a miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises a region of complementarity complementary to the Dicer-excised region of a pri-miRNA hairpin or pre-miRNA hairpin, wherein (a) the region of complementarity is at the 3' end of the miRNA inhibitor and the mer at the 3' end of the region of complementarity anneals to any of the first to fifth nt counting from the 5' end of the Dicer-excised region. In this example, the first to fifth nt counting from the 5' end of the Dicer-excised region are positions 27, 28, 29, 30 and 31. Examples of such a miRNA inhibitor include, without limitation, those with the sequences, in 3' to 5' order: 27, 28, 29, 30, 31, etc.; 28, 29, 30, 31, 32, etc.; 29, 30, 31, 32, 33, etc.; 30, 31, 32, 33, 34, etc.; 31, 32, 33, 34, 35, etc.; where "etc." indicates one or more additional mers at the 5' end.

[00113] In a fifty-third embodiment, the invention provides a miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises a region of complementarity complementary to the Dicer-excised region of a pri-miRNA hairpin or pre-miRNA hairpin, wherein the region of complementarity is at the 5' end of the miRNA inhibitor, and the mer at the 5' end of the region of complementarity anneals to any of the third to seventh nt counting from the 3' end of the Dicer-excised region. In this example, the third to seventh nt counting from the 3' end of the Dicer-excised region are represented by positions 38, 37, 36, 35, and 34. Examples of such a miRNA inhibitor include, without limitation, those with the sequences, in 5' to 3' order: 38, 37, 36, 35, 34, etc.; 37, 36, 35, 34, 33, etc.; 36, 35, 34, 33, 32, etc.; 35, 34, 33, 32, 31, etc.; and 34, 33, 32, 31, etc.; where "etc." indicates one or more additional mers at the 3' end.

[00114] In a fifty-fourth embodiment, the sequence of the miRNA inhibitor can consist of a sequence that is the entire Dicer-excised region or a portion thereof. In such a case, the sequence of the miRNA does not actually comprise a sequence of the miRNA itself. In this example, such a miRNA can comprise a sequence, as non-limiting examples, of: 27-40, 27-39, 27-38, 27-37, 27-36, 27-35, 27-34, 28-40, 28-39, 28-38, 28-37, 28-36, 28-35, 28-34, 29-40, 29-39, 29-38, 29-37, 29-36, 29-35, 29-34, 30-40, 30-39, 30-38, 30-37, 30-36, 30-35, 30-34, 31-40, 31-39, 31-38, 31-37, 31-36, 31-35, or 31-34. Preferably, a miRNA inhibitor comprises at least 6 mers.

[00115] In a fifty-fifth embodiment, the invention provides a miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises a region of complementarity complementary to the Dicer-excised region of a pri-miRNA hairpin or pre-miRNA hairpin, wherein the 3' end of the region of complementarity anneals to any of the first to fifth nt counting from the 5' end of the Dicer-excised region, and the mer at the 5' end of the region of complementarity anneals to any of the third to seventh nt counting from the 3' end of the Dicer-excised region. Non-limiting examples of such miRNAs comprise the sequences of positions: 27-38, 27-37, 27-36, 27-35, 27-34, 28-38, 28-37, 28-36, 28-35, 28-34, 29-38, 29-37, 29-36, 29-35, 29-34, 30-38, 30-37, 30-36, 30-35, 30-34, 31-38, 31-37, 31-36, 31-35, or 31-34. Preferably, the miRNA inhibitor comprises at least 6 mers.

miRNA INHIBITORS

[00116] The miRNA inhibitor of the invention can comprise a sequence which anneals (or is complementary or substantially complementary) to a sequence in the stem and/or the terminal loop of a pre-miRNA or pri-miRNA, such a Dicer-excised region.

[00117] In some miRNAs, the loop (or terminal loop) sequence is the sequence that is between the Dicer cleavage positions in a pre-miRNA hairpin, specifically the sequence extending from the 5' cleavage Dicer cleavage site to the 3'-Dicer cleavage site of a pre-miRNA.

[00118] In a fifty-sixth embodiment, the miRNA inhibitor comprises a sequence (or its complement) which straddles at least one pri-miRNA and/or pre-miRNA cleavage site.

[00119] In a fifty-seventh embodiment, the miRNA inhibitor is a polymer (e.g., a polymer comprising nucleotides or functionally equivalent molecules) comprising a sequence which straddles at least one cleavage site of a pri-miRNA and/or pre-miRNA, therefore interfering with cleavage and production of the miRNA. A miRNA inhibitor comprising a sequence which straddles the cleavage site between nt 5 and 6 can comprise nt 5 and 6 or the complementary sequence (nt 59 and 60). As another, non-limiting example, a miRNA comprising a sequence which straddles the cleavage site between nt 61 and 62 can comprise nt 61 and 62 or the complementary sequence (nt 3 and 4).

[00120] Cleavage of a pre-miRNA generally produces staggered ends. Therefore, the cleavage site on one strand is not generally aligned with the cleavage site on the other strand. If the two cleavage sites were aligned, two blunt-ended molecules would result from cleavage. In the example miRNA directly above, one cleavage site is between nt 5 and 6, but this is not directly aligned with the cleavage site between nt 61 and 62. A miRNA inhibitor comprising a sequence which straddles a cleavage site on one strand may or may not straddle the cleavage site on the other strand. For example, a miRNA with a sequence with a 5' end beginning at nt 4 or 5 (e.g., 4-22, 4-23, 4-24, 4-25, 4-26, or 5-23, 5-24, 5-25, 5-26, etc.) is said to straddle at least one cleavage site in the example pre-miRNA even though it straddles the cleavage site between nt 5 and 6, but not that between nt 61 and 62. Various miRNA inhibitors with sequences complementary to sequences of 4-22, 4-23, 4-24, 4-25, 4-26, or 5-23, 5-24, 5-25, 5-26, etc., would also be termed to straddle a cleavage site.

[00121] A miRNA inhibitor can comprise a sequence which straddles a cleavage site by at least 1 nt (e.g., it comprises a sequence comprising at least 1 nt on either side of a cleavage site on one strand or the other).

[00122] A miRNA inhibitor can comprise a sequence which straddles a cleavage site by at least 2 nt (e.g., it comprises a sequence comprising at least 2 nt on either side of a cleavage site on one strand or the other).

[00123] A miRNA inhibitor can comprise a sequence which straddles a cleavage site by at least 3 nt (e.g., it comprises a sequence comprising at least 3 nt on either side of a cleavage site on one strand or the other).

[00124] A miRNA inhibitor can comprise a sequence which straddles a cleavage site by at least 4 nt (e.g., it comprises a sequence comprising at least 4 nt on either side of a cleavage site on one strand or the other).

[00125] A miRNA inhibitor can comprise a sequence which straddles a cleavage site by at least 5 nt (e.g., it comprises a sequence comprising at least 5 nt on either side of a cleavage site on one strand or the other).

[00126] In a fifty-eighth embodiment, the miRNA inhibitor comprises a sequence complementary to all or a portion of the Dicer-excised region. For example, the miRNA inhibitor might anneal to a portion of the Dicer-excised region (e.g., annealing to at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more nt of the Dicer-excised region). A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 1 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 2 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 3 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 4 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is

complementary to a sequence of at least 5 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 6 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 7 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 8 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 9 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 10 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 11 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 12 nt of the Dicer-excised region. A miRNA inhibitor can comprise a sequence which is complementary to a sequence of at least 12 or more nt of the Dicer-excised region.

[00127] A miRNA inhibitor can comprise a sequence which both straddles a cleavage site (by any measure indicated, e.g., 1, 2, 3, 4, 5, 5 or more nt) and be complementary to a sequence of the Dicer-excised region of any length indicated (at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 12 or more nt) of the Dicer-excised region, or any combination thereof (e.g., straddling a cleavage site by 1 nt, and complementary to 5 nt of the Dicer-excised region; straddling a cleavage site by 3 nt, and complementary to 2 nt of the Dicer-excised region; straddling the cleavage site by 5 nt, and complementary to 5 nt of the Dicer-excised region, etc.).

VARIOUS EMBODIMENTS

[00128] The present invention provides, inter alia, miRNA inhibitors and methods of their use. Various miRNA inhibitors are disclosed in Tables 1 to 10 and the Figures, in addition to elsewhere in the specification.

[00129] Various anti-miR-145 oligos are described and illustrated herein, e.g., in Figures 1 to 3 and Tables 1 to 4. These can be used to treat any disease related to expression or over-expression of miR-145.

[00130] Various anti-miR-208a oligos are described and illustrated herein, e.g., in Figures 4 and 5 and Table 5. These can be used to treat any disease related to expression or over-expression of miR-208a.

[00131] Various anti-miR-137 oligos are described and illustrated herein, e.g., in Figure 6 and Table 6. These can be used to treat any disease associated with miR-137 expression or over-expression.

[00132] Various anti-miR-122 oligos are described and illustrated herein, e.g., in Figure 7 and Table 7. These can be used to treat any disease associated with miR-122 expression or over-expression.

[00133] Some specific inhibitors are listed below.

[00134] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 381, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00135] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 382, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00136] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 383, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00137] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 384, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00138] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 385, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00139] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 386, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00140] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 387, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00141] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 388, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00142] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 389, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00143] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 390, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00144] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: , wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00145] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 391, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00146] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 392, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00147] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 393, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00148] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 394, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00149] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 395, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00150] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 396, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00151] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 397, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00152] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 398, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00153] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 399, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00154] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 400, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00155] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 401, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00156] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: , wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00157] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 402, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00158] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 403, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00159] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 404, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00160] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: , wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00161] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 405, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00162] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 406, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00163] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 407, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00164] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of SEQ ID NO: 408, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00165] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 381, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00166] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 382, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00167] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 383, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00168] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 384, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00169] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 385, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00170] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 386, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00171] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 387, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00172] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 388, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00173] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 389, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00174] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 390, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00175] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: , wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00176] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 391, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00177] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 392, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00178] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 393, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00179] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 394, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00180] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 395, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00181] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 396, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00182] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 397, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00183] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 398, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00184] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 399, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00185] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 400, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00186] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 401, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00187] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: , wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00188] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 402, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00189] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 403, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00190] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 404, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00191] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: , wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00192] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 405, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00193] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 406, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00194] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 407, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00195] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor consists of the sequence of SEQ ID NO: 408, wherein the miRNA inhibitor comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00196] A method of treating or ameliorating a disease associated with the expression or over-expression of miR-145 in an individual, the method comprising the step of administering to the individual a therapeutically effective amount of any one or more of:

[00197] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 381, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00198] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 382, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00199] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 383, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00200] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 384, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00201] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 385, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00202] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 386, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00203] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 387, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00204] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 388, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00205] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 389, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues, and/or

[00206] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 390, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00207] A method of treating or ameliorating a disease associated with the expression or over-expression of miR-208a in an individual, the method comprising the step of administering to the individual a therapeutically effective amount of any one or more of:

[00208] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 391, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00209] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 392, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00210] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 393, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00211] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 394, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00212] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 395, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00213] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 396, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00214] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 397, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00215] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 398, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00216] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 399, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00217] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 400, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues, and/or

[00218] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 401, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00219] A method of treating or ameliorating a disease associated with the expression or over-expression of miR-137 in an individual, the method comprising the step of administering to the individual a therapeutically effective amount of any one or more of:

[00220] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 402, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00221] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 403, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues, and/or

[00222] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 404, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00223] A method of treating or ameliorating a disease associated with the expression or over-expression of miR-122 in an individual, the method comprising the step of administering to the individual a therapeutically effective amount of any one or more of:

[00224] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 405, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00225] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 406, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues,

[00226] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 407, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues, and/or

[00227] A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises or consists of the sequence of SEQ ID NO: 408, wherein the miRNA comprises one or more nucleotides, modified nucleotides and/or nucleotide analogues.

[00228] The miRNA inhibitors of this invention may comprise only a first strand. Alternatively, the miRNA inhibitors can comprise a first and a second strand. The miRNA inhibitors can comprise any combination of one or more nucleotides, modified nucleotides and/or nucleotide analogues, and can further optionally comprise any 5' or 3' end

modification disclosed herein or known in the art, and can be in combination with any delivery vehicle disclosed herein or known in the art, and can be used for any method of treatment or reducing the level, activity or efficacy of any cognate miRNA.

[00229] Compositions for delivery of the miRNA inhibitors of the invention, and methods of their use are described herein, and below.

DELIVERY

[00230] The miRNA inhibitors of the invention can be delivered using any method or composition described herein, or known in the art to be used for delivering miRNA inhibitors or small nucleic acids. For example, miRNA inhibitor can optionally be attached to a ligand selected to improve one or more characteristic, such as, e.g., stability, distribution and/or cellular uptake of the agent. The miRNA inhibitor(s) can be isolated or be part of a pharmaceutical composition used for the methods described herein. Particularly, the pharmaceutical composition can be formulated for delivery to specific tissues (e.g., those afflicted with a miRNA-related disease) or formulated for parenteral administration. The pharmaceutical composition can optionally comprise two or more miRNA inhibitors, each one directed to the same, overlapping or a different segment of the pre-miRNA or pri-miRNA. Optionally, the pharmaceutical composition can further comprise or be used in conjunction with any known treatment for the particular miRNA-related disease.

Pharmaceutical Composition Comprising a miRNA Inhibitor

[00231] Additional components of a pharmaceutical composition comprising a miRNA inhibitor are contemplated to aid in delivery, stability, efficacy, or reduction of immunogenicity.

[00232] Liposomes have been used previously for drug delivery (e.g., delivery of a chemotherapeutic). Liposomes (e.g., cationic liposomes) are described in PCT publications WO02/100435A1, WO03/015757A1, WO04029213A2; and WO/2011/076807; U.S. Pat. Nos. 5,962,016; 5,030,453; and 6,680,068; and U.S. Patent Application 2004/0208921. A process of making liposomes is also described in WO04/002453A1. Furthermore, neutral lipids have been incorporated into cationic liposomes (e.g., Farhood et al. 1995), as well as PEGylated lipids.

[00233] Cationic liposomes have been used to deliver drugs to various cell types (Sioud and Sorensen 2003; U.S. Patent Application 2004/0204377; Duxbury et al., 2004; Donze and Picard, 2002).

[00234] Use of neutral liposomes disclosed in Miller et al. 1998, and U.S. Patent Application 2003/0012812.

[00235] Chemical transfection using lipid-based, amine-based and polymer-based techniques is disclosed in products from Ambion Inc., Austin, Tex.; and Novagen, EMD Biosciences, Inc, an Affiliate of Merck KGaA, Darmstadt, Germany); Ovcharenko D (2003) Ambion TechNotes 10 (5): 15-16). Additionally, Song et al. (Nat Med. published online (Feb 10, 2003) doi: 10.1038/nm828) and others [Caplen et al. 2001 Proc. Natl. Acad. Sci. (USA), 98: 9742-9747; and McCaffrey et al. Nature 414: 34-39] disclose that liver cells can be efficiently transfected by injection of the small nucleic acids into a mammal's circulatory system.

[00236] A variety of molecules have been used for cell-specific nucleic acid delivery. See, for example, WO/2011/076807. For example, the nucleic acid-condensing property of protamine has been combined with specific antibodies to deliver small nucleic acids. Song et al. 2005 Nat Biotech. 23: 709-717. The self-assembly PEGylated polycation polyethylenimine (PEI) has also been used to condense and protect small nucleic acids. Schiffelers et al. 2004 Nucl. Acids Res. 32: e149, 141-1 10.

[00237] The miRNA inhibitors of the invention can be delivered via, for example, Lipid nanoparticles (LNP); neutral liposomes (NL); polymer nanoparticles; or via modification of the miRNA inhibitor (e.g., covalent attachment), or by any method known in the art for delivery of a miRNA inhibitor.

[00238] Lipid nanoparticles (LNP) are self-assembling cationic lipid based systems. These can comprise, for example, a neutral lipid (the liposome base); a cationic lipid (for small nucleic acid loading); cholesterol (for stabilizing the liposomes); and PEG-lipid (for stabilizing the formulation, charge shielding and extended circulation in the bloodstream).

[00239] The cationic lipid can comprise, for example, a headgroup, a linker, a tail and a cholesterol tail. The LNP can have, for example, good delivery to the diseased area, extended circulation in the blood, small particles (e.g., less than 100 nm), and stability in the microenvironment of the diseased area (which may have low pH and/or be hypoxic).

Neutral liposomes (NL) are non-cationic lipid based particles.

[00240] Polymer nanoparticles are self-assembling polymer-based particles. Neutral liposomes known in the art can be used to deliver miRNA inhibitors of the invention.

miRNA inhibitor compositions in a Lipid nanoparticles (LNP) comprising a neutral lipid; a cationic lipid; cholesterol; and PEG-lipid

[00241] Lipid nanoparticles (LNP) are self-assembling cationic lipid based systems. These can comprise, for example, a neutral lipid (the liposome base); a cationic lipid (for nucleic acid loading); cholesterol (for stabilizing the liposomes); and PEG-lipid (for stabilizing the formulation, charge shielding and extended circulation in the bloodstream).

[00242] A neutral lipid is, for example, the liposome base. A cationic lipid is, for example, for nucleic acid loading. Cholesterol is, for example, for stabilizing the liposomes. PEG-lipid is, for example, for stabilizing the formulation, charge shielding and extended circulation in the bloodstream. Lipid nanoparticles known in the art can be used to deliver miRNA inhibitors of the invention.

Additional pharmaceutical compositions

[00243] In an seventh aspect, the miRNA inhibitor is packaged as a monotherapy into a delivery vehicle, or may be further ligated to one or more diagnostic compound, reporter group, cross-linking agent, nuclease-resistance conferring moiety, natural or unusual nucleobase, lipophilic molecule, cholesterol, lipid, lectin, steroid, uvaol, hecigenin, diosgenin, terpene, triterpene, sarsasapogenin, Friedelin, epifriedelanol-derivatized lithocholic acid, vitamin, carbohydrate, dextran, pullulan, chitin, chitosan, synthetic carbohydrate, oligo lactate 15-mer, natural polymer, low- or medium-molecular weight polymer, inulin, cyclodextrin, hyaluronic acid, protein, protein-binding agent, integrin-targeting molecule, polycationic, peptide, polyamine, peptide mimic, and/or transferrin.

[00244] The miRNA inhibitors of the invention can be prepared in a pharmaceutical composition comprising various components appropriate for the particular method of administration of the miRNA inhibitor.

Methods of Use of miRNA Inhibitors

[00245] The invention further provides methods for reducing the level of mature miRNA in a cell, particularly in the case of a disease characterized by expression, over-expression or hyper-activity of a miRNA. The present methods utilize the cellular mechanisms involved in RNA interference to selectively degrade the target RNA in a cell and are comprised of the step of contacting a cell with one of the miRNA inhibitors of the invention.

[00246] The invention also encompasses a method of treating a human subject having a pathological state mediated at least in part by miRNA expression, over-expression or hyper-activity, the method comprising the step of administering to the subject a therapeutically effective amount of a miRNA inhibitor. Additional methods involve preventing, treating, modulating and/or ameliorating a pathological state wherein disease progression requires miRNA, although miRNA is not amplified or over-expressed. Such methods comprise the step of administering one of the miRNA inhibitors of the invention to a subject, as further described below. Such methods can be performed directly on a cell or can be performed on a mammalian subject by administering to a subject one of the miRNA inhibitors/pharmaceutical compositions of the invention. Reduction of target mature miRNA in a cell results in a reduction in the amount of mature miRNA produced. In an organism, this

can result in restoration of balance in a pathway involving miRNA, and/or prevention of miRNA accumulation, and/or a reduction in miRNA activity and/or expression, and/or prevention of miRNA-mediated activation of other genes, and/or amelioration, treatment and/or prevention of a miRNA-related disease. In an eighth aspect, a reduction in miRNA expression, level or activity can limit disease growth.

[00247] The methods and compositions of the invention, e.g., the methods and miRNA inhibitor compositions, can be used in any appropriate dosage and/or formulation described herein or known in the art, as well as with any suitable route of administration described herein or known in the art.

A method of treatment using a composition comprising a miRNA inhibitor described herein.

[00248] In a ninth aspect, the invention relates to a method of treating a miRNA-related disease in an individual, comprising the step of administering to the individual a therapeutically effective amount of a composition comprising a miRNA inhibitor comprising an antisense strand, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by 0, 1, 2, or 3 nucleotides from the antisense strand of a miRNA inhibitor selected from those specific duplexes provided above and as listed in any of Tables 1 to 10. In a tenth aspect, the miRNA inhibitor comprises an antisense strand duplexed with a sense strand, wherein the sense and antisense strands are selected from one or more of the sequences provided in any of Tables 1 to 10.

[00249] Various particular specific aspects of this invention are described below. Any aspects disclosed herein that are not mutually exclusive can be combined.

[00250] In an eleventh aspect, the invention relates to such a method, wherein the composition comprising a miRNA inhibitor further comprises a sense strand, wherein the sense strand comprises at least 15 contiguous nucleotides differing by 0, 1, 2, or 3 nucleotides from the sense strand of a miRNA inhibitor selected from the specific duplexes provided herein and as listed, e.g., in any Table herein.

[00251] In a twelfth aspect of the method, the miRNA inhibitor comprises at least an anti-sense strand, and/or comprises a sense and an anti-sense strand, wherein the sequence of the sense and/or anti-sense strand is the sequence of the sense and/or the anti-sense strand of a miRNA inhibitor selected from those specific duplex provided herein and as listed, e.g., in Table 1, wherein the composition further comprises a pharmaceutically effective formulation.

[00252] In a thirteenth aspect of the method, the miRNA inhibitor comprises at least an anti-sense strand, and/or comprises a sense and an anti-sense strand, wherein the sequence of the sense and/or anti-sense strand comprises the sequence of the sense

and/or the anti-sense strand of a miRNA inhibitor selected from those specific duplex provided herein and as listed, e.g., in any of Tables 1 to 10, wherein the composition further comprises a pharmaceutically effective formulation.

[00253] In a fourteenth aspect, the invention provides a method of inhibiting expression of miRNA in a subject, wherein the method comprises the step of administering to the subject an effective amount of a composition comprising a miRNA inhibitor (e.g., any described herein). In a sixteenth aspect, the subject is human. In one aspect, the subject has a miRNA-related disease.

[00254] In a seventeenth aspect, the method further comprises the step of administering an additional treatment.

[00255] In an eighteenth aspect, the additional treatment is a method (or procedure). In one aspect, the additional treatment is a therapeutically effective dose of a composition.

[00256] In a nineteenth aspect, the additional treatment and the miRNA inhibitor can be administered in any order, or can be administered simultaneously.

[00257] In a twentieth aspect, the method further comprises the step of administering an additional treatment for a miRNA-related disease.

[00258] In a twenty-first aspect, the method further comprises the step of administering an additional treatment. A miRNA inhibitor can be used in conjunction with any additional treatment disclosed herein, as appropriate for the disease, optionally, in further conjunction with one or more additional miRNA inhibitors to miRNA.

[00259] References to any additional treatment are meant to also include the pharmaceutically acceptable salts of any of the active substances.

[00260] In a twenty-second aspect, the composition comprises a second miRNA inhibitor. In a twenty-third aspect, the second miRNA inhibitor is physically distinct from the first, or the two are physically connected (e.g., linked or conjugated). In a twenty-fourth aspect, the first and second miRNA inhibitors are combined within the same composition (e.g., both in the same lipid nanoparticle).

A method of inhibiting the formation of miRNA, using a composition comprising a miRNA inhibitor described herein.

[00261] In fifty-ninth embodiment, the invention provides a method of inhibiting the expression of miRNA in an individual, comprising the step of administering to the individual a therapeutically effective amount of a composition comprising a miRNA inhibitor of the invention. In a twenty-fifth aspect, the miRNA inhibitor comprises a strand, wherein the strand comprises at least 15 contiguous mers differing by 0, 1, 2, or 3 mers from the sequence of a miRNA inhibitor listed in any of Tables 1 to 10, wherein the composition is in a pharmaceutically effective formulation. In a twenty-sixth aspect, the miRNA inhibitor

comprises a strand, wherein the strand comprises at least 15 contiguous mers from the sequence of a miRNA inhibitor listed in any of Tables 1 to 10, wherein the composition is in a pharmaceutically effective formulation. In a twenty-seventh aspect, the miRNA inhibitor comprises a strand, wherein the strand comprises the sequence of a miRNA inhibitor listed in any of Tables 1 to 10, wherein the composition is in a pharmaceutically effective formulation. In a twenty-eighth aspect, the miRNA inhibitor comprises a strand, wherein the strand consists of the sequence of a miRNA inhibitor listed in any of Tables 1 to 10, wherein the composition is in a pharmaceutically effective formulation. In a sixtieth embodiment, the miRNA inhibitor comprises a first strand and a second strand.

[00262] Various particular specific aspects of the uses of the miRNA inhibitors are described below.

[00263] In a twenty-ninth aspect, the individual is afflicted with or susceptible to a miRNA-related disease.

[00264] In a thirtieth aspect, the method further comprises the step of administering an additional treatment.

[00265] In a thirty-first aspect, the additional treatment and the miRNA inhibitor can be administered in any order or can be administered simultaneously.

[00266] In a thirty-second aspect, the composition comprises a second miRNA inhibitor. In a thirty-third aspect, the second miRNA inhibitor is physically separate from the first, or the two are physically connected (e.g., covalently linked or otherwise conjugated). In a thirty-fourth aspect, the first and second miRNA inhibitors are combined within the same composition (e.g., both in the same lipid nanoparticle).

[00267] In a thirty-fifth aspect, the method further comprises the step of administering an additional miRNA inhibitor.

miRNA inhibitor for use in treating various miRNA-related diseases

[00268] In a sixty-first embodiment, the miRNA inhibitor of the invention comprises a sequence disclosed herein and is administered to a patient in need thereof (e.g., a patient suffering from a miRNA-related disease disclosed herein or known in the literature). In a thirty-sixth aspect, the miRNA inhibitor of the invention is administered to a patient in need thereof, along with one or more additional pharmaceutical agent appropriate for that disease. For example, a patient suffering from a miRNA-related disease can be administered a pharmacologically effective amount of one or more miRNA inhibitor along with a pharmacologically effective amount of one or more of any miRNA-related disease treatment listed herein, and/or any other miRNA-related disease treatment known in the art.

[00269] A patient suffering from a miRNA-related disease can be administered one or more miRNA inhibitor and one or more additional miRNA-related disease treatment. This

additional treatment can be selected from the list of any disease treatment listed herein, and/or any anti- miRNA-related disease treatment known in the art.

[00270] The miRNA inhibitors of the invention can be administered along with (as part of the same therapeutic treatment regimen, prior to, simultaneously with, or after) one or more additional therapeutics to treat a miRNA-related disease.

[00271] The patient can also be administered as more than one miRNA inhibitor.

[00272] In the case of miRNA-related diseases, the miRNA inhibitor(s) and additional disease treatment(s) can be administered in any order, simultaneously or sequentially, or in multiple doses over time. Administration of the miRNA inhibitor and the additional treatment can be, for example, simultaneous, concurrent, separate or sequential.

[00273] Simultaneous administration may, e.g., take place in the form of one fixed combination with two or more active ingredients, or by simultaneously administering two or more active ingredients that are formulated independently. Sequential use (administration) preferably means administration of one (or more) components of a combination at one time point, other components at a different time point, that is, in a chronically staggered manner, preferably such that the combination shows more efficiency than the single compounds administered independently (especially showing synergism). Separate use (administration) preferably means administration of the components of the combination independently of each other at different time points, preferably meaning that the components (a) and (b) are administered such that no overlap of measurable blood levels of both compounds are present in an overlapping manner (at the same time).

[00274] Also combinations of two or more of sequential, separate and simultaneous administration are possible, preferably such that the combination component-drugs show a joint therapeutic effect that exceeds the effect found when the combination component-drugs are used independently at time intervals so large that no mutual effect on their therapeutic efficiency can be found, a synergistic effect being especially preferred.

[00275] In a sixty-second embodiment, the miRNA inhibitor inhibits miR-145, miR-208a, miR-137 or miR-122. The invention also pertains to methods of using these miRNA inhibitors to inhibit the miRNA, and/or to treat, prevent or ameliorate a disease related to expression or over-expression of the miRNA. In a sixty-third embodiment, the invention provides for the use of the miR-145 inhibitor in the treatment or amelioration of various diseases related to expression or over-expression of miR-145, including Marfan syndrome, Loeys-Dietz syndrome, Takayasu's arteritis, giant cell arteritis, liver fibrosis, kidney fibrosis, heart fibrosis, respiratory disease, pulmonary arterial hypertension (PAH), idiopathic pulmonary fibrosis (IPF), Asthma, Chronic Obstructive Pulmonary Disease (COPD), chronic/scarless wound healing, and primary open angle glaucoma (POAG). In a sixty-fourth embodiment, the invention provides for the use of the miR-208a inhibitor in the treatment or amelioration of

various diseases related to expression or over-expression of miR-208a, including Non-alcoholic fatty liver disease (NAFLD), Non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, heart disease, heart failure, acute coronary syndrome (ACS), hypertrophic heart dysfunction, acute myocardial infarction, obesity, fatty liver, metabolic syndrome, and Type II diabetes mellitus (T2DM). In a sixty-fifth embodiment, the invention provides for the use of the miRNA inhibitor in the treatment or amelioration of various diseases related to expression or over-expression of miRNA-137, or miRNA-137 variants with a single nucleotide polymorphism, including schizophrenia. In a sixty-seventh embodiment, the invention provides for the use of the miRNA-122 inhibitor in the treatment or amelioration of various diseases related to expression or over-expression of miRNA-122, including viral infections, including HCV infection, and dysregulated homeostasis, including high cholesterol and fatty liver disease.

[00276] The compositions for delivery of a miRNA inhibitor, as described herein and known in the art, can be used with any of the miRNA inhibitors described herein. Similarly, the methods of use described herein and known in the art can be used with any miRNA inhibitor described herein.

NOVEL ANTI-miRNA OLIGONUCLEOTIDES

[00277] This invention presents various novel miRNA inhibitors, including anti-miRNA oligonucleotides. Sequences of these various novel miRNA inhibitors are presented in the Tables below.

[00278] Table 1. Human microRNA stem loop hairpin ligands for SPR binding assays.

[00279] Table 2. Human pre-microRNA hairpin substrates for *in vitro* Dicer processing assays

[00280] Table 3. Human miR-145 AMO hairpin walk biochemical screening data

[00281] Table 4. Human miR-145 PNA

[00282] Table 5. Human miR-208a AMO hairpin walk biochemical screening data

[00283] Table 6. Human miR-137 AMO data

[00284] Table 7. Human miR-122 AMO hairpin walk biochemical screening data

[00285] Table 8. Chemistry of AMOs

[00286] Table 9. A list of some miRNA inhibitors to miR-145, miR-208a, miR-137 and miR-122.

[00287] Table 10. Activity of various AMOs to miR-145.

[00288] Table 1 shows miRNA stem loop hairpin ligands used in SPR binding assays, including those described in Example 1. "hsa" indicates *Homo sapiens*.

Table 1. Human microRNA stem loop hairpin ligands for SPR binding assays

<u>Ligand</u>	<u>Chemical Composition</u>	<u>SEQ ID NO:</u>
hsa-miR-145	5'-Biotin-CACCUUGUCCUCACGGUCCAGUUUUUCCCAGGAAUCCCUUAGAUGCUAAGAUGGGGAUCCUGGAAAUA CUGUUCUUGAGGUCAUGGUU-3'	1
hsa-miR-208a	5'-Biotin-UGACGGGCGAGCUUUUGGCCCCGGGUUAUACCUGAUGCUCACGUAAUAGACGAGCAAAAAGCUUGUUGGUCA-3'	2

[00289] Table 2 shows human pre-microRNA hairpin substrates for in vitro Dicer processing assays, as detailed in Example 1.

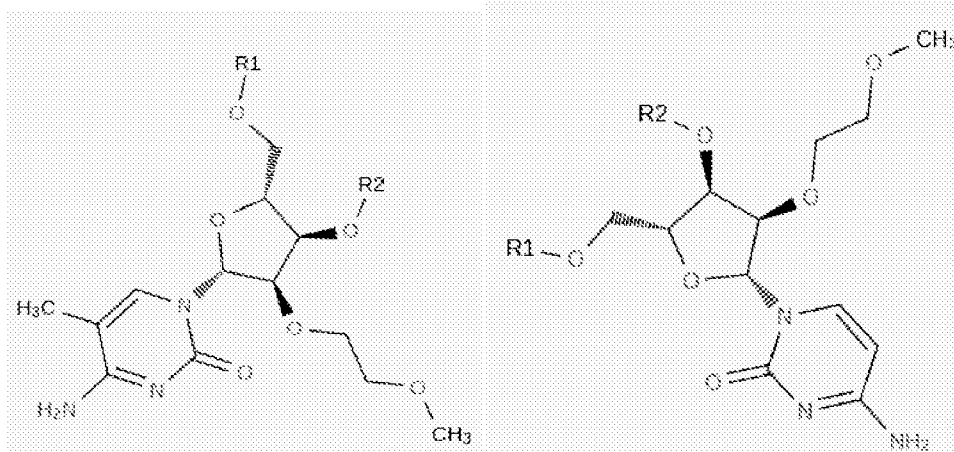
Table 2. Human pre-microRNA hairpin substrates for in vitro Dicer processing assays

<u>Substrate</u>	<u>Chemical Composition</u>	<u>SEQ ID NO:</u>
hsa-pre-miR-145	5'-Phosphate-GUCCAGUUUUUCCCAGGAAUCCCUUAGAUGCUAAGAUGGGGAUCCUGGAAAUA CUGUUCU-3'	3
hsa-pre-miR-208a	5'-Phosphate-GAGCUUUUGGCCCCGGGUUAUACCUGAUGCUCACGUAAAGACGAGCAAAAAGCUUGU-3'	4
hsa-pre-miR-137	5'-phosphate-ACGGGUUUUCUUGGGUGGAUAAUACGGAUUACGUUGUUAUUGCUUAAGAAUACGCGUAG-3'	5
hsa-pre-miR-122	5'-phosphate-UGGAGUGUGACAAUGGUGUUUGUGUCUAAACUAUCAAACGCCAUUAUCACACUAAAUA-3'	6

[00290] In the Tables herein:

[00291] "005" indicates a 2'-MOE modification. As examples, "A005", "G005", or "C005", etc., represent a modified nucleotide wherein the base is, respectively, A, G, or C, respectively, and the sugar has a 2'-MOE modification.

[00292] "006" is also MOE. It refers to a MOE-C without the 5-Me modification on the nucleobase. Below, C006 is on the left and C005 is on the right. Both are MOE-C but C005 has the 5-Me group.



[00293] R1 and R2 are where the molecule attaches to the rest of the oligonucleotide via the PO (phosphate) or PS (phosphorothioate) linkages.

[00294] "007" indicates a 2'-F. As examples, "A007", "G007", or "C007", etc., represent a modified nucleotide wherein the base is, respectively, A, G, or C, respectively, and the sugar has a 2'-F modification.

[00295] "p" indicates a phosphate (a component of a nucleotide).

[00296] "X003" indicates a C6 3' end cap, which is a C6 modification at the 3' carbon of the sugar. U.S. Pat. No. 8,404,831.

[00297] In the Tables, "ALL MOE" indicates that all or almost all the mers are 2'MOE; "Alternating" indicates that, in general, two different modifications alternate (e.g., 007 and 006).

[00298] Table 3 shows Human miR-145 AMO hairpin walk biochemical screening data. In this Table, those molecules with a higher SPR and lower miR-145 processing can be considered more efficacious. Processing can be considered more efficacious, as the masses are roughly equivalent/comparable. In the final column, for example, for 36XD, "1.17" indicates 1.17% cleavage of the miR-145 pre-miRNA by Dicer in the presence of 36XD compared to the negative control treatment (100% cleavage).

[00299] "Man" is short for Manual, meaning the modifications are assigned by hand (manually) rather than according to a predetermined pattern.

Table 3. Human miR-145 AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
36XD	AATCCCCATCTTAGCATCTAA	144	A005 p A007 p U005 p C007 p C006 p C007 p C006 p A007 p U005 p C007 p U005 p U007 p A005 p G007 p C006 p A007 p U005 p C007 p U005 p A007 p A005 p X003	234	Alternating	106	1.17
54IA	GTATTTCCAGGAATCCCCATC	145	G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p A005 p U005 p C006 p X003	235	All MOE	1	119.49
19VF	CTTAGCATCTAAGGGATTCT	146	C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p X003	236	All MOE	52	8.22
20HB	TTCCAGGAATCCCCATCTTAG	147	U005 p U007 p C006 p C007 p A005 p G007 p G005 p A007 p A005 p U007 p C006 p C007 p C006 p C007 p A005 p U007 p C006 p U007 p U005 p A007 p G005 p X003	237	Alternating	43	33.93
94LF	GGAATCCCCATCTTAGCATCT	148	G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p X003	238	All MOE	104	8.51
35RA	ACCATGACCTCAAGAACAGTA	149	A005 p C006 p C006 p A005 p U005 p G005 p A005 p C006 p C006 p U005 p C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p X003	239	All MOE	15	99.04

Table 3. Human miR-145 AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO.	Modified Sequence	SEQ ID NO.	Chemistry	SPR Report Point	miR-145 Processing (% Control)
52XB	ACCTCAAGAACAGTATTTCCA	150	A005 p C006 p C006 p U005 p C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p X003	240	All MOE	2	56.97
68HA	GAACAGTATTTCCAGGAATCC	151	G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p X003	241	All MOE	3	157.8 9
16WE	TCCTGGGAAAACCTGGACCGTG	152	U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p G005 p X003	242	All MOE	31	18.02
08KF	ATTTCAGGAATCCCCATCTT	153	A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p X003	243	All MOE	3	92.50
22GD	AAAACCTGGACCGTGAGGACAA	154	A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p G005 p A005 p G005 p G005 p A005 p C006 p A005 p A005 p X003	244	All MOE	41	34.61
66WB	CCATGACCTCAAGAACAGTAT	155	C006 p C006 p A005 p U005 p G005 p A005 p C006 p C006 p U005 p C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p X003	245	All MOE	7	80.20

Table 3. Human miR-145 AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO.	Modified Sequence	SEQ ID NO.	Chemistry	SPR Report Point	miR-145 Processing (% Control)
05LF	AGCATCTAAGGGATTCCTGGG	156	A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p X003	246	All MOE	110	4.02
10VE	AAGGGATTCCTGGGAAAAGT	157	A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p X003	247	All MOE	13	103.8 3
90BF	CCATCTTAGCATCTAAGGGAT	158	C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p X003	248	All MOE	34	23.27
21HA	TTCCAGGAATCCCCATCTTAG	159	U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p X003	249	All MOE	18	6.39
21HA	TTCCAGGAATCCCCATCTTAG	160	U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p X003	250	All MOE	24	13.04
63XA	CAGTATTTCCAGGAATCCCCA	161	C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p X003	251	All MOE	3	145.9 5

Table 3. Human miR-145 AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO.	Modified Sequence	SEQ ID NO.	Chemistry	SPR Report Point	miR-145 Processing (% Control)
19KF	ATCTTAGCATCTAAGGGATTC	162	A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p X003	252	All MOE	30	1.13
29WB	TATTTCCAGGAATCCCCATCT	163	U005 p A007 p U005 p U007 p U005 p C007 p C006 p A007 p G005 p G007 p A005 p A007 p U005 p C007 p C006 p C007 p C006 p A007 p U005 p C007 p U005 p X003	253	Alternating	1	82.28
04AF	CAGGAATCCCCATCTTAGCAT	164	C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p X003	254	All MOE	88	2.37
52MB	TGACCTCAAGAACAGTATTTTC	165	U005 p G005 p A005 p C006 p C006 p U005 p C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p X003	255	All MOE	6	61.79
67WA	AAGAACAGTATTTCCAGGAAT	166	A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p X003	256	All MOE	3	82.77
01BF	ATCTAAGGGATTCTGGGAAA	167	A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p X003	257	All MOE	3	68.36

Table 3. Human miR-145 AMO hairpin walk biochemical screening data

Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
16LE	ATTCTGGGAAACTGGACCG	168	A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p X003	258	All MOE	33	14.31
26XA	AATCCCCATCTTAGCATCTAA	169	A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p X003	259	All MOE	91	0.52
26XA	AATCCCCATCTTAGCATCTAA	170	A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p X003	260	All MOE	113	4.90
21VD	GGAAACTGGACCGTGAGGAC	171	G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p G005 p A005 p G005 p G005 p A005 p C006 p X003	261	All MOE	76	8.69
05AF	TTAGCATCTAAGGGATTCCTG	172	U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p X003	262	All MOE	118	3.11
15MB	GAATCCCCATCTTAGCATCTA	173	G005 p A007 p A005 p U007 p C006 p C007 p C006 p C007 p A005 p U007 p C006 p U007 p U005 p A007 p G005 p C007 p A005 p U007 p C006 p U007 p A005 p X003	263	Alternating	108	0.38

Table 3. Human miR-145 AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
20KE	CTAAGGGATTCTGGGAAAC	174	C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p X003	264	All MOE	9	73.35
99QF	CCCCATCTTAGCATCTAAGGG	175	C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p X003	265	All MOE	44	7.71
30WA	TATTTCCAGGAATCCCCATCT	176	U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p X003	266	All MOE	0	101.8 0
30WA	TATTTCCAGGAATCCCCATCT	177	U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p X003	267	All MOE	1	117.2 6
58CB	CCTCAAGAACAGTATTTCCAG	178	C006 p C006 p U005 p C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p X003	268	All MOE	4	64.71
63MA	AACAGTATTTCCAGGAATCCC	179	A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p X003	269	All MOE	2	101.7 9

Table 3. Human miR-145 AMO hairpin walk biochemical screening data

Antisense Concept Id	Sequence	SEQ ID NO.	Modified Sequence	SEQ ID NO.	Chemistry	SPR Report Point	miR-145 Processing (% Control)
14BC	AGGGATTCCTGGGAAAAC	180	A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p A005 p C006 p U005 p X019	270	Man 1	11	116.09
29LB	GGGATTCCTGGGAAAAC	181	G005 p G007 p G005 p A007 p U005 p U007 p C006 p C007 p U005 p G007 p G005 p G007 p A005 p A007 p A005 p A007 p C006 p U007 p G005 p G007 p A005 p X003	271	Man 2	27	155.51
02BE	CCTGGGAAAAC	182	C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p G005 p A005 p X003	272	All MOE	43	16.09
03PF	TTCCAGGAATCCCCATCTTA	183	U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p X003	273	All MOE	2	99.17
27LD	AAACTGGACCGTGAGGACAAG	184	A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p G005 p A005 p G005 p G005 p A005 p C006 p A005 p A005 p G005 p X003	274	All MOE	44	40.54
52BB	CATGACCTCAAGAACAGTATT	185	C006 p A005 p U005 p G005 p A005 p C006 p C006 p U005 p C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p X003	275	All MOE	6	98.63

Table 3. Human miR-145 AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
77LA	TCAAGAACAGTATTTCCAGGA	186	U005 p C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p X003	276	All MOE	5	89.02
00QF	GCATCTAAGGGATTCTGGGA	187	G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p X003	277	All MOE	57	0.15
11CB	TCCTGGGAAAACCTGGA	188	U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p A005 p X003	278	All MOE	20	81.89
16AE	GGATTCTGGGAAAACCTGGAC	189	G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p X003	279	All MOE	13	70.20
95GF	CATCTTAGCATCTAAGGGATT	190	C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p X003	280	All MOE	35	4.24
26MA	GAATCCCCATCTTAGCATCTA	191	G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p X003	281	All MOE	87	1.56
26MA	GAATCCCCATCTTAGCATCTA	192	G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p X003	282	All MOE	106	2.57

Table 3. Human miR-145 AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
21KD	TGGGAAAACCTGGACCGTGAGG	193	U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p G005 p A005 p G005 p G005 p X003	283	All MOE	67	27.27
41RD	AGGGATTCTCTGGGAAAACCTGGAC	194	A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p U027 p X058	284	Man 3	62	48.73
59CA	AGTATTTCCAGGAATCCCCAT	195	A005 p G005 p U005 p A005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p X003	285	All MOE	0	158.18
19RC	TCCTGGGAAAACCTGGA	196	U005 p C007 p C006 p U007 p G005 p G007 p G005 p A007 p A005 p A007 p A005 p C007 p U005 p G007 p G005 p A007 p X003	286	Alternating	24	82.71
14PF	TCTTAGCATCTAAGGGATTCC	197	U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p X003	287	All MOE	32	10.92
25BB	CCAGGAATCCCCATCTTAGCA	198	C006 p C007 p A005 p G007 p G005 p A007 p A005 p U007 p C006 p C007 p C006 p C007 p A005 p U007 p C006 p U007 p U005 p A007 p G005 p C007 p A005 p X003	288	Alternating	112	13.83

Table 3. Human miR-145 AMO hairpin walk biochemical screening data

Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
09FF	AGGAATCCCCATCTTAGCATC	199	A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p X003	289	All MOE	85	11.69
30LA	GGGATTCTGGGAAAACCTGGA	200	G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p X003	290	All MOE	16	101.2 7
30LA	GGGATTCTGGGAAAACCTGGA	201	G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p X003	291	All MOE	15	117.7 0
13BD	CTGGACCGTGAGGACAAGGTG	202	C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p G005 p A005 p G005 p G005 p A005 p C006 p A005 p A005 p G005 p G005 p U005 p G005 p X003	292	All MOE	60	49.99
57RB	GACCTCAAGAACAGTATTTCC	203	G005 p A005 p C006 p C006 p U005 p C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p X003	293	All MOE	8	32.90
63BA	AGAACAGTATTTCCAGGAATC	204	A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p X003	294	All MOE	2	107.7 3

Table 3. Human miR-145 AMO hairpin walk biochemical screening data

Antisense Concept Id	Sequence	SEQ ID NO.	Modified Sequence	SEQ ID NO.	Chemistry	SPR Report Point	miR-145 Processing (% Control)
06GF	TCTAAGGGATTCTGGGAAAA	205	U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p X003	295	All MOE	5	86.31
11QE	TTCCTGGGAAAACCTGGACCGT	206	U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p X003	296	All MOE	41	35.24
22CA	ATCCCCATCTTAGCATCTAAG	207	A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p X003	297	All MOE	58	1.95
22CA	ATCCCCATCTTAGCATCTAAG	208	A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p X003	298	All MOE	69	0.03
27AD	GAAAACCTGGACCGTGAGGACA	209	G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p G005 p A005 p G005 p G005 p A005 p C006 p A005 p X003	299	All MOE	47	0.07
61QB	AACCATGACCTCAAGAACAGT	210	A005 p A005 p C006 p C006 p A005 p U005 p G005 p A005 p C006 p C006 p U005 p C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p X003	300	All MOE	9	73.03

Table 3. Human miR-145 AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
05HC	AGGGATTCCTGGGAAAAGTGG	211	A005 p G007 p G005 p G007 p A005 p U007 p U005 p C007 p C006 p U007 p G005 p G007 p G005 p A007 p A005 p A007 p A005 p C007 p U005 p G007 p G005 p X003	301	Alternating	17	203.69
00FF	TAGCATCTAAGGGATTCCTGG	212	U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p X003	302	All MOE	105	4.58
10RB	TCCCCATCTTAGCATCTAAGG	213	U005 p C007 p C006 p C007 p C006 p A007 p U005 p C007 p U005 p U007 p A005 p G007 p C006 p A007 p U005 p C007 p U005 p A007 p A005 p G007 p G005 p X003	303	Alternating	44	0.00
15PE	TAAGGGATTCCTGGGAAAAGT	214	U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p X003	304	All MOE	13	97.76
94VF	CCCATCTTAGCATCTAAGGGA	215	C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p X003	305	All MOE	54	5.30
26BA	CCAGGAATCCCCATCTTAGCA	216	C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p X003	306	All MOE	81	8.44

Table 3. Human miR-145 AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
26BA	CCAGGAATCCCCATCTTAGCA	217	C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p X003	307	All MOE	93	7.76
53HB	CTCAAGAACAGTATTTCCAGG	218	C006 p U005 p C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p X003	308	All MOE	3	103.34
68RA	ACAGTATTTCCAGGAATCCCC	219	A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p X003	309	All MOE	0	122.17
19GC	AGGGATTCTCTGGGAAAACCTGGAC	220	A005 p G007 p G005 p G007 p A005 p U007 p U005 p C007 p C006 p U007 p G005 p G007 p G005 p A007 p A005 p A007 p A005 p C007 p U005 p G007 p G005 p A007 p C006 p U027 p X058	310	Man 2	48	55.29
24QB	ACCATGACCTCAAGAACAGTA	221	A005 p C007 p C006 p A007 p U005 p G007 p A005 p C007 p C006 p U007 p C006 p A007 p A005 p G007 p A005 p A007 p C006 p A007 p G005 p U007 p A005 p X003	311	Alternating	16	66.02
07GE	CTGGGAAAACCTGGACCGTGAG	222	C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p G005 p A005 p G005 p X003	312	All MOE	52	29.39

Table 3. Human miR-145 AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
08UF	TCCAGGAATCCCCATCTTAGC	223	U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p X003	313	All MOE	52	8.78
12QD	AACTGGACCGTGAGGACAAGG	224	A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p G005 p A005 p G005 p G005 p A005 p C006 p A005 p A005 p G005 p G005 p X003	314	All MOE	61	25.94
57GB	ATGACCTCAAGAACAGTATTT	225	A005 p U005 p G005 p A005 p C006 p C006 p U005 p C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p X003	315	All MOE	5	66.30
62QA	CAAGAACAGTATTTCCAGGAA	226	C006 p A005 p A005 p G005 p A005 p A005 p C006 p A005 p G005 p U005 p A005 p U005 p U005 p U005 p C006 p C006 p A005 p G005 p G005 p A005 p A005 p X003	316	All MOE	6	48.89
05VF	CATCTAAGGGATTCTGGGAA	227	C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p X003	317	All MOE	7	35.64
16HB	AGGGATTCTGGGAAAACCTGG	228	A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p X003	318	All MOE	16	101.20

Table 3. Human miR-145 AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO.	Modified Sequence	SEQ ID NO.	Chemistry	SPR Report Point	miR-145 Processing (% Control)
16HB	AGGGATTCCTGGGAAAACCTGG	229	A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p X003	319	All MOE	21	131.2 8
11FE	GATTCCTGGGAAAACCTGGACC	230	G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p X003	320	All MOE	24	28.11
21RA	TCCCCATCTTAGCATCTAAGG	231	U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p X003	321	All MOE	39	0.13
21RA	TCCCCATCTTAGCATCTAAGG	232	U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p X003	322	All MOE	61	3.23
26PD	GGGAAAACCTGGACCGTGAGGA	233	G005 p G005 p G005 p A005 p A005 p A005 p A005 p C006 p U005 p G005 p G005 p A005 p C006 p C006 p G005 p U005 p G005 p A005 p G005 p G005 p A005 p X003	323	All MOE	74	21.06

[00300] LXH874 is a Human miR-145 PNA used in Figure 3.

Table 4. Human miR-145 PNA		
Antisense Concept Id	Sequence	SEQ ID NO:
LXH874	GAATCCCCATCTTAGCATCT A	7

[00301] Table 5 shows Human miR-208a AMO hairpin walk biochemical screening data. The final column shows the amount of pre-miRNA remaining uncleaved by Dicer after addition of the miRNA inhibitor.

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
10TA	CGGGCCAAA AGCTCGCCC GTC	8	C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p C006 p U005 p C006 p G005 p C006 p C006 p C006 p G005 p U005 p C006 p X003	59	All MOE	18	509.88
43YF	CTTATACGT GAGCATCAG GTA	9	C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p X003	60	All MOE	17	0.77

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>SPR Report Point</u>	<u>miR-145 Processing (% Control)</u>
59IE	TGCTCGTCT TATACGTGA GCA	10	U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p X003	61	All MOE	11	48.96
74SD	AGCTTTTTG CTCGTCTTA TAC	11	A005 p G005 p C006 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p A005 p U005 p A005 p C006 p X003	62	All MOE	41	247.32

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
02KA	GTATAACCC GGGCCAAAA GCT	12	G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p C006 p U005 p X003	63	All MOE	16	531.50
57XF	ACGTGAGCA TCAGGTATA ACC	13	A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p X003	64	All MOE	15	113.19

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
42NF	CATCAGGTA TAACCCGGG CCA	14	C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p X003	65	All MOE	18	44.72
56JD	GACCAACAA GCTTTTGC TCG	15	G005 p A005 p C006 p C006 p A005 p A005 p C006 p A005 p A005 p G005 p C006 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p X003	66	All MOE	13	696.18

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
06JA	ACCCGGGC CAAAAGCTC GCCC	16	A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p C006 p U005 p C006 p G005 p C006 p C006 p C006 p X003	67	All MOE	10	253.30
54YE	TTTTGCTCG TCTTATACG TGA	17	U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p X003	68	All MOE	20	11.07

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
56MF	GGTATAACC CGGGCCAAA AGC	18	G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p C006 p X003	69	All MOE	2	145.74
60ID	ACAAGCTTT TTGCTCGTC TTA	19	A005 p C006 p A005 p A005 p G005 p C006 p U005 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p X003	70	All MOE	5	500.25

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>SPR Report Point</u>	<u>miR-145 Processing (% Control)</u>
53NF	TATACGTGA GCATCAGGT ATA	20	U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p X003	71	All MOE	6	680.44
68XE	CTCGTCTTA TACGTGAGC ATC	21	C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p X003	72	All MOE	23	5.88

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
38DF	GAGCATCAG GTATAACCC GGG	22	G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p X003	73	All MOE	43	5.50
01ZA	ATAACCCGG GCCAAAAGC TCG	23	A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p C006 p U005 p C006 p G005 p X003	74	All MOE	9	522.01

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
57MF	GTGAGCATC AGGTATAAC CCG	24	G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p X003	75	All MOE	61	9.02
42CF	TCAGGTATA ACCCGGGC CAAA	25	U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p X003	76	All MOE	13	21.53

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>SPR Report Point</u>	<u>miR-145 Processing (% Control)</u>
65YD	CCAACAAGC TTTTTGCTC GTC	26	C006 p C006 p A005 p A005 p C006 p A005 p A005 p G005 p C006 p U005 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p X003	77	All MOE	5	439.92
15YA	CCGGGCCAA AAGCTCGCC CGT	27	C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p C006 p U005 p C006 p G005 p C006 p C006 p C006 p G005 p U005 p X003	78	All MOE	10	477.89

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>SPR Report Point</u>	<u>miR-145 Processing (% Control)</u>
49DF	TCTTATACG TGAGCATCA GGT	28	U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p X003	79	All MOE	17	1.20
54NE	TTGCTCGTC TTATACGTG AGC	29	U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p X003	80	All MOE	16	13.98

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
69XD	AAGCTTTT GCTCGTCTT ATA	30	A005 p A005 p G005 p C006 p U005 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p X003	81	All MOE	26	201.32
53CF	TACGTGAGC ATCAGGTAT AAC	31	U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p X003	82	All MOE	8	194.76

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>SPR Report Point</u>	<u>miR-145 Processing (% Control)</u>
68ME	CGTCTTATA CGTGAGCAT CAG	32	C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p X003	83	All MOE	60	2.40
47SF	GCATCAGGT ATAACCCGG GCC	33	G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p X003	84	All MOE	19	167.57

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
010A	AACCCGGGC CAAAAGCTC GCC	34	A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p C006 p U005 p C006 p G005 p C006 p C006 p X003	85	All MOE	9	295.98
50DE	TTTTTGCTC GTCTTATAC GTG	35	U005 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p X003	86	All MOE	16	58.22

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
41RF	AGGTATAAC CCGGGCCAA AAG	36	A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p X003	87	All MOE	19	420.73
65ND	AACAAGCTT TTTGCTCGT CTT	37	A005 p A005 p C006 p A005 p A005 p G005 p C006 p U005 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p U005 p X003	88	All MOE	2	615.40

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
15NA	GGGCCAAAA GCTCGCCCG TCA	38	G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p C006 p U005 p C006 p G005 p C006 p C006 p C006 p G005 p U005 p C006 p A005 p X003	89	All MOE	35	403.27
48SF	TTATACGTG AGCATCAGG TAT	39	U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p X003	90	All MOE	1	135.97

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
64CE	GCTCGTCTT ATACGTGAG CAT	40	G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p X003	91	All MOE	15	49.49
33IF	TGAGCATCA GGTATAACC CGG	41	U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p X003	92	All MOE	21	90.50

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>SPR Report Point</u>	<u>miR-145 Processing (% Control)</u>
79MD	GCTTTTGC TCGTCTTAT ACG	42	G005 p C006 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p X003	93	All MOE	33	298.13
07EA	TATAACCCG GGCCAAAAG CTC	43	U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p C006 p U005 p C006 p X003	94	All MOE	18	613.47

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
52RF	CGTGAGCAT CAGGTATAA CCC	44	C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p X003	95	All MOE	19	110.08
47HF	ATCAGGTAT AACCCGGGC CAA	45	A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p X003	96	All MOE	16	7.97

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
61DD	ACCAACAAG CTTTTGCT CGT	46	A005 p C006 p C006 p A005 p A005 p C006 p A005 p A005 p G005 p C006 p U005 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p X003	97	All MOE	7	533.98
11DA	CCCGGGCC AAAAGCTCG CCCG	47	C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p C006 p U005 p C006 p G005 p C006 p C006 p C006 p G005 p X003	98	All MOE	10	207.19

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
44IF	GTCTTATAC GTGAGCATC AGG	48	G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p X003	99	All MOE	26	1.74
70MD	TGACCAACA AGCTTTTTG CTC	49	U005 p G005 p A005 p C006 p C006 p A005 p A005 p C006 p A005 p A005 p G005 p C006 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p X003	100	All MOE	10	204.21

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
59SE	TTTGCTCGT CTTATACGT GAG	50	U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p X003	101	All MOE	11	11.30
65CD	CAAGCTTTT TGCTCGTCT TAT	51	C006 p A005 p A005 p G005 p C006 p U005 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p X003	102	All MOE	14	521.98

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>SPR Report Point</u>	<u>miR-145 Processing (% Control)</u>
58HF	ATACGTGAG CATCAGGTA TAA	52	A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p X003	103	All MOE	6	744.86
63RE	TCGTCTTAT ACGTGAGCA TCA	53	U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p X003	104	All MOE	30	1.23

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
42XF	AGCATCAGG TATAACCCG GGC	54	A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p X003	105	All MOE	0	94.03
06TA	TAACCCGGG CCAAAAGCT CGC	55	U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p G005 p C006 p U005 p C006 p G005 p C006 p X003	106	All MOE	0	185.50

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	SPR Report Point	miR-145 Processing (% Control)
55IE	CTTTTGGCT CGTCTTATA CGT	56	C006 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p X003	107	All MOE	35	333.42
46WF	CAGGTATAA CCCGGGCC AAAA	57	C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p A005 p A005 p A005 p A005 p X003	108	All MOE	19	53.56

Table 5. Human miR-208a AMO hairpin walk biochemical screening data							
<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>SPR Report Point</u>	<u>miR-145 Processing (% Control)</u>
60SD	CAACAAGCT TTTTGCTCG TCT	58	C006 p A005 p A005 p C006 p A005 p A005 p G005 p C006 p U005 p U005 p U005 p U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p X003	109	All MOE	4	534.49

[00302] Table 6 shows Human miR-137 AMO hairpin walk biochemical screening data. The final column shows the amount of pre-miRNA remaining uncleaved by Dicer after addition of the miRNA inhibitor. For example, in the first row, 0.90 or 90% of the test miRNA pre-miRNA remained uncleaved in the presence of miRNA inhibitor 58VE.

<u>Table 6. Human miR-137 AMO data</u>						
<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>miR-137 Processing (% hairpin remaining)</u>
58VE	TAATCCGTAT TATCCACCC	110	U005 p A007 p A005 p U007 p C006 p C007 p G005 p U007 p A005 p U007 p U005 p A007 p U005 p C007 p C006 p A007 p C006 p C007 p C006 p X003	116	Alternating	0.90
72ZE	CGTAATCCGT ATTATCCAC	111	C006 p G007 p U005 p A007 p A005 p U007 p C006 p C007 p G005 p U007 p A005 p U007 p U005 p A007 p U005 p C007 p C006 p A007 p C006 p X003	117	Alternating	0.72

<u>Table 6. Human miR-137</u> <u>AMO data</u>						
<u>Antisense</u> <u>Concept</u> <u>Id</u>	<u>Sequence</u>	<u>SEQ</u> <u>ID</u> <u>NO:</u>	<u>Modified</u> <u>Sequence</u>	<u>SEQ ID</u> <u>NO:</u>	<u>Chemistry</u>	<u>miR-137</u> <u>Processing</u> <u>(% hairpin</u> <u>remaining)</u>
78EE	AACAACGTAA TCCGTATTA	112	A005 p A007 p C006 p A007 p A005 p C007 p G005 p U007 p A005 p A007 p U005 p C007 p C006 p G007 p U005 p A007 p U005 p U007 p A005 p X003	118	Alternating	0.70
73JE	AAGCAATAAC TTCGTAATC	113	A005 p A007 p G005 p C007 p A005 p A007 p U005 p A007 p A005 p C007 p U005 p U007 p C006 p G007 p U005 p A007 p A005 p U007 p C006 p X003	119	Alternating	0.63

<u>Table 6. Human miR-137</u> <u>AMO data</u>						
<u>Antisense</u> <u>Concept</u> <u>Id</u>	<u>Sequence</u>	<u>SEQ</u> <u>ID</u> <u>NO:</u>	<u>Modified</u> <u>Sequence</u>	<u>SEQ ID</u> <u>NO:</u>	<u>Chemistry</u>	<u>miR-137</u> <u>Processing</u> <u>(% hairpin</u> <u>remaining)</u>
68PE	ATAACAACGT AATCCGTAT	114	A005 p U007 p A005 p A007 p C006 p A007 p A005 p C007 p G005 p U007 p A005 p A007 p U005 p C007 p C006 p G007 p U005 p A007 p U005 p X003	120	Alternating	0.84
53QE	CGCGTATTCT TAAGCAATA	115	C006 p G007 p C006 p G007 p U005 p A007 p U005 p U007 p C006 p U007 p U005 p A007 p A005 p G007 p C006 p A007 p A005 p U007 p A005 p X003	121	Alternating	0.49

[00303] Table 7 shows the results of the human miR-122 AMO hairpin walk biochemical screening data. In Table 7, a more negative number in the last column indicates a more active compound. The processing here is listed as the % of guide strand that is produced relative to having no inhibitor. So, the more negative the number, the more inhibition. "-100.00" would indicate no guide strand produced at all, or complete inhibition.

Table 7. Human miR-122 AMO hairpin walk biochemical screening data

Antisense Concept Id	Sequence	SEQ ID NO:	Modified Sequence	SEQ ID NO:	Chemistry	miR-122 Processing (% Control)
56LE	AACACCATGT CACACTCC	122	A005 p A007 p C006 p A007 p C006 p C007 p A005 p U007 p U005 p G007 p U005 p C007 p A005 p C007 p A005 p C007 p U005 p C007 p C006 p X003	133	Alternating	-58.97
51FE	GGCGTTTGAT AGTTTAGAC	123	G005 p G007 p C006 p G007 p U005 p U007 p U005 p G007 p A005 p U007 p A005 p G007 p U005 p U007 p U005 p A007 p G005 p A007 p C006 p X003	134	Alternating	-48.65

Table 7. Human miR-122 AMO hairpin walk biochemical screening data

<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>miR-122 Processing (% Control)</u>
56AE	GCGTTTGATA GTTTAGACA	124	G005 p C007 p G005 p U007 p U005 p U007 p G005 p A007 p U005 p A007 p G005 p U007 p U005 p U007 p A005 p G007 p A005 p C007 p A005 p X003	135	Alternating	-42.01
50VE	GTTTGATAGTT TAGACACA	125	G005 p U007 p U005 p U007 p G005 p A007 p U005 p A007 p G005 p U007 p U005 p U007 p A005 p G007 p A005 p C007 p A005 p C007 p A005 p X003	136	Alternating	-41.81

Table 7. Human miR-122 AMO hairpin walk biochemical screening data

<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>miR-122 Processing (% Control)</u>
55PE	TATTTAGTGTG ATAATGGC	126	U005 p A007 p U005 p U007 p U005 p A007 p G005 p U007 p G005 p U007 p G005 p A007 p U005 p A007 p A005 p U007 p G005 p G007 p C006 p X003	137	Alternating	-57.25
50KE	TGGCGTTTGA TAGTTAGAC	127	U005 p G007 p G005 p C007 p G005 p U007 p U005 p U007 p G005 p A007 p U005 p A007 p G005 p U007 p U005 p A007 p G005 p A007 p C006 p X003	138	Alternating	-77.43

Table 7. Human miR-122 AMO hairpin walk biochemical screening data

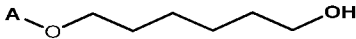
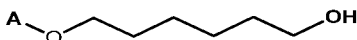
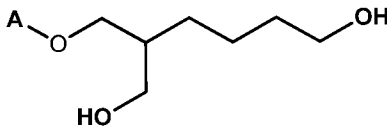
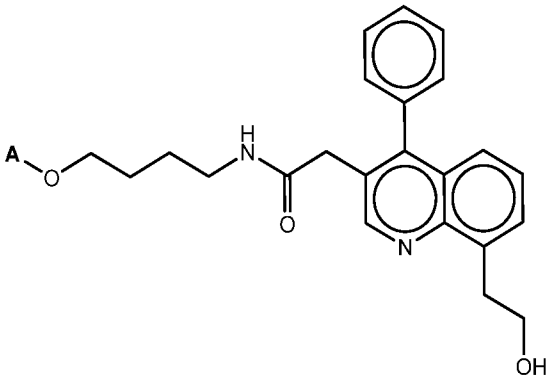
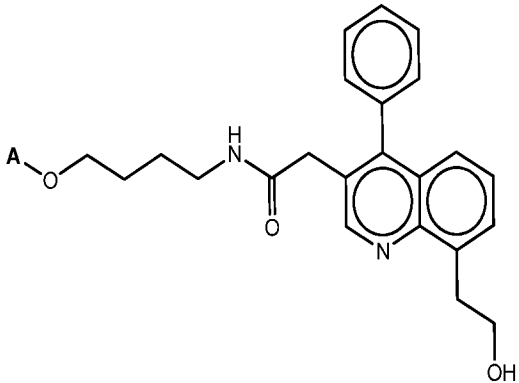
<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>miR-122 Processing (% Control)</u>
55EE	AATGGCGTTT GATAGTTAG	128	A005 p A007 p U005 p G007 p G005 p C007 p G005 p U007 p U005 p U007 p G005 p A007 p U005 p A007 p G005 p U007 p U005 p A007 p G005 p X003	139	Alternating	-75.26
31BF	ATAATGGCGT TTGATAGTT	129	A005 p U007 p A005 p A007 p U005 p G007 p G005 p C007 p G005 p U007 p U005 p U007 p G005 p A007 p U005 p A007 p G005 p U007 p U005 p X003	140	Alternating	-77.25

Table 7. Human miR-122 AMO hairpin walk biochemical screening data

<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>miR-122 Processing (% Control)</u>
45VF	TGATAATGGC GTTTGATAG	130	U005 p G007 p A005 p U007 p A005 p A007 p U005 p G007 p G005 p C007 p G005 p U007 p U005 p U007 p G005 p A007 p U005 p A007 p G005 p X003	141	Alternating	-54.49
40QF	TGTGATAATG GCGTTTGAT	131	U005 p G007 p U005 p G007 p A005 p U007 p A005 p A007 p U005 p G007 p G005 p C007 p G005 p U007 p U005 p U007 p G005 p A007 p U005 p X003	142	Alternating	-30.64

Table 7. Human miR-122 AMO hairpin walk biochemical screening data

<u>Antisense Concept Id</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>	<u>Modified Sequence</u>	<u>SEQ ID NO:</u>	<u>Chemistry</u>	<u>miR-122 Processing (% Control)</u>
45LF	TGATAGTTTAG ACACAAAC	132	U005 p G007 p A005 p U007 p A005 p G007 p U005 p U007 p U005 p A007 p G005 p A007 p C006 p A007 p C006 p A007 p A005 p A007 p C006 p X003	143	Alternating	-59.55

Table 8. Chemistry of AMOs		
Chemistry	Description	R =
Alternating	[Xp(MOE)Xp]n-R	
All MOE	[(MOE)Xp]n-R	
Man 1	[(MOE)Xp]n-R	
Man 2	[Xp(MOE)Xp]n-R	
Man 3	[(MOE)Xp]n-X058	

[00304] In a sixty-eighth embodiment, the sequence of the miRNA inhibitor comprises a region of complementarity complementary to the Dicer-excised region (or a portion thereof) of a pri-miRNA hairpin or pre-miRNA hairpin, wherein (a) the region of complementarity is at the 3' end of the miRNA inhibitor and the mer at the 3' end of the region of complementarity anneals to any of the first to fifth nt counting from the 5' end of the Dicer-excised region; or (b) the region of complementarity is at the 5' end of the miRNA inhibitor, and the mer at the 5' end of the region of complementarity anneals to any of the third to seventh nt counting from the 3' end of the Dicer-excised region. In a sixty-ninth embodiment, the invention provides a miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises a region of complementarity complementary to the Dicer-excised region (or a portion thereof) of a pri-miRNA hairpin or pre-miRNA hairpin, wherein the region of complementarity is at the 3' end of the miRNA inhibitor and the mer at the 3' end of the region of complementarity anneals to any of the third to seventh nt counting from the 3' end of the Dicer-excised region. Many such efficacious miRNA inhibitors are listed in Table 9. Table 9 represents some, but not necessarily all, of the particularly efficacious miRNA inhibitors disclosed herein.

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.							
Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
miR-145							
19VF	C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p X003	325	A237	CTTAGCATCTA AGGGATTCCT	353	CUUAGCAU CUAAGGGA UUCCU	381

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
29WB	U005 p A007 p U005 p U007 p U005 p C007 p C006 p A007 p G005 p G007 p A005 p A007 p U005 p C007 p C006 p C007 p C006 p A007 p U005 p C007 p U005 p X003	326	MAN	TATTTCCAGGA ATCCCCATCT	354	UAUUUCCA GGAAUCCC CAUCU	382
01BF	A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p G005 p A005 p A005 p A005 p X003	327	A237	ATCTAAGGGAT TCCTGGGAAA	355	AUCUAAGG GAUCCUG GGAAA	383

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
05AF	U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p X003	328	A237	TTAGCATCTAA GGGATTCCTG	356	UUAGCAUC UAAGGGAU UCCUG	384
15MB	G005 p A007 p A005 p U007 p C006 p C007 p C006 p C007 p A005 p U007 p C006 p U007 p U005 p A007 p G005 p C007 p A005 p U007 p C006 p U007 p A005 p X003	329	MAN	GAATCCCCATC TTAGCATCTA	357	GAAUCCCC AUCUUAGC AUCUA	385

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
26MA	G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p X003	330	A237	GAATCCCCATC TTAGCATCTA	358	GAAUCCCC AUCUUAGC AUCUA	386
14PF	U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p X003	331	A237	TCTTAGCATCT AAGGGATTCC	359	UCUUAGCA UCUAAGGG AUUCC	387

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
25BB	C006 p C007 p A005 p G007 p G005 p A007 p A005 p U007 p C006 p C007 p C006 p C007 p A005 p U007 p C006 p U007 p U005 p A007 p G005 p C007 p A005 p X003	332	MAN	CCAGGAATCCC CATCTTAGCA	360	CCAGGAAU CCCCAUCU UAGCA	388
00FF	U005 p A005 p G005 p C006 p A005 p U005 p C006 p U005 p A005 p A005 p G005 p G005 p G005 p A005 p U005 p U005 p C006 p C006 p U005 p G005 p G005 p X003	333	A237	TAGCATCTAAG GGATTCCTGG	361	UAGCAUCU AAGGGAUU CCUGG	389

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
26BA	C006 p C006 p A005 p G005 p G005 p A005 p A005 p U005 p C006 p C006 p C006 p C006 p A005 p U005 p C006 p U005 p U005 p A005 p G005 p C006 p A005 p X003	334	A237	CCAGGAATCCC CATCTTAGCA	362	CCAGGAU CCCCAUCU UAGCA	390
miR- 208a 59IE	U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p X003	335	A237	TGCTCGTCTTA TACGTGAGCA	363	UGCUCGUC UUAUACGU GAGCA	391

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
68XE	C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p X003	336	A237	CTCGTCTTATA CGTGAGCATC	364	CUCGUCUU AUACGUGA GCAUC	392
38DF	G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p X003	337	A237	GAGCATCAGGT ATAACCCGGG	365	GAGCAUCA GGUAUAAC CCGGG	393

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
57MF	G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p X003	338	A237	GTGAGCATCAG GTATAACCCG	366	GUGAGCAU CAGGUUAU ACCCG	394
68ME	C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p X003	339	A237	CGTCTTATACG TGAGCATCAG	367	CGUCUUAU ACGUGAGC AUCAG	395

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
47SF	G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p C006 p X003	340	A237	GCATCAGGTAT AACCCGGGCC	368	GCAUCAGG UAUAACCC GGGCC	396
64CE	G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p X003	341	A237	GCTCGTCTTAT ACGTGAGCAT	369	GCUCGUCU UAUACGUG AGCAU	397

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
33IF	U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p X003	342	A237	TGAGCATCAGG TATAACCCGG	370	UGAGCAUC AGGUAUAA CCCGG	398
63RE	U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p A005 p U005 p C006 p A005 p X003	343	A237	TCGTCTTATAC GTGAGCATCA	371	UCGUCUUA UACGUGAG CAUCA	399

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
42XF	A005 p G005 p C006 p A005 p U005 p C006 p A005 p G005 p G005 p U005 p A005 p U005 p A005 p A005 p C006 p C006 p C006 p G005 p G005 p G005 p C006 p X003	344	A237	AGCATCAGGTA TAACCCGGGC	372	AGCAUCAG GUAUAACC CGGGC	400
54NE	U005 p U005 p G005 p C006 p U005 p C006 p G005 p U005 p C006 p U005 p U005 p A005 p U005 p A005 p C006 p G005 p U005 p G005 p A005 p G005 p C006 p X003	345	A237	TTGCTCGTCTT ATACGTGAGC	373	UUGCUCGU CUUAUACG UGAGC	401

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
miR-137							
73JE	A005 p A007 p G005 p C007 p A005 p A007 p U005 p A007 p A005 p C007 p U005 p U007 p C006 p G007 p U005 p A007 p A005 p U007 p C006 p X003	346	A239	AAGCAATAACT TCGTAATC	374	AAGCAAUA ACUUCGUA AUC	402
72ZE	C006 p G007 p U005 p A007 p A005 p U007 p C006 p C007 p G005 p U007 p A005 p U007 p U005 p A007 p U005 p C007 p C006 p A007 p C006 p X003	347	A239	CGTAATCCGTA TTATCCAC	375	CGUAAUCC GUAUUAUC CAC	403

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
58VE	U005 p A007 p A005 p U007 p C006 p C007 p G005 p U007 p A005 p U007 p U005 p A007 p U005 p C007 p C006 p A007 p C006 p C007 p C006 p X003	348	A239	TAATCCGTATT ATCCACCC	376	UAAUCCGU AUUAUCCA CCC	404
miR-122							
50KE	U005 p G007 p G005 p C007 p G005 p U007 p U005 p U007 p G005 p A007 p U005 p A007 p G005 p U007 p U005 p A007 p G005 p A007 p C006 p X003	349	A239	TGGCGTTTGAT AGTTAGAC	377	UGGCGUUU GAUAGUUA GAC	405

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

Target / Concept ID	Antisense Modified Sequence String	SEQ ID NO:	Rule set	Antisense Sequence (DNA)	SEQ ID NO:	Generic Antisense Sequence	SEQ ID NO:
56AE	G005 p C007 p G005 p U007 p U005 p U007 p G005 p A007 p U005 p A007 p G005 p U007 p U005 p U007 p A005 p G007 p A005 p C007 p A005 p X003	350	A239	GCGTTTGATAG TTTAGACA	378	GCGUUUGA UAGUUUAG ACA	406
51FE	G005 p G007 p C006 p G007 p U005 p U007 p U005 p G007 p A005 p U007 p A005 p G007 p U005 p U007 p U005 p A007 p G005 p A007 p C006 p X003	351	A239	GGCGTTTGATA GTTTAGAC	379	GGCGUUUG AUAGUUUA GAC	407

TABLE 9. SOME EFFICACIOUS miRNA INHIBITORS.

<u>Target / Concept ID</u>	<u>Antisense Modified Sequence String</u>	<u>SEQ ID NO:</u>	<u>Rule set</u>	<u>Antisense Sequence (DNA)</u>	<u>SEQ ID NO:</u>	<u>Generic Antisense Sequence</u>	<u>SEQ ID NO:</u>
55EE	A005 p A007 p U005 p G007 p G005 p C007 p G005 p U007 p U005 p U007 p G005 p A007 p U005 p A007 p G005 p U007 p U005 p A007 p G005 p X003	352	A239	AATGGCGTTTG ATAGTTAG	380	AAUGGCGU UUGAUAGU UAG	408

[00305] In Table 10, the activity of various AMOs is shown. The name of the molecule is given in the first column, followed by the start and end positions on the miR-145 map, length in nucleotides (nt) and molecular weight (Mwt) in Daltons (Da). "Band 3%" is the % of mature product in the lane in the gel analysis for that particular sample. The whole reaction from the dicer assay is loaded onto an agarose gel. The banding pattern is examined and quantified to determine how much of each particular is in each lane. Higher Band 3% indicates more mature miR-145, and thus less inhibition by the AMO. A good inhibitor, for example, will lower the band 3%.

[00306] "Report Point" (RP) is a snapshot quantifier for the surface plasmon resonance (SPR) assay. The Report Point is proportional to ligand binding measured in a small window in time in the assay, just toward the end of the injection. The higher the Report Point, the more mass (or ligand) is bound to the surface of the chip that has the RNA on its surface. AMOs that bind to the target RNA (e.g., miR-145) will bind to the RNA, then the mass increases at the surface of the chip, and the Report Point will go up. Less binding of the AMO indicates a lower Report Point.

[00307] "RP/Length" is the ratio of "Report Point/Length" and is a way to normalize the data to account for different masses of the ligands (AMOs). Smaller (in this case, shorter) AMOs will have less effect on the mass change on the surface of the SPR chip when they bind, relative to larger (e.g., longer) ones. Thus, this is a computational way to correct for the difference using the AMO length.

[00308] As an alternative to the ratio of "Report Point/Length", the ratio of Report Point and Molecular Weight can be used. The relative results of "Report Point/Length" and "Report Point/Molecular Weight" of a series of AMOs should be similar, though normalizing to Molecular Weight will be more rigorous.

[00309] The final column presents RP/Mwt*1000. RP/Mwt is multiplied by 1000 to change the denominator units to kDa, rather than the Da that the molecular weights are recorded in.

TABLE 10. ADDITIONAL miRNA INHIBITORS								
AMO	miR-145 map start	miR-145 map end	Length (nt)	Mol. Weight (Mwt)	Band 3 %	Report Point (RP)	RP/ Length	RP/ Mwt*1000
78JB	54	39	16	6141.52	0.04	61	3.8	9.9
68UB	56	39	18	6883.05	0.06	50	2.8	7.3
67JC	59	42	18	6932.09	0.07	43	2.4	6.2
85PA	59	43	17	6063.03	0.26	47	2.7	7.7
88ZA	56	42	15	5347.59	0.33	42	2.8	7.8
26MA	59	39	21	8060.91	0.48	52	2.5	6.4
67UC	59	40	20	7673.62	0.49	57	2.8	7.4

TABLE 10. ADDITIONAL miRNA INHIBITORS

AMO	miR- 145 map start	miR- 145 map end	Length (nt)	Mol. Weight (Mwt)	Band 3 %	Report Point (RP)	RP/ Length	RP/ Mwt*1000
93YA	51	39	13	4659.10	0.57	40	3.1	8.5
79UA	49	39	11	3963.66	0.65	38	3.5	9.6
74PA	47	39	9	3292.24	0.68	30	3.4	9.2
73EB	53	39	15	5778.26	0.72	56	3.7	9.6
62PC	59	41	19	7295.35	0.78	51	2.7	7.0
77ZB	52	39	14	5415.00	0.79	51	3.6	9.4
79KA	58	40	19	6751.52	0.87	52	2.7	7.7
15MB	59	39	21	7429.89	0.90	66	3.1	8.8
82IB	49	39	11	4286.18	1.07	39	3.5	9.0
65DC	61	39	23	8851.48	1.14	75	3.3	8.5
84EA	57	41	17	5999.97	1.19	51	3.0	8.4
77OB	50	39	12	4664.45	1.24	43	3.6	9.3
80UA	59	41	19	6734.45	1.26	51	2.7	7.6
99OA	57	39	19	6695.41	1.27	54	2.8	8.1
73PB	55	39	17	6504.78	1.41	55	3.2	8.4
88DA	58	39	20	7657.62	1.47	57	2.9	7.5
72TB	51	39	13	5051.74	1.58	53	4.1	10.5
94JA	55	39	17	5999.97	1.73	59	3.5	9.9
99DA	53	39	15	5329.54	1.75	58	3.9	10.9
92YA	57	39	19	7270.34	1.83	56	2.9	7.7
60IC	62	39	24	9214.74	1.83	78	3.2	8.4

EXAMPLE**Surface plasmon resonance binding assay.**

[00310] SPR assays were run on the ProteOn XPR36 system (BioRad) using a neutravidin-coated NLC Sensor Chip (BioRad, #176-5021). Human microRNA stem loop hairpin ligands (5' biotinylated, RNase-free HPLC purification; Table 1) were synthesized by Integrated DNA Technologies (IDT) or TriLink BioTechnologies and diluted to 800 nM in Buffer A (20 mM Tris-HCl, pH 7.5, 140 mM NaCl, 5 mM MgCl₂, 2 mM KCl, 2 mM CaCl₂ and 0.05% P20). Ligands were heated for 5 min at 90°C and then cooled to 37°C at a rate of 3.5°C/min to ensure proper folding. Ligands (25 µl total) were bound to the NLC chip by injection at a rate of 50 µl/min. The coupled surface was washed sequentially with 1 M NaCl, Buffer A and Regeneration Buffer. Regeneration Buffer was composed of a 1:1:1 mix of water, acid solution (37.5 mM oxalic acid, 37.5 mM phosphoric acid, 37.5 mM formic acid and 37.5 mM malonic acid), and ionic solution (0.4 M KCl, 1.8 M MgCl₂, 0.9 M urea, 1.8M guanidine HCl). Anti-miR-oligonucleotide (AMO; Novartis Biologics Center) or peptide nucleic acid (PNA; PNA Bio) analytes were prepared as a 1 µM working stock in Buffer A. Analytes were heated to 85°C for 5 min, cooled to 37°C at a rate of 3.2°C/min and diluted to a final concentration of 100 nM in Buffer A. Sample injections (60 µl total) were performed for 6 analytes/round at a rate of 50 µl/min at 37°C. The surface was regenerated between injections by washing with Regeneration Buffer (30 µl total) at a rate of 100 µl/min. For the initial SPR screen report point values were collected at 63-65.7 s post-injection. To determine binding kinetics, surfaces were generated with between 1,000 and 1,200 RU (relative units) of the microRNA stem loop hairpin ligands. The anti-miR analytes were diluted in three-fold serial dilution and injected over the chip simultaneously, along with a buffer blank, at 50 µL/min for 2 min. Dissociation was monitored for 20 min. The top analyte concentrations were chosen empirically, ranging from 100 to 300 nM. Data were analyzed using the ProteOn Manager v. 3.1.0.6. After alignment at the injection point, data were double referenced against the interspot, which has no bound ligand, and to the simultaneous buffer blank. Data were fit using a Langmuir 1:1 binding model, with association and dissociation constants fit globally and Rmax fit locally.

***In vitro* Dicer processing assay.**

[00311] Human pre-microRNA hairpin substrates (5' phosphorylated, RNase-free HPLC purification; Table 2) were synthesized by Integrated DNA Technologies (IDT) or TriLink BioTechnologies. Substrates were diluted to a final concentration of 12.5 µM in nuclease-free Buffer B (30 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM DTT). AMOs and PNAs were resuspended at 25 µM in Buffer B, heated to 85°C for 5 min and cooled to 25°C at a rate of 3°C/min. *In vitro* processing assays were performed using the Recombinant Dicer

Enzyme Kit (Genlantis, #T510008) according to the manufacturer's protocol. AMOs and substrates were pre-incubated for 20 min at a molar stoichiometry of 1:1 or 2:1. Reaction samples were prepared by addition of 12 µl of 2X Loading Buffer (9.7 ml deionized formamide, 0.2 ml 0.5 M EDTA, 0.1 ml 100 mg/ml tartrazine) and subjected to electrophoresis at 200V for 1.25 h on 20% TBE gels (Life Technologies, #EC63152BOX) in 1X TBE buffer (Life Technologies, #LC6678) using an XCell Surelock Mini-Cell (Life Technologies, #EI0001). Gels were rinsed with milliQ H₂O and stained for 20 min with 5 µl SYBR gold (Life Technologies, #S11494) in 50 ml 1X TBE buffer. Gels were scanned and densitometry was performed using the BioRad ChemiDoc imaging system. Processing was quantitated by calculating the percent intensity of the miRNA duplex band relative to the total intensity in the lane and normalized to the 'no AMO' control treatment group.

***In vitro* Dicer processing assay - protection.**

[00312] Human pre-microRNA hairpin substrates (5' phosphorylated, RNase-free HPLC purification; Table 2) were synthesized by Integrated DNA Technologies (IDT) or TriLink BioTechnologies. Substrates were diluted to a final concentration of 25 µM in RNase free water. AMOs were suspended to a final concentration of 80 µM in 1X PBS. *In vitro* processing assays were performed using the Recombinant Dicer Enzyme Kit (Genlantis, #T510008). An assay mixture was prepared by mixing of 25 µL of hairpin solution, 10 µL of 10 mM ATP, 5 µL of 50 mM MgCl₂, and 40 µL of reaction buffer. Each assay mixture was made by mixing 8 µL of the assay mixture, 2 µL of the AMO solution, and 1 µL of Dicer solution. A control wells used 2 µL of 1X PBS in place of the AMO solution and another control well used RNase free water in place of the Dicer solution and 1X PBS in place of the AMO solution. Samples were incubated at 37°C for 24 h and then 2 µL of each sample was loaded onto a 1.2% agarose SybrSafe gel. Gels were imaged using a ChemiDoc MP imager. The bands were quantitated using the accompanying software (version 4.1). The percent hairpin remaining was calculated by comparing band intensities to the sample from the control well which did not include AMO or Dicer. Experiments were performed in triplicate and average results are reported.

Immunofluorescence assay.

[00313] On Day 1 transfection master mix was prepared by mixing 0.05 μ l/well Lipofectamine RNAiMAX (Life Technologies, #13778150) in 8.95 μ l/well Opti-MEM Reduced Serum Medium (Life Technologies, #31985070) followed by incubation for 5 min at room temperature. Transfection master mix (9 μ l/well) was transferred to a black, clear-bottom collagen-coated 384-well destination plate (Greiner BioOne, #781956) using a Matrix WellMate (Thermo Scientific). Plates were centrifuged for 1 min at 300xg. AMOs (1.5 μ l at 1 μ M) in 1X siRNA Buffer (Dharmacon/GE Healthcare, #B-002000-UB-100) were transferred from a 384-well clear, v-bottom source plate (Greiner BioOne, #781271) to the destination plate using a Matrix Platemate 2x2 (Thermo Scientific) and incubated for 20 min at room temperature to allow complex formation (50 nM final AMO concentration). Pulmonary arterial smooth muscle cell (PASMCs; Lonza, #CC-2581) were trypsinized (ReagentPack; Lonza, CC-5034), centrifuged at 300xg for 5 min at room temperature and resuspended in complete growth medium (SmGM-2; Lonza, #CC-3182) without antibiotics at 6.0×10^4 viable cells/ml. Reverse transfection was performed by transferring 20 μ l of PASMC cell suspension (1200 cells/well) to the 384-well destination plate using a Matrix WellMate. The destination plates were centrifuged for 1 min at 300xg at room temperature and incubated at 37°C + 5% CO₂ overnight. On Day 2 30 μ l of complete growth medium was added to the destination plates to dilute the transfection complexes and cells were allowed to recover for ~6 hrs. Plates were washed 4X with basal medium (SmGM-2; Lonza, CC-3181) using a BioMek FX (Beckman Coulter) and cells were starved overnight. On Day 3 and Day 5 cells were stimulated with human recombinant TGF β 1 (20 ng/ml final concentration; PeproTech, #100-21) in fresh basal media. On Day 8 cells were fixed with 4% paraformaldehyde (Electron Microscopy Sciences, #15710) for 20 min at room temperature. Plates were washed 3X with Triton Wash Buffer (PBS + 0.1% Triton X-100) using a ELx405 plate washer (BioTek) and then incubated with LiCOR blocking solution (LI-COR Biosciences, #927-40000) + 0.1% Triton X-100 for 1 h at room temperature. Cells were stained overnight with a directly-conjugated anti-smooth muscle actin-Cy3 mouse monoclonal (1A4) primary antibody (1:200 dilution; Sigma-Aldrich, #C6198) in blocking buffer. On Day 9 plates were washed 3X with Triton Wash Buffer and nuclei were stained with Hoechst 33258 (5 μ g/ml; Life Technologies, H3569) in filtered PBS for 10 min at room temperature. Cells were washed 3X with filtered PBS. Plates were sealed with aluminum foil plate covers (VWR, 60941-076) and imaged on an IN Cell Analyzer 1000 (GE Healthcare).

EMBODIMENTS

[00314] The following are embodiments of the invention.

[00315] 1. A miRNA inhibitor comprising a polymer, wherein the sequence of the miRNA inhibitor comprises a region of complementarity complementary to the Dicer-excised region or a portion thereof of a pri-miRNA hairpin or pre-miRNA hairpin, wherein (a) the region of complementarity is at the 3' end of the miRNA inhibitor and the mer at the 3' end of the region of complementarity anneals to any of the first to fifth nt counting from the 5' end of the Dicer-excised region; or (b) the region of complementarity is at the 5' end of the miRNA inhibitor, and the mer at the 5' end of the region of complementarity anneals to any of the third to seventh nt counting from the 3' end of the Dicer-excised region.

[00316] 2. The miRNA inhibitor of embodiment 1, wherein the polymer comprises a nucleotide, a modified nucleotide, and/or a nucleotide analogue.

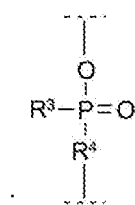
[00317] 3. The miRNA inhibitor of embodiment 1, wherein the nucleotide is RNA or DNA.

[00318] 4. The miRNA inhibitor of embodiment 1, wherein the modified nucleotide comprises a modification of the nucleotide at the base, sugar and/or phosphate.

[00319] 5. The miRNA inhibitor of embodiment 4, wherein the modification of the sugar is at the 2' carbon.

[00320] 6. The miRNA inhibitor of embodiment 4, wherein the modification of the sugar is a halo, a C1-10 alkyl, or a C1-10 alkoxy at the 2' carbon, 2'-OMe, 2'-MOE, 2'H, or 2'F.

[00321] 7. The miRNA inhibitor of claim 4, wherein the modification at the phosphate is selected from: phosphorothioate, phosphorodithioate, phosphoramidate, boranophosphonoate, an amide linker, C₁₋₆ alkyl, and a compound of formula (I)



(I), where R³ is selected from O⁻, S⁻, NH₂, BH₃, CH₃, C₁₋₆ alkyl, C₆₋₁₀ aryl, C₁₋₆ alkoxy and C₆₋₁₀ aryl-oxy, wherein C₁₋₆ alkyl and C₆₋₁₀ aryl are unsubstituted or optionally independently substituted with 1 to 3 groups independently selected from halo, hydroxyl and NH₂; and R⁴ is selected from O, S, NH, or CH₂.

[00322] 8. The miRNA inhibitor of embodiment 1, wherein the modified nucleotide is selected from: 2-aminopurine, (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

[00323] 9. The miRNA inhibitor of embodiment 1, wherein the nucleotide analogue is: peptide nucleic acid (PNA), PNA carrying an alternating a/b amino acid backbone consisting of (2'R,4'R)-nucleobase-substituted proline and (1S,2S)-2-aminocyclopentanecarboxylic acid (acpcPNA), locked nucleic acid (LNA), morpholino nucleotide, threose nucleic acid (TNA), glycol nucleic acid (GNA), arabinose nucleic acid (ANA), 2'-fluoroarabinose nucleic acid (FANA), cyclohexene nucleic acid (CeNA), anhydrohexitol nucleic acid (HNA), and/or unlocked nucleic acid (UNA), or xeno nucleic acid (XNA), or xylo nucleic acid (XNA).

[00324] 10. The miRNA inhibitor of embodiment 1, wherein the sequence of the miRNA inhibitor comprises 15 contiguous positions of the sequence of any miRNA inhibitor sequence disclosed herein.

[00325] 11. The miRNA inhibitor of embodiment 1, wherein the sequence of the miRNA inhibitor comprises the sequence of any miRNA inhibitor sequence disclosed herein.

[00326] 12. The miRNA inhibitor of claim 1, wherein the sequence of the miRNA inhibitor is the sequence of any miRNA inhibitor sequence disclosed herein.

[00327] 13. A miRNA inhibitor comprising a polymer which can anneal to a pri-miRNA and/or pre-miRNA in a sequence-dependent manner and comprises a sequence which can straddle a cleavage site by at least 3 to 7 nucleotides, and/or anneal to a Dicer-excised region or Drosha-excised region in the pri-miRNA and/or pre-miRNA.

[00328] 14. The miRNA inhibitor of embodiment 13, wherein the polymer comprises a nucleotide, a modified nucleotide, and/or a nucleotide analogue.

[00329] 15. A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises 15 contiguous positions of the sequence of any miRNA inhibitor sequence disclosed herein.

- [00330] 16. A miRNA inhibitor, wherein the sequence of the miRNA inhibitor comprises the sequence of any miRNA inhibitor sequence disclosed herein.
- [00331] 17. A miRNA inhibitor to miR-145, miR-137, miR-208a, or miR-122, wherein the sequence of the miRNA inhibitor comprises at least 15 contiguous positions of, comprises, or is the sequence of any miRNA inhibitor sequence disclosed herein.
- [00332] 18. A method of reducing the level, expression and/or activity of miR-145, miR-137, miR-208a, or miR-122 in a patient, the method comprising the step of administering a therapeutically effective amount of a miRNA inhibitor of claim 17.
- [00333] 19. A method of treating or ameliorating a disease or condition related to expression or over-expression of miR-145, miR-137, miR-208a, or miR-122, the method comprising the step of administering a therapeutically effective amount of a miRNA inhibitor of claim 17.
- [00334] 20. A method of treating a patient afflicted with Marfan syndrome, Loeys-Dietz syndrome, Takayasu's arteritis, giant cell arteritis, liver fibrosis, kidney fibrosis, heart fibrosis, respiratory disease, pulmonary arterial hypertension (PAH), idiopathic pulmonary fibrosis (IPF), Asthma, Chronic Obstructive Pulmonary Disease (COPD), chronic/scarless wound healing, and/or primary open angle glaucoma (POAG), wherein the method comprises the step of administering a therapeutically effective amount of an inhibitor to miR-145 disclosed herein.
- [00335] 21. A method of treating a patient afflicted with Non-alcoholic fatty liver disease (NAFLD), Non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, heart disease, heart failure, acute coronary syndrome (ACS), hypertrophic heart dysfunction, acute myocardial infarction, obesity, fatty liver, metabolic syndrome, and/or Type II diabetes mellitus (T2DM), wherein the method comprises the step of administering a therapeutically effective amount of an inhibitor to miR-208a disclosed herein.
- [00336] 22. A method of treating a patient afflicted with schizophrenia, wherein the method comprises the step of administering a therapeutically effective amount of an inhibitor to miR-137 disclosed herein.
- [00337] 23. A method of treating a patient afflicted with a viral infection, HCV infection, and/or dysregulated homeostasis, including high cholesterol and fatty liver disease, wherein the method comprises the step of administering a therapeutically effective amount of an inhibitor to miR-122 disclosed herein.

[00338] Unless defined otherwise, the technical and scientific terms used herein have the same meaning as that usually understood by a specialist familiar with the field to which the invention belongs.

[00339] Unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known per se, as will be clear to the skilled person. Reference is for example again made to the standard handbooks and the general background art mentioned herein and to the further references cited therein. Unless indicated otherwise, each of the references cited herein is incorporated in its entirety by reference.

[00340] Claims are non-limiting and provided below.

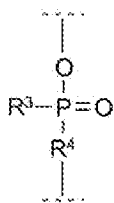
[00341] Although particular embodiments and claims have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, or is not intended to be limiting with respect to the scope of the appended claims, or the scope of subject matter of claims of any corresponding future application. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other embodiments, advantages, and modifications considered to be within the scope of the following claims. Those skilled in the art will recognize or be able to ascertain, using no more than routine experimentation, many equivalents of the specific embodiments described herein. Such equivalents are intended to be encompassed by the following claims. Redrafting of claim scope in later filed corresponding applications may be due to limitations by the patent laws of various countries and should not be interpreted as giving up subject matter of the claims.

CLAIMS

1. A miRNA inhibitor comprising a polymer, wherein the sequence of the miRNA inhibitor comprises a region of complementarity that is complementary to the Dicer-excised region or a portion thereof of a pri-miRNA hairpin or pre-miRNA hairpin, wherein
 - (a) the region of complementarity is at the 3' end of the miRNA inhibitor and the mer at the 3' end of the region of complementarity anneals to any of the first to fifth nt counting from the 5' end of the Dicer-excised region; or
 - (b) the region of complementarity is at the 5' end of the miRNA inhibitor, and the mer at the 5' end of the region of complementarity anneals to any of the third to seventh nt counting from the 3' end of the Dicer-excised region.
2. The miRNA inhibitor of claim 1, wherein the polymer comprises a nucleotide, a modified nucleotide, and/or a nucleotide analogue.
3. The miRNA inhibitor of claim 1, wherein the modified nucleotide comprises a modification of the nucleotide at the base, sugar and/or phosphate.
4. The miRNA inhibitor of claim 4, wherein the modification of the sugar is at the 2' carbon.
5. The miRNA inhibitor of claim 4, wherein the modification of the sugar is a halo, a C1-10 alkyl, or a C1-10 alkoxy at the 2' carbon, 2'-OMe, 2'-MOE, 2'H, or 2'F.

6. The miRNA inhibitor of claim 4, wherein the modification at the phosphate is selected from:

phosphorothioate, phosphorodithioate, phosphoramidate, boranophosphonoate, an amide linker, C1-6 alkyl, and a compound of formula (I):



(I), where R3 is selected from O-, S-, NH2, BH3, CH3, C1-6 alkyl, C6-10 aryl, C1-6 alkoxy and C6-10 aryl-oxy, wherein C1-6 alkyl and C6-10 aryl are unsubstituted or optionally independently substituted with 1 to 3 groups independently selected from halo, hydroxyl and NH2; and R4 is selected from O, S, NH, or CH2.

7. The miRNA inhibitor of claim 1, wherein the modified nucleotide is selected from: 2-aminopurine, (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.
8. The miRNA inhibitor of claim 1, wherein the nucleotide analogue is: peptide nucleic acid (PNA), PNA carrying an alternating a/b amino acid backbone consisting of (2'R,4'R)-nucleobase-substituted proline and (1S,2S)-2-aminocyclopentanecarboxylic acid (acpcPNA), locked nucleic acid (LNA), morpholino nucleotide, threose nucleic acid (TNA), glycol nucleic acid (GNA), arabinose nucleic acid (ANA), 2'-fluoroarabinose nucleic acid (FANA), cyclohexene nucleic acid (CeNA), anhydrohexitol nucleic acid (HNA), and/or unlocked nucleic acid (UNA), or xeno nucleic acid (XNA), or xylo nucleic acid (XNA).

9. The miRNA inhibitor of claim 1, which can anneal to a pri-miRNA and/or pre-miRNA in a sequence-dependent manner and comprises a sequence which can straddle a cleavage site by at least 3 to 7 nucleotides, and/or anneal to a Dicer-excised region or Drosha-excised region in the pri-miRNA and/or pre-miRNA.
10. The miRNA inhibitor of claim 1,
wherein the miRNA inhibitor is complementary to miR-145, miR-137, miR-208a, or miR-122, and
wherein the sequence of the miRNA inhibitor comprises at least 15 contiguous positions of, comprises, or is the sequence of any miRNA inhibitor sequence disclosed herein.
11. A method of reducing the level, expression or activity of miR-145, miR-137, miR-208a, or miR-122 in a patient,
the method comprising the step of administering a therapeutically effective amount of a miRNA inhibitor of claim 1.
12. A method of treating or ameliorating a disease or condition related to expression or over-expression of miR-145, miR-137, miR-208a, or miR-122,
the method comprising the step of administering a therapeutically effective amount of a miRNA inhibitor of claim 1.
13. A method of treating a patient afflicted with Marfan syndrome, Loeys-Dietz syndrome, Takayasu's arteritis, giant cell arteritis, liver fibrosis, kidney fibrosis, heart fibrosis, respiratory disease, pulmonary arterial hypertension (PAH), idiopathic pulmonary fibrosis (IPF), Asthma, Chronic Obstructive Pulmonary Disease (COPD), chronic/scarless wound healing, and/or primary open angle glaucoma (POAG),
wherein the method comprises the step of administering a therapeutically effective amount of an inhibitor to miR-145 disclosed herein.
14. A method of treating a patient afflicted with Non-alcoholic fatty liver disease (NAFLD), Non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, heart disease, heart failure, acute coronary syndrome (ACS), hypertrophic heart dysfunction, acute myocardial infarction, obesity, fatty liver, metabolic syndrome, and/or Type II diabetes mellitus (T2DM), wherein the method comprises the step of administering a therapeutically effective amount of an inhibitor to miR-208a disclosed herein.

15. A method of treating a patient afflicted with schizophrenia, wherein the method comprises the step of administering a therapeutically effective amount of an inhibitor to miR-137 disclosed herein.
16. A method of treating a patient afflicted with a viral infection, HCV infection, and/or dysregulated homeostasis, including high cholesterol and fatty liver disease, wherein the method comprises the step of administering a therapeutically effective amount of an inhibitor to miR-122 disclosed herein.

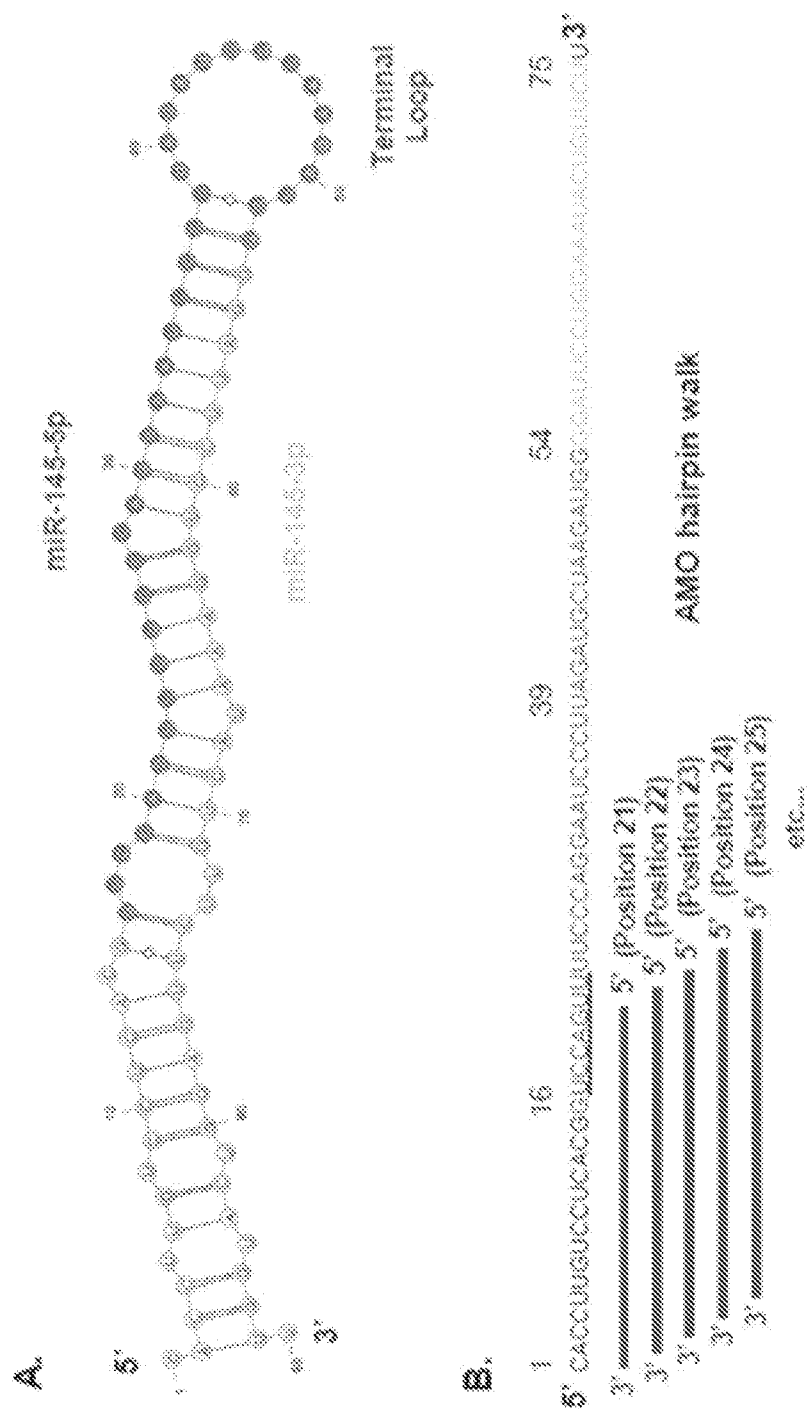


FIG. 1

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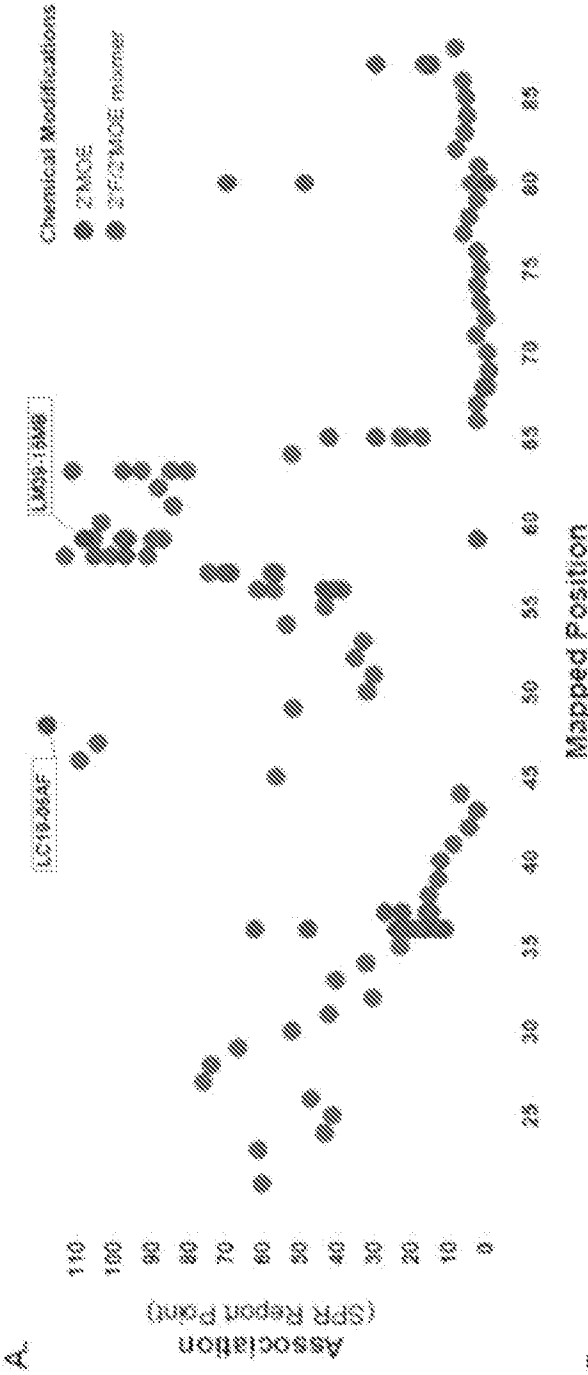


FIG. 2A

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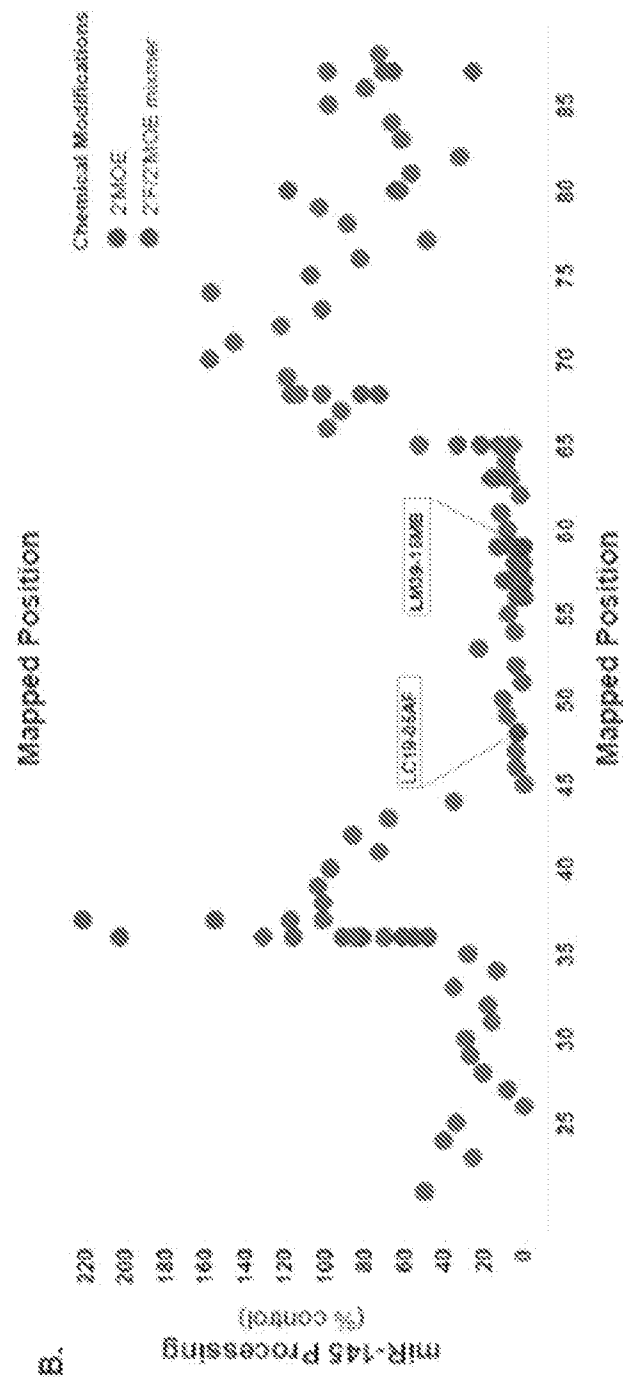


FIG. 2B

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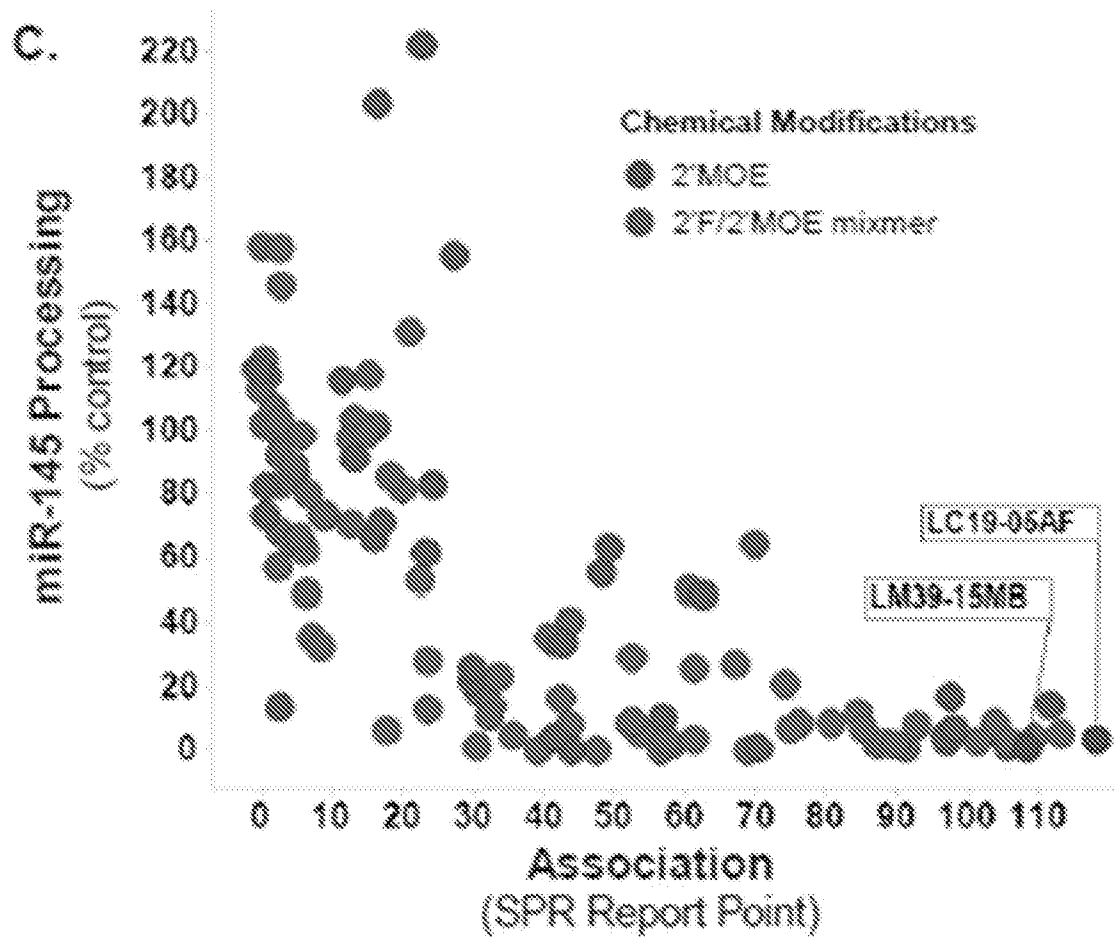
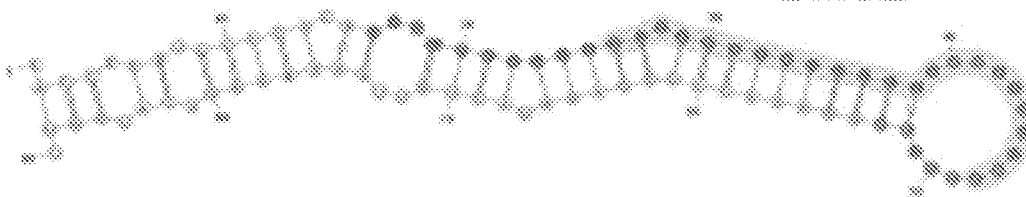


FIG. 2C

5/18

D.

LC19-05AF
2'MOE



E.

LM39-15MB
2'F/2'MOE



FIGS. 2D-E

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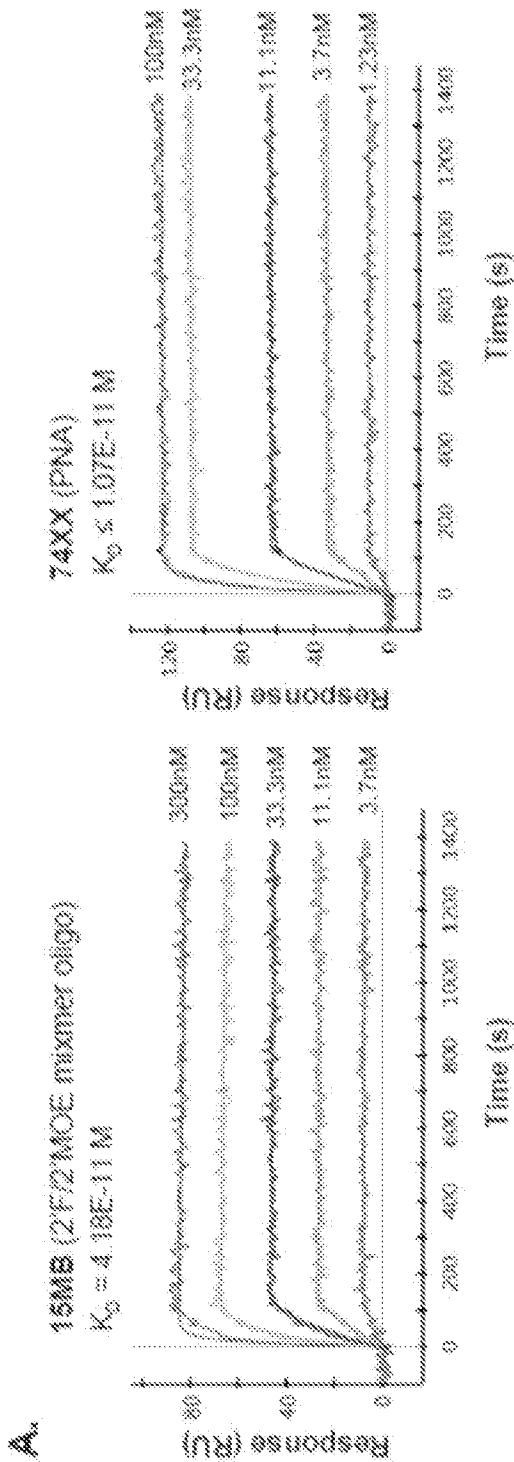


FIG. 3A

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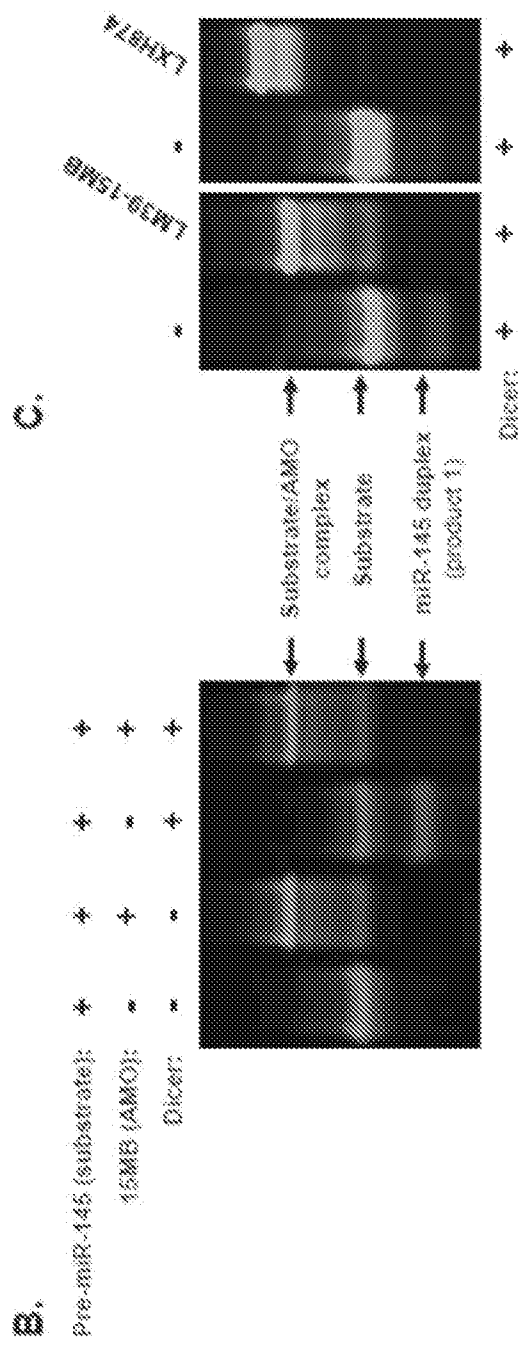


FIG. 3B-C

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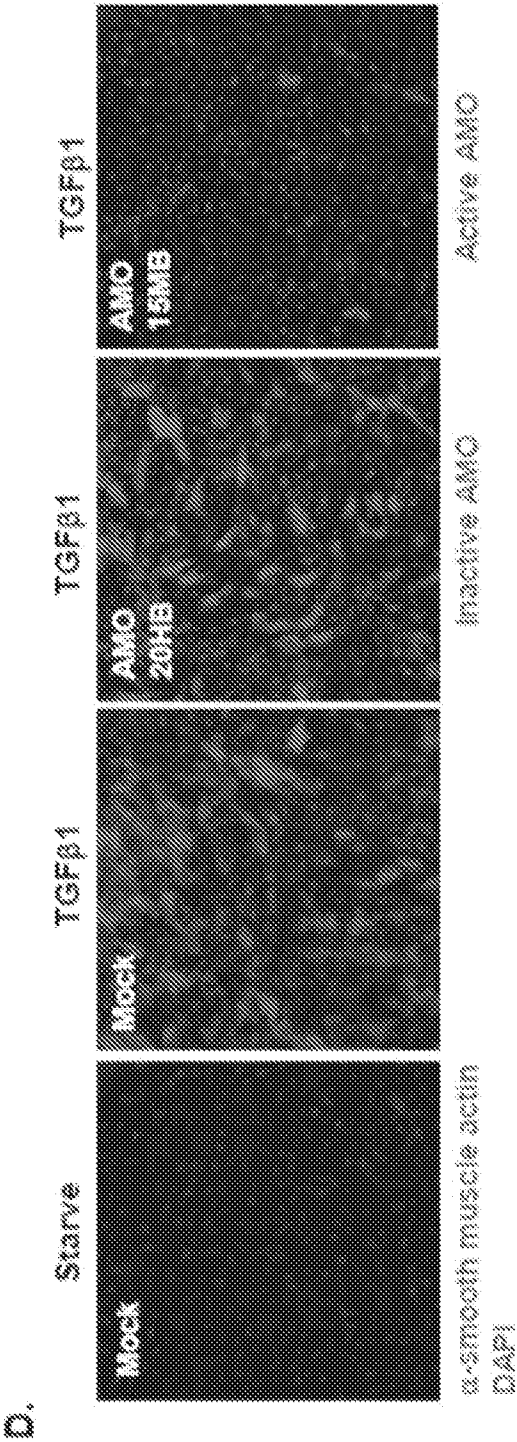


FIG. 3D

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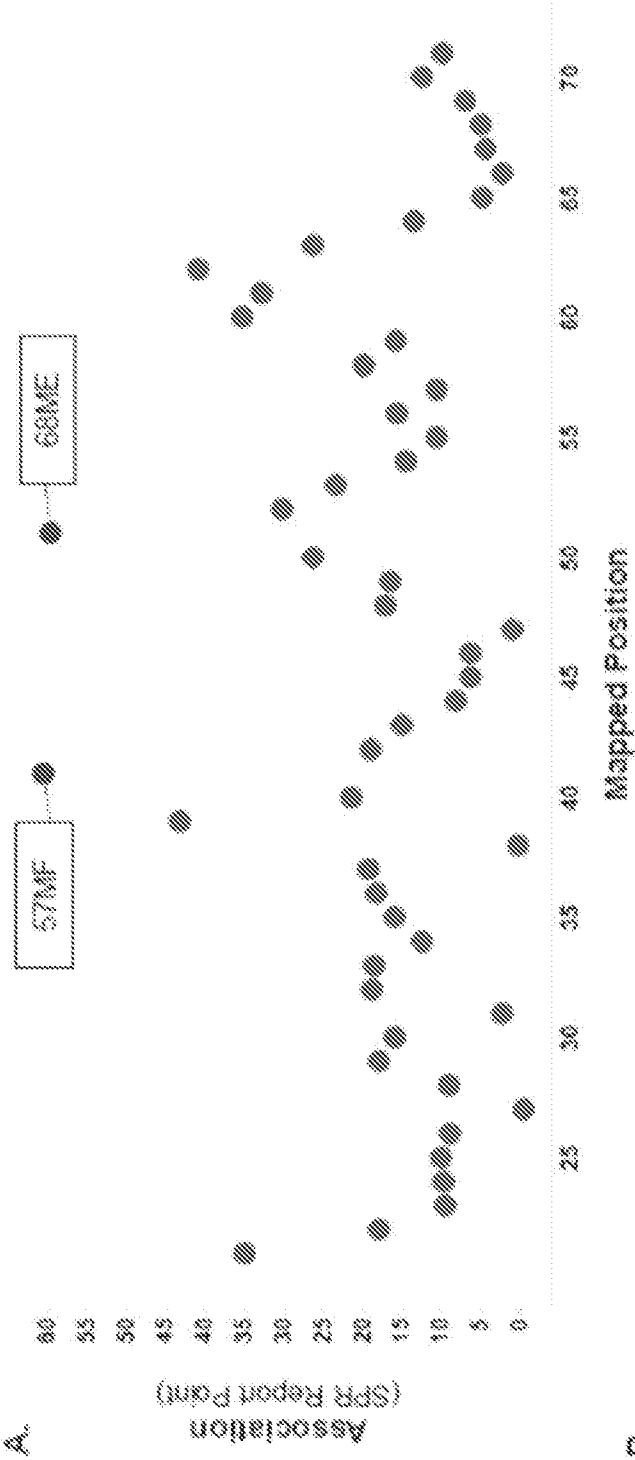


FIG. 4A

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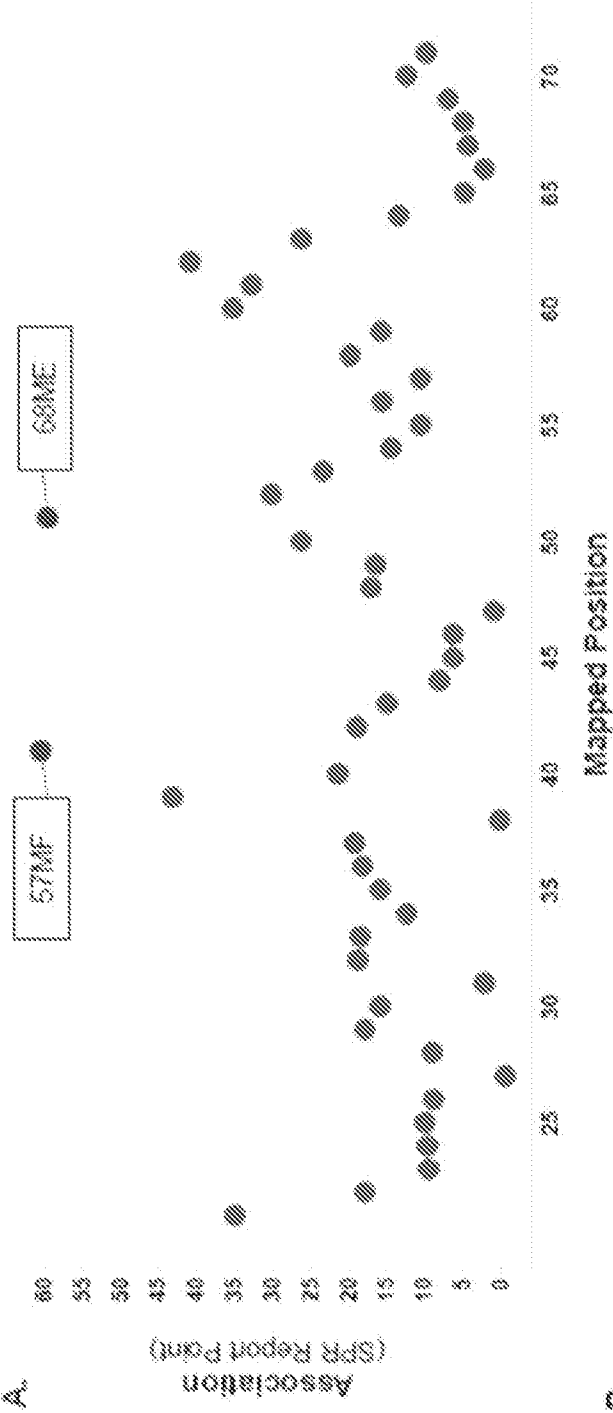


FIG. 4B

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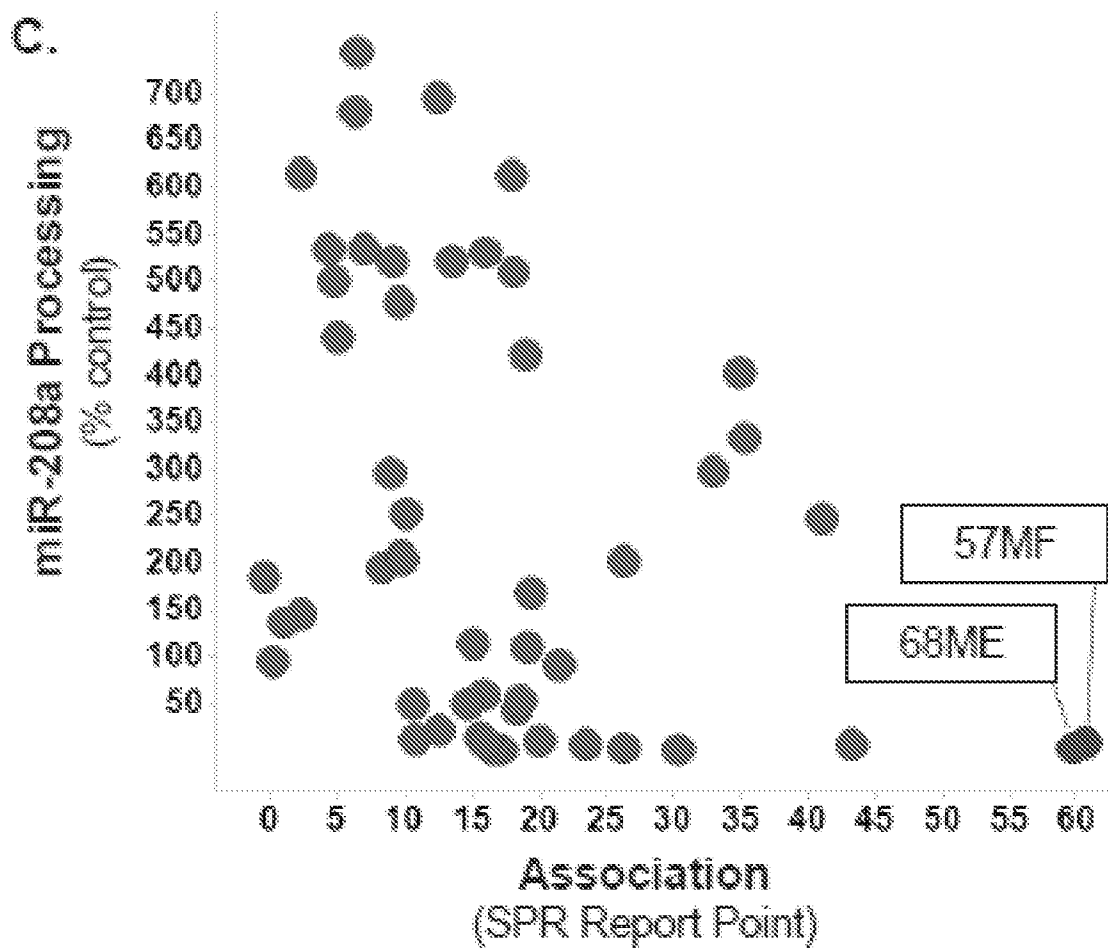
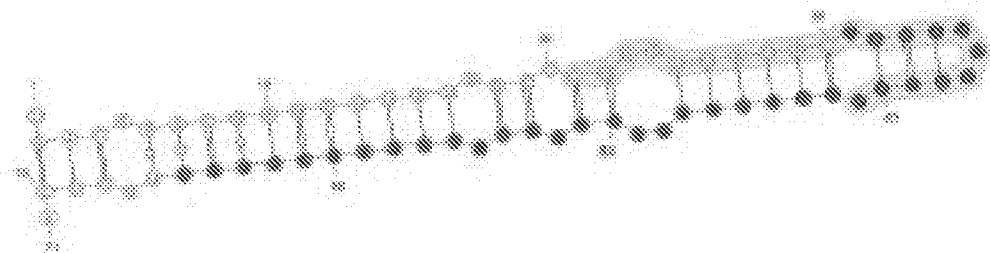


FIG. 4C

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D.

57MF



E.

68ME

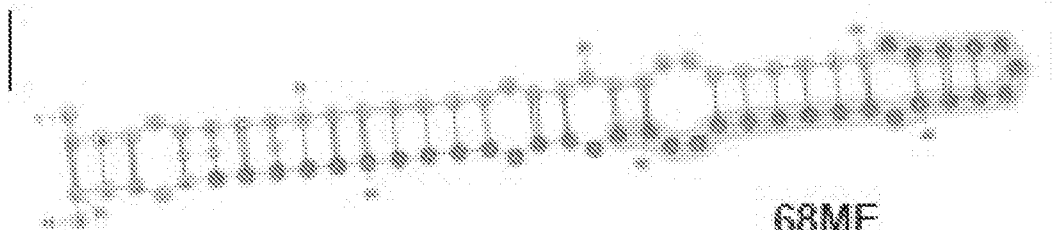


FIG. 4D-E

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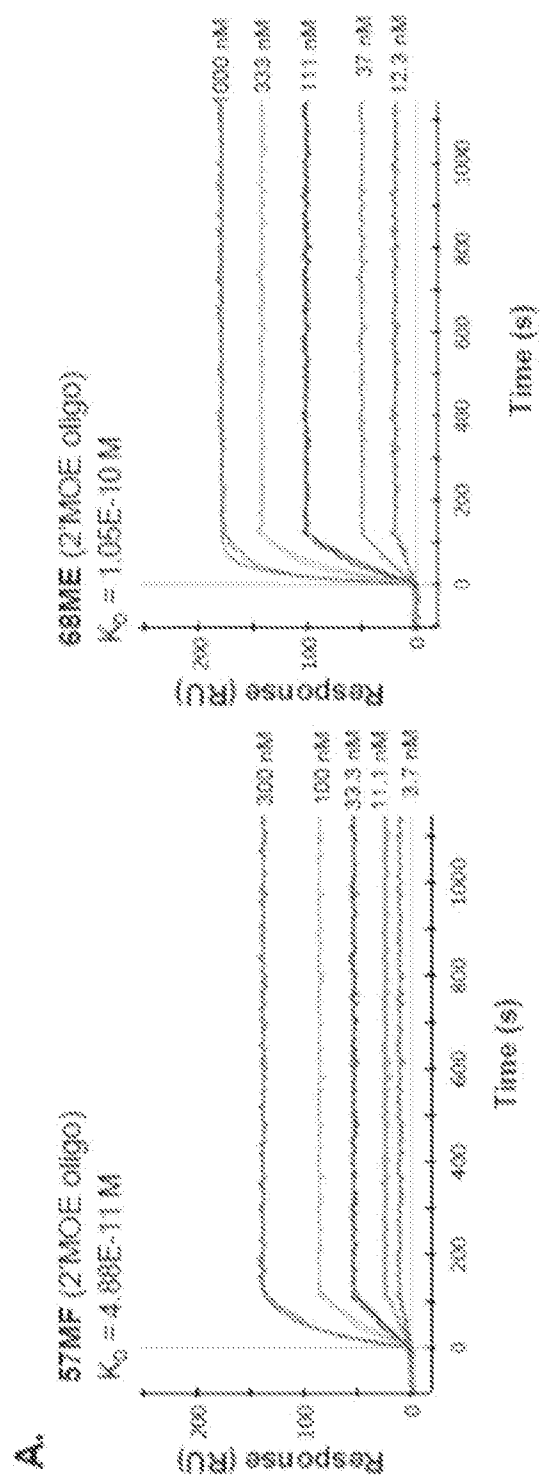


FIG. 5A

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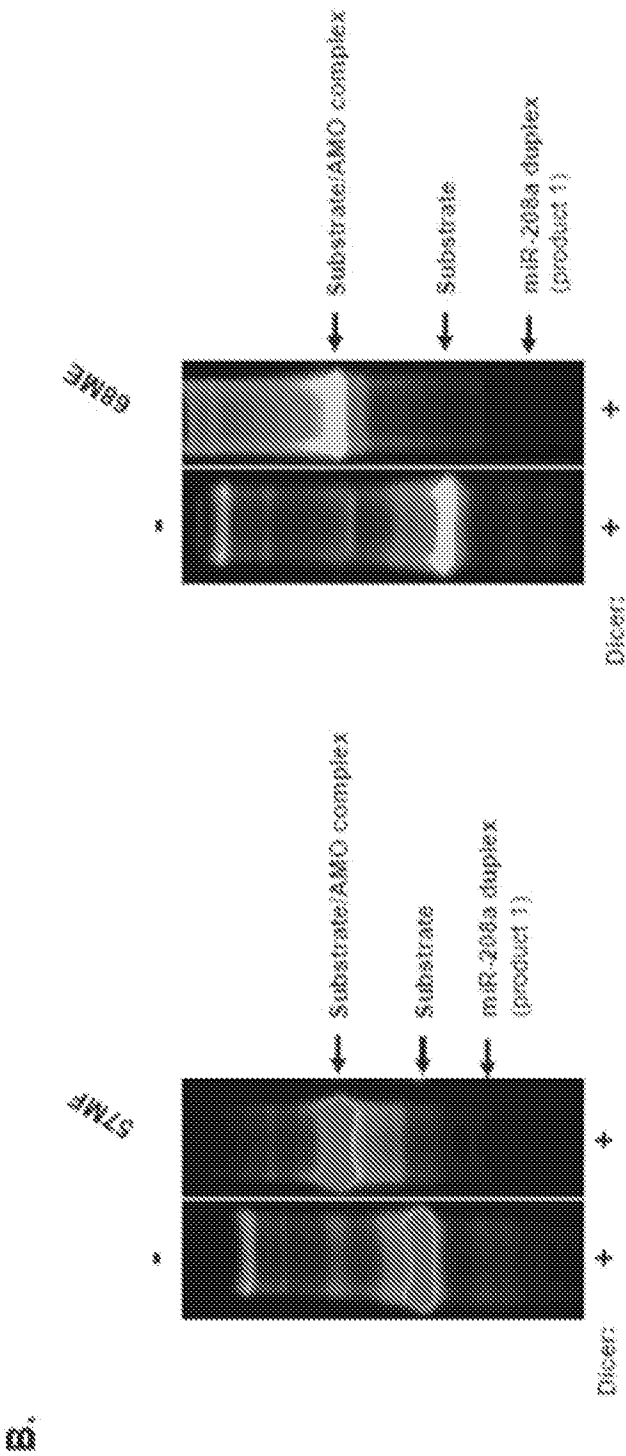


FIG. 5B

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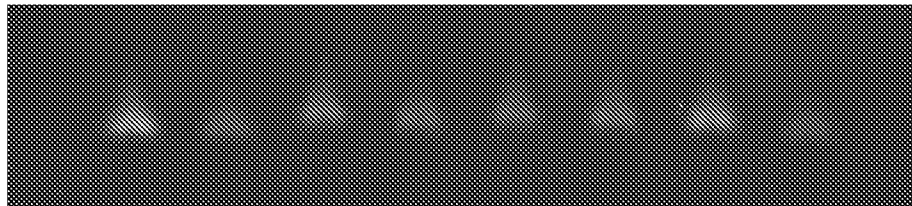
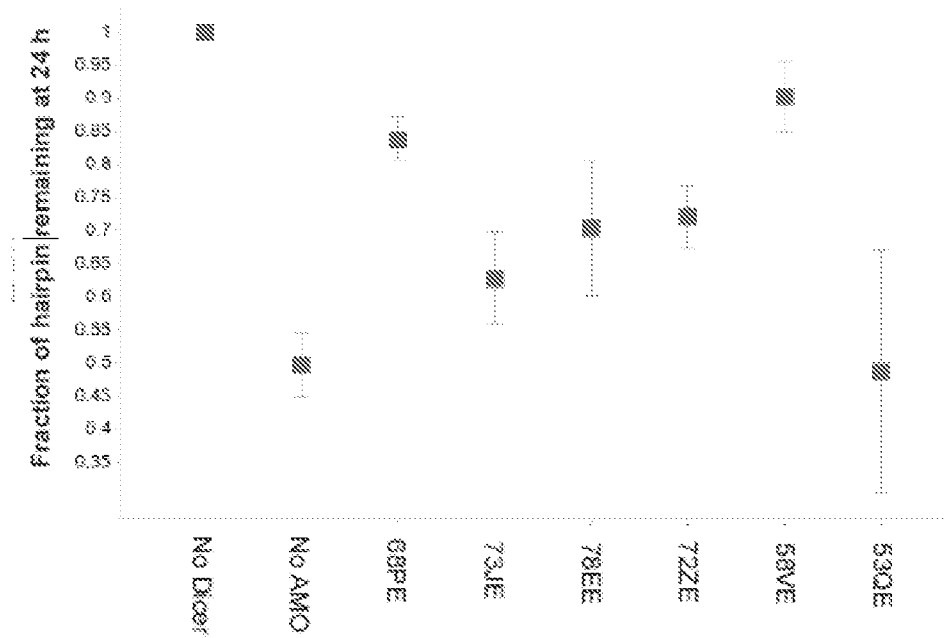


FIG. 6A

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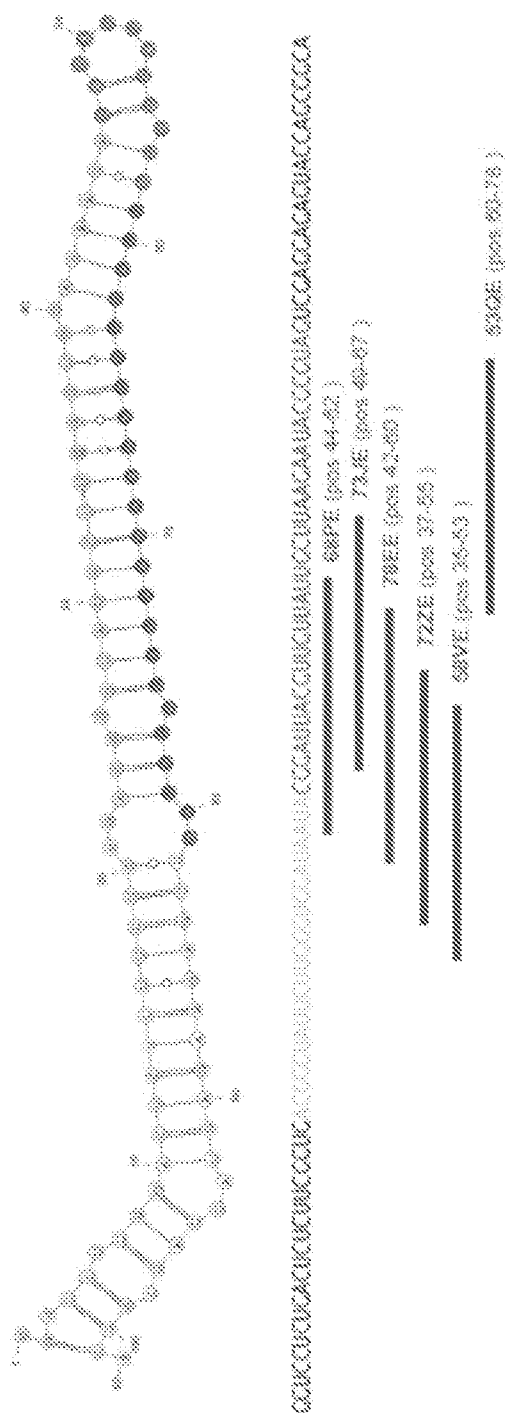


FIG. 6B-C

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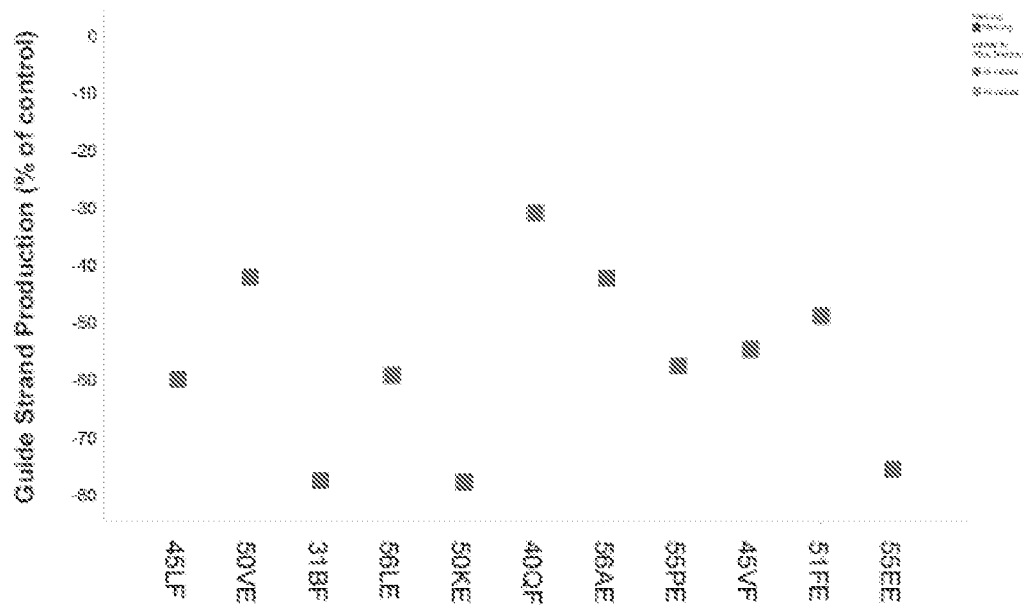


FIG. 7A

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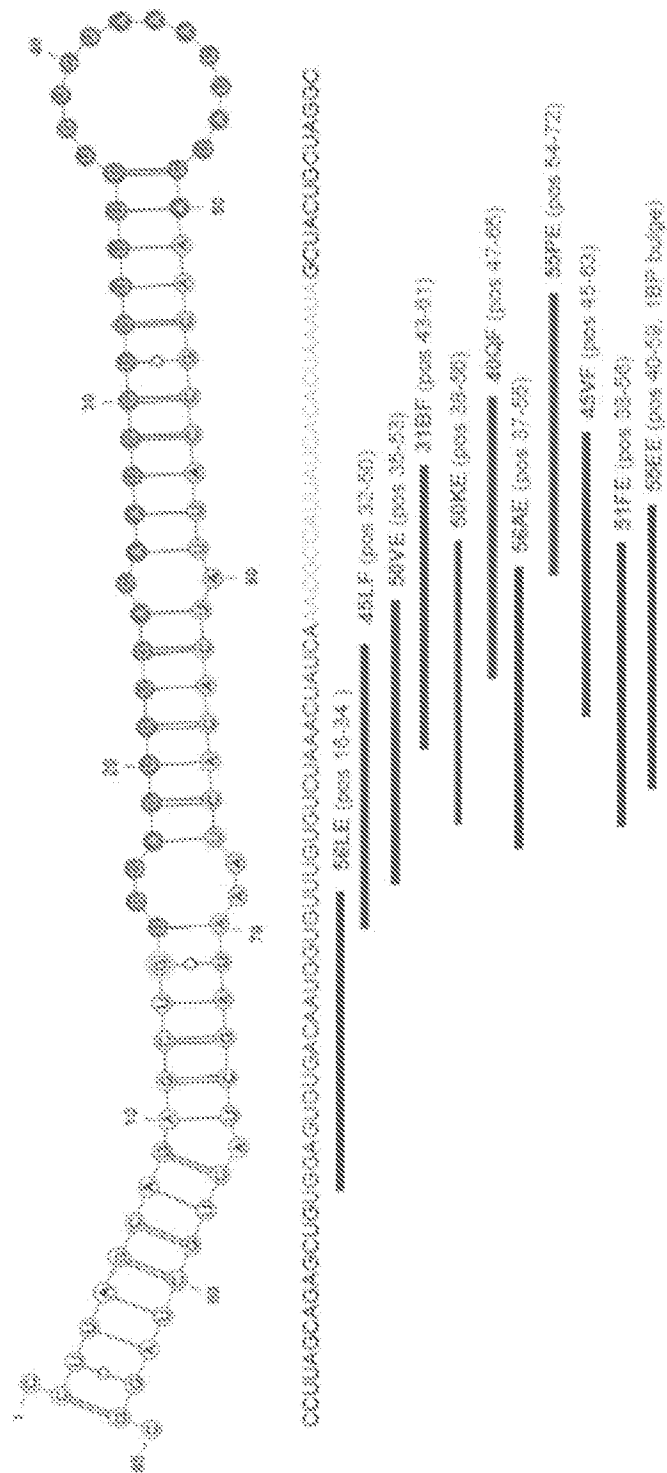


FIG. 7B-C

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2017/055499

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

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

B. FIELDS SEARCHED

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

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

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/149646 A1 (SUNNYBROOK RES INST [CA]; YANG BURTON [CA]) 8 November 2012 (2012-11-08) page 30, line 16 - line 21 page 31, line 30 - line 32; claims 1,7 page 13, line 12 - page 14, line 30 -----	1-16
X	WO 2013/125967 A1 (INST CHEMII BIOORG PAN [PL]) 29 August 2013 (2013-08-29) page 19, paragraph 1 - paragraph 2; claim 1; figures 3,4 Legend to figure 4; page 10 -----	1-16
A	WO 2012/006181 A2 (SINAI SCHOOL MEDICINE [US]; LLOVET JOSEP M [ES]; TOFFANIN SARA [US]; S) 12 January 2012 (2012-01-12) paragraph [0117] -----	1



Further documents are listed in the continuation of Box C.



See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

20 December 2017

Date of mailing of the international search report

03/01/2018

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2017/055499

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012149646 A1	08-11-2012	NONE	
WO 2013125967 A1	29-08-2013	EP 2817408 A1 WO 2013125967 A1	31-12-2014 29-08-2013
WO 2012006181 A2	12-01-2012	NONE	