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(57) Abstract: Provided herein are methods and compositions for the treatment of cardiac disease using an inhibitor of miR-21.



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TARGETING MICRORNAS FOR THE TREATMENT OF CARDIAC DISORDERS**FIELD OF INVENTION**

Provided herein are methods and compositions for the treatment of cardiac diseases.

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DESCRIPTION OF RELATED ART

MicroRNAs (miRNAs), also known as “mature miRNA” are small (approximately 18-24 nucleotides in length), non-coding RNA molecules encoded in the genomes of plants and animals. In certain instances, highly conserved, endogenously expressed miRNAs regulate the expression of genes by binding to the 3'-untranslated regions (3'-UTR) of specific mRNAs. More than 1000 different miRNAs have been identified in plants and animals. Certain mature miRNAs appear to originate from long endogenous primary miRNA transcripts (also known as pri-miRNAs, pri-mirs, pri-miRs or pri-pre-miRNAs) that are often hundreds of nucleotides in length (Lee, et al., EMBO J., 2002, 21(17), 4663-4670).

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Functional analyses of miRNAs have revealed that these small non-coding RNAs contribute to different physiological processes in animals, including developmental timing, organogenesis, differentiation, patterning, embryogenesis, growth control and programmed cell death. Examples of particular processes in which miRNAs participate include stem cell differentiation, neurogenesis, angiogenesis, hematopoiesis, and exocytosis (reviewed by Alvarez-Garcia and Miska, Development, 2005, 132, 4653-4662). MiRNAs have also been linked to heart disease.

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SUMMARY OF INVENTION

Provided herein are methods and compositions for the treatment, prevention, and or amelioration of cardiac disease.

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Provided herein are methods comprising administering to a subject having a cardiac disease a compound comprising a modified oligonucleotide consisting of 8 to 25 linked nucleosides, wherein the nucleobase sequence of the modified oligonucleotide is complementary to the nucleobase sequence of miR-21, wherein the modified oligonucleotide is not conjugated to a cholesterol moiety.

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Provided herein are methods comprising preventing or delaying the onset of a cardiac disease comprising administering to a subject having a cardiac disease a compound comprising a modified oligonucleotide consisting of 8 to 25 linked nucleosides, wherein the nucleobase sequence of the modified oligonucleotide is complementary to the nucleobase sequence of

miR-21, wherein the modified oligonucleotide is not conjugated to a cholesterol moiety, thereby preventing or delaying the onset of the cardiac disease.

Provided herein are methods for treating cardiac disease comprising administering to a subject having a cardiac disease a compound comprising a modified oligonucleotide consisting of 8 to 25 linked nucleosides, wherein the nucleobase sequence of the modified oligonucleotide is complementary to the nucleobase sequence of miR-21, wherein the modified oligonucleotide is not conjugated to a cholesterol moiety, thereby treating the cardiac disease.

In certain embodiments, the methods provided herein comprise selecting a subject having a cardiac disease. In certain embodiments, the cardiac disease is cardiac fibrosis. In certain embodiments, the cardiac disease is cardiac enlargement. In certain embodiments, the administering delays the transition from cardiac enlargement to cardiac failure. In certain embodiments, the cardiac enlargement is cardiac hypertrophy. In certain embodiments, the cardiac enlargement is cardiac dilation. In certain embodiments, the cardiac hypertrophy is hypertrophic cardiomyopathy.

In certain embodiments, the methods provided herein comprise selecting a subject having cardiac fibrosis.

In certain embodiments, the cardiac disease is heart failure, post-myocardial infarction remodeling, or myocardial infarction.

In certain embodiments, the cardiac disease is cardiomyopathy. In certain embodiments, the cardiomyopathy is hypertrophic cardiomyopathy, restrictive cardiomyopathy, dilated cardiomyopathy (DCM), idiopathic dilated cardiomyopathy, or dilated cardiomyopathy with arrhythmias.

In certain embodiments, the cardiac disease is diastolic heart failure, chronic atrial fibrillation, primary pulmonary hypertension, acute respiratory distress syndrome, brugada syndrome, progressive cardiac conduction disease, uremic pericarditis, anthracycline cardiomyopathy, arterial fibrosis, post-radiation lymphatic fibrosis, sarcoidosis, scleroderma, endocardial fibroelastosis, serotonergic excess, cardiac valvulopathy, atrial fibrosis, atrial fibrillation, mitral valvular disease, hypertension, chronic ventricular dysfunction, pressure and volume overload, or myocardial fibrosis.

In certain embodiments, the methods provided herein comprise improving one or more symptoms of cardiac disease.

In certain embodiments, the administering increases exercise tolerance. In certain embodiments, the administering increases cardiac ejection volume. In certain embodiments, the administering increases cardiac output. In certain embodiments, the administering reduces

ventricular end diastolic blood pressure. In certain embodiments, the administering increases ventricular compliance.

In certain embodiments, the administering reduces cardiac fibrosis.

5 In certain embodiments, the methods provided herein comprise selecting a subject having elevated serum troponin levels. In certain embodiments, serum troponin levels are reduced. In certain embodiments, an increase in serum troponin levels is prevented. In certain embodiments, the troponin is troponin I. In certain embodiments, the troponin is troponin T.

10 In certain embodiments, the methods provided herein comprise selecting a subject having elevated serum natriuretic peptides levels. In certain embodiments, the natriuretic peptide is brain natriuretic peptide. In certain embodiments, the natriuretic peptide is atrial natriuretic peptide. In certain embodiments, serum natriuretic peptide levels are reduced. In certain embodiments, an increase in serum natriuretic peptide levels is prevented.

15 In certain embodiments, the methods provided herein comprise administering at least one additional therapy. In certain embodiments, the at least one additional therapy comprises a therapeutic agent. In certain embodiments, the at least one additional therapeutic agent is selected from an ACE inhibitor, an aldosterone antagonist, an angiotensin II receptor blocker, a calcium channel blocker, a sodium channel blocker, an antiarrhythmic agent, an antihypertensive agent, an alpha blocker, a beta blocker, and a diuretic. In certain embodiments, the at least one additional therapy comprises surgery.

20 In certain embodiments, provided herein are methods for contacting a cardiac cell with a compound comprising a modified oligonucleotide consisting of 8 to 25 linked nucleosides, wherein the nucleobase sequence of the modified oligonucleotide is complementary to the nucleobase sequence of miR-21, provided that the modified oligonucleotide is not conjugated to a cholesterol moiety. In certain embodiments, the cardiac cell is a cardiac fibroblast. In certain
25 embodiments, the cardiac cell is a cardiac myocyte. In certain embodiments, the cardiac cell is *in vivo*.

In certain embodiments, the modified oligonucleotide is not an antagomir.

In certain embodiments, the compound consists of the modified oligonucleotide.

30 In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 85% complementary to a nucleobase sequence selected from SEQ ID NO: 1 and 2. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to a nucleobase sequence selected from SEQ ID NO: 1 and 2. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 95% complementary to a nucleobase sequence selected from SEQ ID NO: 1 and 2. In certain

embodiments, the nucleobase sequence of the modified oligonucleotide is fully complementary to a nucleobase sequence selected from SEQ ID NO: 1 and 2.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide has no more than two mismatches to a nucleobase sequence selected from SEQ ID NO: 1 and 2. In certain embodiments, the nucleobase sequence of the modified oligonucleotide has no more than one mismatch to a nucleobase sequence selected from SEQ ID NO: 1 and 2. In certain embodiments, the nucleobase sequence of the modified oligonucleotide has one mismatch to a nucleobase sequence selected from SEQ ID NO: 1 and 2. In certain embodiments, the nucleobase sequence of the modified oligonucleotide has no mismatches to a nucleobase sequence selected from SEQ ID NO: 1 and 2.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide comprises a nucleobase sequence selected from SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, GAUAAGCUA, AUAAGCUA, and UAAGCUA. In certain embodiments, the nucleobase sequence of the modified oligonucleotide consists of a nucleobase sequence selected from SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, GAUAAGCUA, AUAAGCUA, and UAAGCUA.

In certain embodiments, the modified oligonucleotide consists of 8 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 9 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 10 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 11 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 12 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 13 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 14 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 15 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 17 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 18 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 19 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 21 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 22 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 23 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 24 linked nucleosides.

In certain embodiments, the modified oligonucleotide comprises at least one modified internucleoside linkage. In certain embodiments, the modified oligonucleotide comprises at least two modified internucleoside linkages. In certain embodiments, the modified oligonucleotide comprises at least three modified internucleoside linkages. In certain embodiments, the first and last internucleoside linkages of the modified oligonucleotide are modified internucleoside linkages. In certain embodiments, each internucleoside linkage of the modified oligonucleotide is a modified internucleoside linkage. In certain embodiments, at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage.

In certain embodiments, the modified oligonucleotide comprises at least one nucleoside comprising a modified sugar. In certain embodiments, the modified oligonucleotide comprises at least two nucleosides comprising a modified sugar. In certain embodiments, the modified oligonucleotide comprises at least three nucleosides comprising a modified sugar. In certain embodiments, each nucleoside of the modified oligonucleotide comprises a modified sugar. In certain embodiments, each nucleoside of the modified oligonucleotide comprises a 2'-O-methoxyethyl sugar. In certain embodiments, a plurality of nucleosides comprising a 2'-O-methoxyethyl sugar and a plurality of nucleosides comprising a 2'-fluoro sugar modification. In certain embodiments, each modified sugar is independently selected from a 2'-O-methoxyethyl sugar, a 2'-fluoro sugar, 2'-O-methyl sugar, and a bicyclic sugar moiety. In certain embodiments, the bicyclic sugar moiety is bridged between the 2' and 4' carbon atoms with a -O-CH₂- biradical group.

In certain embodiments, the compound consists of the modified oligonucleotide; the modified oligonucleotide has the entire nucleobase sequence of SEQ ID NO: 3; each internucleoside linkage of the modified oligonucleotide is a phosphorothioate internucleoside linkage; nucleosides 1, 2, 12, 13, 14, 21, and 22 are 2'-O-methoxyethyl nucleosides; nucleosides 3, 4, 5, 6, 7, 8, 9, 10, 11, 15, 16, 17, 18, 19, and 20 are 2'-fluoro nucleosides; and nucleosides 13 and 21 comprise 5-methylcytosines.

DETAILED DESCRIPTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the arts to which the invention belongs. Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. In the event that there is a plurality of definitions for terms herein, those in this section

prevail. Standard techniques may be used for chemical synthesis, chemical analysis, pharmaceutical preparation, formulation and delivery, and treatment of subjects. Certain such techniques and procedures may be found for example in "Carbohydrate Modifications in Antisense Research" Edited by Sangvi and Cook, American Chemical Society, Washington D.C., 1994; and "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., 18th edition, 1990; and which is hereby incorporated by reference for any purpose. Where permitted, all patents, patent applications, published applications and publications, GENBANK sequences, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can command go, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

Before the present compositions and methods are disclosed and described, it is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

"Cardiac disease" means a disease affecting the heart.

"Cardiac enlargement" means abnormal increase in the size or mass of the heart. In certain embodiments, cardiac enlargement is cardiac hypertrophy. In certain embodiments, cardiac enlargement is cardiac dilation.

"Cardiac hypertrophy" means an abnormal thickening of heart muscle.

"Cardiac dilation" means abnormal increase in the interior volume of one or more chambers of the heart.

"Myocardial infarction" means the interruption of the supply of blood to part of the heart, which results in the death of heart cells. Myocardial infarction is also known as a heart attack.

"Ischemia" means restriction of blood supply in cells or tissues.

"Myocardium" means heart muscle tissue.

"Fibrosis" means the formation or development of excess fibrous connective tissue in an organ or tissue. In certain embodiments, fibrosis occurs as a reparative or reactive process. In certain embodiments, fibrosis occurs in response to damage or injury. The term "fibrosis" is to be understood as the formation or development of excess fibrous connective tissue in an organ or

tissue as a reparative or reactive process, as opposed to a formation of fibrous tissue as a normal constituent of an organ or tissue.

“Subject suspected of having” means a subject exhibiting one or more clinical indicators of a disease.

5 “Compound comprising an oligonucleotide consisting of” a number of linked nucleosides means a compound that includes an oligonucleotide having the specified number of linked nucleosides. Thus, the compound may include additional substituents or conjugates. Unless otherwise indicated, the compound does not include any additional nucleosides beyond those of the oligonucleotide.

10 “Anti-miR” means an oligonucleotide having a nucleobase sequence complementary to a microRNA. In certain embodiments, an anti-miR is a modified oligonucleotide.

“Anti-miR-21” means an oligonucleotide having a nucleobase sequence complementary to miR-21. In certain embodiments, an anti-miR-21 is a modified oligonucleotide.

“Subject” means a human or non-human animal selected for treatment or therapy.

15 “Subject in need thereof” means the state in which a subject is identified as in need of a therapy or treatment.

“Administering” means providing a pharmaceutical agent or composition to a subject, and includes, but is not limited to, administering by a medical professional and self-administering.

20 “Parenteral administration” means administration through injection or infusion. Parenteral administration includes, but is not limited to, subcutaneous administration, intravenous administration, or intramuscular administration.

“Subcutaneous administration” means administration just below the skin.

“Intravenous administration” means administration into a vein.

25 “Peripheral intravenous administration” means administration into a peripheral vein of the body.

“Peripheral vein” means a vein that is not in the chest or abdomen of the body.

30 “Intracardial administration” means administration into the heart. In certain embodiments, intracardial administration occurs by way of a catheter. In certain embodiments, intracardial administration occurs by way of open heart surgery.

“Pulmonary administration” means administration to the lungs.

“Administered concomitantly” refers to the administration of at least two agents in any manner in which the pharmacological effects of both are manifest in the patient at the same time. Concomitant administration does not require that both agents be administered in a single

pharmaceutical composition, in the same dosage form, or by the same route of administration. The time periods during which the effects of each agent occur need not be identical. The effects need only be overlapping for a period of time and need not be coextensive.

“Duration” means the period of time during which an activity or event continues. In certain embodiments, the duration of treatment is the period of time during which doses of a pharmaceutical agent or pharmaceutical composition are administered.

“Therapy” means a disease treatment method. In certain embodiments, therapy includes, but is not limited to, chemotherapy, radiation therapy, or administration of a pharmaceutical agent.

“Treatment” means the application of one or more specific procedures used for the cure or amelioration of a disease. In certain embodiments, the specific procedure is the administration of one or more pharmaceutical agents.

“Amelioration” means a lessening of severity of at least one indicator of a condition or disease. In certain embodiments, amelioration includes a delay or slowing in the progression of one or more indicators of a condition or disease. The severity of indicators may be determined by subjective or objective measures which are known to those skilled in the art.

“At risk for developing” means the state in which a subject is predisposed to developing a condition or disease. In certain embodiments, a subject at risk for developing a condition or disease exhibits one or more symptoms of the condition or disease, but does not exhibit a sufficient number of symptoms to be diagnosed with the condition or disease. In certain embodiments, a subject at risk for developing a condition or disease exhibits one or more symptoms of the condition or disease, but to a lesser extent required to be diagnosed with the condition or disease.

“Prevent the onset of” means to prevent the development a condition or disease in a subject who is at risk for developing the disease or condition. In certain embodiments, a subject at risk for developing the disease or condition receives treatment similar to the treatment received by a subject who already has the disease or condition.

“Delay the onset of” means to delay the development of a condition or disease in a subject who is at risk for developing the disease or condition. In certain embodiments, a subject at risk for developing the disease or condition receives treatment similar to the treatment received by a subject who already has the disease or condition.

“Therapeutic agent” means a pharmaceutical agent used for the cure, amelioration or prevention of a disease.

“Dose” means a specified quantity of a pharmaceutical agent provided in a single administration. In certain embodiments, a dose may be administered in two or more boluses, tablets, or injections. For example, in certain embodiments, where subcutaneous administration is desired, the desired dose requires a volume not easily accommodated by a single injection. In
5 such embodiments, two or more injections may be used to achieve the desired dose. In certain embodiments, a dose may be administered in two or more injections to minimize injection site reaction in an individual.

“Dosage unit” means a form in which a pharmaceutical agent is provided. In certain embodiments, a dosage unit is a vial containing lyophilized oligonucleotide. In certain
10 embodiments, a dosage unit is a vial containing reconstituted oligonucleotide.

“Therapeutically effective amount” refers to an amount of a pharmaceutical agent that provides a therapeutic benefit to an animal.

“Pharmaceutical composition” means a mixture of substances suitable for administering to an individual that includes a pharmaceutical agent. For example, a pharmaceutical
15 composition may comprise a sterile aqueous solution.

“Pharmaceutical agent” means a substance that provides a therapeutic effect when administered to a subject.

“Active pharmaceutical ingredient” means the substance in a pharmaceutical composition that provides a desired effect.

20 “Improved liver function” means the change in liver function toward normal limits and/or function. In certain embodiments, liver function is assessed by measuring molecules found in a subject’s blood. For example, in certain embodiments, improved liver function is measured by a reduction in blood liver transaminase levels.

25 “Acceptable safety profile” means a pattern of side effects that is within clinically acceptable limits.

“Side effect” means a physiological response attributable to a treatment other than desired effects. In certain embodiments, side effects include, without limitation, injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, and myopathies. Such side effects may be
30 detected directly or indirectly. For example, increased aminotransferase levels in serum may indicate liver toxicity or liver function abnormality. In addition, increased bilirubin may indicate liver toxicity or liver function abnormality.

“Injection site reaction” means inflammation or abnormal redness of skin at a site of injection in an individual.

“Subject compliance” means adherence to a recommended or prescribed therapy by a subject.

“Comply” means the adherence with a recommended therapy by a subject.

“Recommended therapy” means a treatment recommended by a medical professional
5 for the treatment, amelioration, or prevention of a disease.

“Target nucleic acid” means a nucleic acid to which an oligomeric compound is designed to hybridize.

“Targeting” means the process of design and selection of nucleobase sequence that will hybridize to a target nucleic acid.

10 “Targeted to” means having a nucleobase sequence that will allow hybridization to a target nucleic acid.

“Target engagement” means the interaction of a drug with its target molecule in a manner that changes the activity, expression or level of the target. In certain embodiments, target engagement means an anti-miR interacting with its microRNA target, such that the activity of the
15 microRNA is inhibited.

“Modulation” means to a perturbation of function or activity. In certain embodiments, modulation means an increase in activity. In certain embodiments, modulation means a decrease in activity.

20 “Expression” means any functions and steps by which a gene’s coded information is converted into structures present and operating in a cell.

“5’ target site” refers to the nucleobase of a target nucleic acid which is complementary to the 5’-most nucleobase of a particular oligonucleotide.

“3’ target site” means the nucleobase of a target nucleic acid which is complementary to the 3’-most nucleobase of a particular oligonucleotide.

25 “Region” means a portion of linked nucleosides within a nucleic acid. In certain embodiments, an oligonucleotide has a nucleobase sequence that is complementary to a region of a target nucleic acid. For example, in certain such embodiments an oligonucleotide is complementary to a region of a miRNA stem-loop sequence. In certain such embodiments, an oligonucleotide is fully complementary to a region of a miRNA stem-loop sequence.

30 “Segment” means a smaller or sub-portion of a region.

“Nucleobase sequence” means the order of contiguous nucleobases, in a 5’ to 3’ orientation, independent of any sugar, linkage, and/or nucleobase modification.

“Contiguous nucleobases” means nucleobases immediately adjacent to each other in a nucleic acid.

“Nucleobase complementarity” means the ability of two nucleobases to pair non-covalently via hydrogen bonding.

“Complementary” means that an oligomeric compound is capable of hybridizing to a target nucleic acid under stringent hybridization conditions.

5 “Fully complementary” means each nucleobase of an oligomeric compound is capable of pairing with a nucleobase at each corresponding position in a target nucleic acid. For example, in certain embodiments, an oligomeric compound wherein each nucleobase has complementarity to a nucleobase within a region of a miRNA stem-loop sequence is fully complementary to the miRNA stem-loop sequence.

10 “Percent complementarity” means the percentage of nucleobases of an oligomeric compound that are complementary to an equal-length portion of a target nucleic acid. Percent complementarity is calculated by dividing the number of nucleobases of the oligomeric compound that are complementary to nucleobases at corresponding positions in the target nucleic acid by the total length of the oligomeric compound. In certain embodiments, percent
15 complementarity of an means the number of nucleobases that are complementary to the target nucleic acid, divided by the length of the modified oligonucleotide.

“Percent identity” means the number of nucleobases in first nucleic acid that are identical to nucleobases at corresponding positions in a second nucleic acid, divided by the total number of nucleobases in the first nucleic acid.

20 “Hybridize” means the annealing of complementary nucleic acids that occurs through nucleobase complementarity.

“Mismatch” means a nucleobase of a first nucleic acid that is not capable of pairing with a nucleobase at a corresponding position of a second nucleic acid.

“Identical” means having the same nucleobase sequence.

25 “mir-21” means the mature miRNA having the nucleobase sequence set forth in SEQ ID NO: 1 (UAGCUUAUCAGACUGAUGUUGA).

“mir-21 stem-loop sequence” means the mir-21 precursor having the nucleobase sequence set forth in SEQ ID NO: 2 (UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACCAGUCGAUGGGCUGUCUGACA).

30 “MicroRNA” means an endogenous non-coding RNA between 18 and 25 nucleobases in length, which is the product of cleavage of a pre-miRNA by the enzyme Dicer. Examples of mature miRNAs are found in the miRNA database known as miRBase (available via the internet at the http address of “microrna.sanger.ac.uk/”). In certain embodiments, microRNA is abbreviated as “miRNA” or “miR.”

“Pre-miRNA” or “pre-miR” means a non-coding RNA having a hairpin structure, which is the product of cleavage of a pri-miR by the double-stranded RNA-specific ribonuclease known as Drosha.

5 “Stem-loop sequence” means an RNA having a hairpin structure and containing a mature miRNA sequence. Pre-miRNA sequences and stem-loop sequences may overlap. Examples of stem-loop sequences are found in the miRNA database known as miRBase.

“Pri-miRNA” or “pri-miR” means a non-coding RNA having a hairpin structure that is a substrate for the double-stranded RNA-specific ribonuclease Drosha.

10 “miRNA precursor” means a transcript that originates from a genomic DNA and that comprises a non-coding, structured RNA comprising one or more miRNA sequences. For example, in certain embodiments a miRNA precursor is a pre-miRNA. In certain embodiments, a miRNA precursor is a pri-miRNA.

“Monocistronic transcript” means a miRNA precursor containing a single miRNA sequence.

15 “Polycistronic transcript” means a miRNA precursor containing two or more miRNA sequences.

“Seed sequence” means a nucleobase sequence comprising from 6 to 8 contiguous nucleobases of nucleobases 1 to 8 of the 5'-end of a mature microRNA sequence.

20 “Seed match sequence” means a nucleobase sequence that is complementary to a seed sequence, and is the same length as the seed sequence.

“Oligomeric compound” means a compound comprising a polymer of linked monomeric subunits.

“Oligonucleotide” means a polymer of linked nucleosides, each of which can be modified or unmodified, independent from one another.

25 “Naturally occurring internucleoside linkage” means a 3' to 5' phosphodiester linkage between nucleosides.

“Natural sugar” means a sugar found in DNA (2'-H) or RNA (2'-OH).

“Natural nucleobase” means a nucleobase that is unmodified relative to its naturally occurring form.

30 “Internucleoside linkage” means a covalent linkage between adjacent nucleosides.

“Linked nucleosides” means nucleosides joined by a covalent linkage.

“Nucleobase” means a heterocyclic moiety capable of non-covalently pairing with another nucleobase.

“Nucleoside” means a nucleobase linked to a sugar.

“Nucleotide” means a nucleoside having a phosphate group covalently linked to the sugar portion of a nucleoside.

“Modified oligonucleotide” means an oligonucleotide having one or more modifications relative to a naturally occurring terminus, sugar, nucleobase, and/or internucleoside linkage.

5 “Single-stranded modified oligonucleotide” means an which is not hybridized to a complementary strand.

“Modified internucleoside linkage” means any change from a naturally occurring internucleoside linkage.

10 “Phosphorothioate internucleoside linkage” means a linkage between nucleosides where one of the non-bridging atoms is a sulfur atom.

“Modified sugar” means substitution and/or any change from a natural sugar.

“Modified nucleobase” means any substitution and/or change from a natural nucleobase.

15 “5-methylcytosine” means a cytosine modified with a methyl group attached to the 5’ position.

“2’-O-methyl sugar” or “2’-OMe sugar” means a sugar having a O-methyl modification at the 2’ position.

“2’-O-methoxyethyl sugar” or “2’-MOE sugar” means a sugar having a O-methoxyethyl modification at the 2’ position.

20 “2’-O-fluoro” or “2’-F” means a sugar having a fluoro modification of the 2’ position.

“Bicyclic sugar moiety” means a sugar modified by the bridging of two non-geminal ring atoms.

“2’-O-methoxyethyl nucleoside” means a 2’-modified nucleoside having a 2’-O-methoxyethyl sugar modification.

25 “2’-fluoro nucleoside” means a 2’-modified nucleoside having a 2’-fluoro sugar modification.

“2’-O-methyl” nucleoside means a 2’-modified nucleoside having a 2’-O-methyl sugar modification.

“Bicyclic nucleoside” means a 2’-modified nucleoside having a bicyclic sugar moiety.

30 “Motif” means a pattern of modified and/or unmodified nucleobases, sugars, and/or internucleoside linkages in an oligonucleotide.

A “fully modified oligonucleotide” means each nucleobase, each sugar, and/or each internucleoside linkage is modified.

A “uniformly modified oligonucleotide” means each nucleobase, each sugar, and/or each internucleoside linkage has the same modification throughout the modified oligonucleotide.

A “gapmer” means a modified oligonucleotide having an internal region of linked nucleosides positioned between two external regions of linked nucleosides, where the
5 nucleosides of the internal region comprise a sugar moiety different than that of the nucleosides of each external region.

A “gap segment” is an internal region of a gapmer that is positioned between the external regions.

A “wing segment” is an external region of a gapmer that is located immediately
10 adjacent to the 5’ or 3’ end of the internal region.

A “symmetric gapmer” means each nucleoside of each external region comprises the same sugar modification.

An “asymmetric gapmer” means each nucleoside of one external region comprises a first sugar modification, and each nucleoside of the other external region comprises a second
15 sugar modification.

A “stabilizing modification” means a modification to a nucleoside that provides enhanced stability to a modified oligonucleotide, in the presence of nucleases, relative to that provided by 2’-deoxynucleosides linked by phosphodiester internucleoside linkages. For example, in certain embodiments, a stabilizing modification is a stabilizing nucleoside
20 modification. In certain embodiments, a stabilizing modification is an internucleoside linkage modification.

A “stabilizing nucleoside” means a nucleoside modified to provide enhanced nuclease stability to an oligonucleotide, relative to that provided by a 2’-deoxynucleoside. In one embodiment, a stabilizing nucleoside is a 2’-modified nucleoside.

A “stabilizing internucleoside linkage” means an internucleoside linkage that provides improved nuclease stability to an oligonucleotide relative to that provided by a phosphodiester internucleoside linkage. In one embodiment, a stabilizing internucleoside linkage is a phosphorothioate internucleoside linkage.

30 Cardiac disease and its manifestations are life-threatening conditions that present a major health risk throughout the world. As such, there is a medical need for methods and compositions useful for the prevention and/or treatment of cardiac diseases.

One form of cardiac disease is cardiac enlargement, which refers to an increase in the size of the heart. Regular aerobic exercise may lead to cardiac enlargement, which is generally

not a health concern. However, most forms of cardiac enlargement involve an abnormal increase in the size of the heart, which leads to additional cardiovascular problems. The types of cardiac enlargement includes cardiac hypertrophy and cardiac dilation.

Cardiac hypertrophy is an abnormal thickening of the heart muscle that occurs in response to increased stress on the heart. Such stress can be caused by hypertension, heart valve stenosis, mechanical load, myocardial infarction, cardiac arrhythmias, endocrine disorders, and genetic mutations in cardiac contractile genes. The thickened heart muscle cannot function effectively in the pumping of blood, thus cardiac hypertrophy can lead to a cardiac arrhythmia (abnormal heart beat) or cardiac failure (impaired pumping action of the heart), in addition to an increased risk for angina (chest pain due to ischemia of the heart muscle) and myocardial infarction (heart attack). Cardiac hypertrophy may also lead to cardiac arrest and sudden cardiac death. Symptoms of cardiac hypertrophy include shortness of breath, chest pressure or pain, heart palpitations, swelling of the feet, ankles or legs, dizziness or lightheadedness, and loss of consciousness. Subjects having mild forms of cardiac hypertrophy may only experience these symptoms as a result of physical exertion. In more severe forms of the disease, the symptoms may manifest at any time, including while the subject is at rest.

Cardiac dilation is characterized by an increase in the inside cavity of a chamber of the heart. Dilation may occur in one or all of the chambers of the heart. Cardiac dilation is most commonly caused by direct damage to the heart muscle. Such types of damage include infection, heart attack, myocarditis, chemotherapy, long term alcohol abuse, excess iron in the body, drug abuse, anti-viral medications, and excessive exposure to metals such as cobalt, lead, and mercury.

Cardiac fibrosis may develop as the body is responding to injury or damage in the heart, and as such is associated with many types of cardiac disease, including cardiac hypertrophy, cardiac dilation, post-myocardial infarction remodeling, and cardiac infection. Cardiac fibrosis contributes to abnormal heart function.

Myocardial infarction, or heart attack, is a condition caused by the interruption of the blood supply to part of the heart. If the blood supply is not restored quickly, myocardial infarction can lead to the death of heart muscle tissue. Classical symptoms of acute myocardial infarction include sudden chest pain (typically radiating to the left arm or left side of the neck), shortness of breath, nausea, vomiting, palpitations, sweating, and anxiety. Early treatment is essential to minimize damage to the heart, however, if left untreated or if very extreme, myocardial infarction can lead to sudden death. Myocardial infarction may be followed by left ventricular (LV) remodeling, during which is a pathological changes occur in the heart, such as

change in size, shape and volumes, wall thickness and function of the heart. Post-myocardial infarction remodeling can lead to cardiac failure.

Several mouse models are used to model human cardiac disease, and are useful for evaluating potential treatments for human cardiac disease. Such models include isoproterenol-induced cardiac hypertrophy, transverse aortic banding, hormone treatment, and chronic exercise training. Both the isoproterenol and transverse aortic banding models are characterized by abnormalities including an enlarged heart, fibrosis, decreased fractional shortening, and increase left ventricular dilatation.

Elevations in miR-21 have been observed in mouse models of human cardiac disease, as well as in disease human heart tissue, thus miR-21 is potentially involved in cardiac disease. A cholesterol-conjugated, 2'-O-methyl modified anti-miR-21 (antagomiR-21), when administered intra-jugularly to mice subjected to transverse aortic banding or isoproterenol treatment, results in reduced heart weight, reduced interstitial fibrosis, and improvements in cardiac function. While these results with a cholesterol-conjugated anti-miR-21 are encouraging, there is a need for additional anti-miR compounds that can be delivered systemically and that do not comprise a cholesterol conjugate.

It is demonstrated herein that a modified oligonucleotide without a cholesterol conjugate can be delivered to the heart via peripheral intravenous administration. Further, similar efficiency of delivery is achieved with both intra-jugular and peripheral intravenous administration, thus the modified oligonucleotide described herein may be delivered to subjects through multiple routes of administration. It is further demonstrated herein that inhibition of miR-21 in a mouse model of human cardiac disease, via intravenous delivery of a modified oligonucleotide without a cholesterol conjugate, decreases both heart size and cardiac fibrosis. Accordingly, provided herein are methods and compositions for the prevention and/or treatment of cardiac diseases, such as cardiac enlargement, including cardiac hypertrophy and cardiac dilation, cardiac failure, and myocardial infarction.

Provided herein are compositions and methods for treating, preventing, or delaying the onset of a cardiac disease, comprising administering a modified oligonucleotide complementary to miR-21 to a subject. The subject may have received a diagnosis of cardiac disease, may be at risk for a cardiac disease, or may be suspected of having a cardiac disease.

In certain embodiments, a cardiac disease is cardiac enlargement. In certain embodiments, cardiac enlargement is cardiac hypertrophy. In certain embodiments, cardiac enlargement is cardiac dilation.

In certain embodiments, a cardiac disease or condition is cardiac hypertrophy. The cardiac hypertrophy may be caused by one or more factors, including but not limited to hypertension, heart valve stenosis, mechanical load, myocardial infarction, cardiac arrhythmias, endocrine disorders, and genetic mutations in cardiac contractile genes. A subject having cardiac hypertrophy may be diagnosed by the presence of one or more symptoms of cardiac hypertrophy, including but not limited to shortness of breath, chest pressure or pain, heart palpitations, swelling of the feet, ankles or legs, dizziness or lightheadedness, and loss of consciousness. Additional diagnostic criteria include an enlarged heart size as visualized by chest x-ray, increased wave forms as measured by an echocardiogram. An echocardiogram can visualize the size and thickness of heart muscle in each chamber of the heart.

In certain embodiments, cardiac enlargement is cardiac dilation. The cardiac dilation may be caused by one or more factors, including but not limited to infection, heart attack, myocarditis, chemotherapy, long term alcohol abuse, excess iron in the body, drug abuse, anti-viral medications, thyroid disease, heart valve defects, and excessive exposure to metals such as cobalt, lead, and mercury. A subject having cardiac hypertrophy may be diagnosed by the presence of one or more symptoms of cardiac hypertrophy, including but not limited to shortness of breath, chest pressure or pain, heart palpitations, swelling of the feet, ankles or legs, dizziness or lightheadedness, and loss of consciousness. Additional diagnostic criteria include an enlarged heart size as visualized by chest x-ray, increased wave forms as measured by an echocardiogram. An echocardiogram can visualize the size and thickness of heart muscle in each chamber of the heart.

In certain embodiments, a cardiac disease is myocardial infarction. A subject experiencing myocardial infarction may be diagnosed by the presence of one or more symptoms of myocardial infarction, such as sudden chest pain (typically radiating to the left arm or left side of the neck), shortness of breath, nausea, vomiting, palpitations, sweating, and anxiety. Subjects may also be diagnosed using tests to detect heart damage, such as electrocardiogram, echocardiogram, chest x-ray and blood tests. Frequently used blood markers of myocardial infarction include the MB subtype of creatine kinase (CK-MB) and troponin levels. The cardiac troponins T and I are released within 4 to 6 hours of myocardial infarction onset, and may remain elevated for up to two weeks.

In certain embodiments, a cardiac disease is cardiac fibrosis. Cardiac fibrosis may be found in connection with a variety of cardiac diseases, such as, for example, cardiac hypertrophy and myocardial infarction. Cardiac fibrosis may be diagnosed by cardiac biopsy, or by measuring blood markers of cardiac fibrosis.

In certain embodiments, a cardiac disease or condition is cardiomyopathy. In certain embodiments, cardiomyopathy is dilatative cardiomyopathy, hypertrophic cardiomyopathy with obstruction, hypertrophic cardiomyopathy without obstruction, restrictive cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, or diabetic cardiomyopathy.

5 In certain embodiments, a cardiac disease or condition is cardiac failure. In certain embodiments, cardiac failure is hypertensive heart failure, diastolic heart failure, systolic heart failure, or right heart failure.

10 In certain embodiments, a cardiac disease is heart valve disease. In certain embodiments, heart valve disease is mitral valve stenosis, aortic valve stenosis, tricuspidal valve stenosis, or pulmonary valve stenosis. In certain embodiments, the heart valve disease is mitral valve insufficiency, aortic valve insufficiency, tricuspidal valve insufficiency, or pulmonary valve insufficiency.

15 In certain embodiments, a cardiac disease is coronary artery disease. In certain embodiments, a cardiac disease or condition is heart-related storage disease, constrictive pericarditis, acute myocardial infarction, chronic myocardial infarction, cardiac arrhythmia, or myocarditis-related fibrosis.

In certain embodiments, administration comprises peripheral intravenous administration. In certain embodiments, administration comprises administration through a central venous catheter.

20 In certain embodiments, administration of the compounds or methods provided herein result in one or more clinically desirable outcomes in a subject having or suspected of having cardiac disease. Such improvements may be used to determine the extent to which a subject is responding to treatment for cardiac disease.

25 In certain embodiments, a clinically desirable outcome is the stopping or slowing of heart size increase. In certain embodiments, heart size increase is prevented. In certain embodiments, a clinically desirable outcome is the amelioration of left ventricular dilation. In certain embodiments, a clinically desirable outcome is the amelioration of impaired fractional shortening. In certain embodiments, a clinically desirable outcome is the prevention of heart weight increase. In certain such embodiments, a clinically desirable outcome is the prevention of left ventricular dilation. In certain embodiments, a clinically desirable outcome is the prevention of impaired fractional shortening.

30 In certain embodiments, a clinically desirable outcome is the reduction of serum cardiac troponin levels. In certain embodiments, a clinically desirable outcome is the prevention of an increase in serum cardiac troponin levels. The cardiac troponin may be troponin I or troponin T.

In certain embodiments, a clinically desirable outcome is the shortening of time during which serum cardiac troponin levels are elevated following injury to the heart.

In certain embodiments, a clinically desirable outcome is reduction of serum elevated serum natriuretic peptides levels. In certain embodiments, a clinically desirable outcome is a prevention of elevation of serum natriuretic peptide levels. In certain such embodiments, the serum natriuretic peptide is atrial natriuretic peptide. In certain such embodiments, the serum natriuretic peptide is brain natriuretic peptide.

In certain embodiments a clinically desirable outcome is improved cardiac function. In certain embodiments a clinically desirable outcome is the amelioration of cardiac fibrosis. In certain embodiments a clinically desirable outcome is the slowing of further progression of fibrosis. In certain embodiments a clinically desirable outcome is the halting of further progression of fibrosis. In certain embodiments a clinically desirable outcome is a reduction in fibrosis. In certain embodiments a clinically desirable outcome is a reduction in collagen content.

Treatments for cardiac diseases may comprise more than one therapy. As such, in certain embodiments provided herein are methods for treating a subject having or suspected of having a cardiac disease comprising administering at least one therapy in addition to administering a modified oligonucleotide having a nucleobase sequence complementary to a miR-21.

In certain embodiments, the methods provided herein comprise administering one or more additional pharmaceutical agents. In certain embodiments, additional pharmaceutical agents include, but are not limited to, diuretics (e.g. spironolactone, eplerenone, furosemide), inotropes (e.g. dobutamine, milrinone), digoxin, vasodilators, angiotensin II converting enzyme (ACE) inhibitors (e.g. are captopril, enalapril, lisinopril, benazepril, quinapril, fosinopril, and ramipril), angiotensin II receptor blockers (ARB) (e.g. candesartan, irbesartan, olmesartan, losartan, valsartan, telmisartan, eprosartan), calcium channel blockers, isosorbide dinitrate, hydralazine, nitrates (e.g. isosorbide mononitrate, isosorbide dinitrate), hydralazine, beta-blockers (e.g. carvedilol, metoprolol), and natriuretic peptides (e.g. nesiritide).

In certain embodiments, an additional therapy may be a pharmaceutical agent that enhances the body's immune system, including low-dose cyclophosphamide, thymostimulin, vitamins and nutritional supplements (e.g., antioxidants, including vitamins A, C, E, beta-carotene, zinc, selenium, glutathione, coenzyme Q-10 and echinacea), and vaccines, e.g., the immunostimulating complex (ISCOM), which comprises a vaccine formulation that combines a multimeric presentation of antigen and an adjuvant.

In certain embodiments, the additional therapy is selected to treat or ameliorate a side effect of one or more pharmaceutical compositions of the present invention. Such side effects include, without limitation, injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, and myopathies. For example, increased aminotransferase levels in serum may indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

Further examples of additional pharmaceutical agents include, but are not limited to, corticosteroids, including but not limited to prednisone; immunoglobulins, including, but not limited to intravenous immunoglobulin (IVIg); analgesics (e.g., acetaminophen); anti-inflammatory agents, including, but not limited to non-steroidal anti-inflammatory drugs (e.g., ibuprofen, COX-I inhibitors, and COX-2, inhibitors); salicylates; antibiotics; antivirals; antifungal agents; antidiabetic agents (e.g., biguanides, glucosidase inhibitors, insulins, sulfonylureas, and thiazolidenediones); adrenergic modifiers; diuretics; hormones (e.g., anabolic steroids, androgen, estrogen, calcitonin, progestin, somatostan, and thyroid hormones); immunomodulators; muscle relaxants; antihistamines; osteoporosis agents (e.g., biphosphonates, calcitonin, and estrogens); prostaglandins, antineoplastic agents; psychotherapeutic agents; sedatives; poison oak or poison sumac products; antibodies; and vaccines.

In certain embodiments, administering to a subject comprises parenteral administration. In certain embodiments, administering to a subject comprises intravenous administration. In certain embodiments, administering to a subject comprises subcutaneous administration.

In certain embodiments, administering to a subject comprises intraarterial administration. In certain embodiments, administering to a subject comprises intracardial administration. Suitable means for intracardial administration include the use of a catheter, or administration during open heart surgery. In certain embodiments, administration comprises use of a stent.

In certain embodiments, administration includes pulmonary administration. In certain embodiments, pulmonary administration comprises delivery of aerosolized oligonucleotide to the lung of a subject by inhalation. Following inhalation by a subject of aerosolized oligonucleotide, oligonucleotide distributes to cells of both normal and inflamed lung tissue, including alveolar macrophages, eosinophils, epithelium, blood vessel endothelium, and bronchiolar epithelium. A suitable device for the delivery of a pharmaceutical composition comprising a modified oligonucleotide includes, but is not limited to, a standard nebulizer device. Formulations and methods for modulating the size of droplets using nebulizer devices to target specific portions of

the respiratory tract and lungs are well known to those skilled in the art. Additional suitable devices include dry powder inhalers or metered dose inhalers.

In certain embodiments, pharmaceutical compositions are administered to achieve local rather than systemic exposures. For example, pulmonary administration delivers a

5 pharmaceutical composition to the lung, with minimal systemic exposure.

Additional suitable administration routes include, but are not limited to, oral, rectal, transmucosal, intestinal, enteral, topical, suppository, intrathecal, intraventricular, intraperitoneal, intranasal, intraocular, intramuscular, intramedullary, and intratumoral.

The compounds provided herein are useful for the treatment, amelioration and/or
10 prevention of cardiac diseases. In certain embodiments, the compound comprises an oligonucleotide. In certain such embodiments, the compound consists of an oligonucleotide. In certain embodiments, the oligonucleotide is a modified oligonucleotide. In certain embodiments, a modified oligonucleotide is complementary to miR-21.

In certain such embodiments, the compound comprises an oligonucleotide hybridized to
15 a complementary strand, i.e. the compound comprises a double-stranded oligomeric compound. In certain embodiments, the hybridization of an oligonucleotide to a complementary strand forms at least one blunt end. In certain such embodiments, the hybridization of an oligonucleotide to a complementary strand forms a blunt end at each terminus of the double-stranded oligomeric compound. In certain embodiments, a terminus of an oligonucleotide comprises one or more
20 additional linked nucleosides relative to the number of linked nucleosides of the complementary strand. In certain embodiments, the one or more additional nucleosides are at the 5' terminus of an oligonucleotide. In certain embodiments, the one or more additional nucleosides are at the 3' terminus of an oligonucleotide. In certain embodiments, at least one nucleobase of a nucleoside of the one or more additional nucleosides is complementary to the target RNA. In certain
25 embodiments, each nucleobase of each one or more additional nucleosides is complementary to the target RNA. In certain embodiments, a terminus of the complementary strand comprises one or more additional linked nucleosides relative to the number of linked nucleosides of an oligonucleotide. In certain embodiments, the one or more additional linked nucleosides are at the 3' terminus of the complementary strand. In certain embodiments, the one or more additional
30 linked nucleosides are at the 5' terminus of the complementary strand. In certain embodiments, two additional linked nucleosides are linked to a terminus. In certain embodiments, one additional nucleoside is linked to a terminus.

In certain embodiments, the compound comprises an oligonucleotide conjugated to one or more moieties which enhance the activity, cellular distribution or cellular uptake of the

resulting antisense oligonucleotides. In certain such embodiments, the moiety is a cholesterol moiety. In certain embodiments, the moiety is a lipid moiety. Additional moieties for conjugation include carbohydrates, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. In certain embodiments, a conjugate group is attached directly to an oligonucleotide. In certain embodiments, a conjugate group is attached to an oligonucleotide by a linking moiety selected from amino, hydroxyl, carboxylic acid, thiol, unsaturations (e.g., double or triple bonds), 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), 6-aminohexanoic acid (AHEx or AHA), substituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, and substituted or unsubstituted C₂-C₁₀ alkynyl. In certain such embodiments, a substituent group is selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl. In certain embodiments, the compound comprises an oligonucleotide which is not conjugated to one or more moieties which enhance the activity, cellular distribution or cellular uptake of the resulting antisense oligonucleotides. In certain such embodiments, the compound comprises an oligonucleotide which is not conjugated to a cholesterol moiety.

In certain such embodiments, the compound comprises an oligonucleotide having one or more stabilizing groups that are attached to one or both termini of an oligonucleotide to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect an oligonucleotide from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap), or at the 3'-terminus (3'-cap), or can be present on both termini. Cap structures include, for example, inverted deoxy abasic caps.

Suitable cap structures include a 4',5'-methylene nucleotide, a 1-(beta-D-erythrofuransyl) nucleotide, a 4'-thio nucleotide, a carbocyclic nucleotide, a 1,5-anhydrohexitol nucleotide, an L-nucleotide, an alpha-nucleotide, a modified base nucleotide, a phosphorodithioate linkage, a threo-pentofuransyl nucleotide, an acyclic 3',4'-seco nucleotide, an acyclic 3,4-dihydroxybutyl nucleotide, an acyclic 3,5-dihydroxypentyl nucleotide, a 3'-3'-inverted nucleotide moiety, a 3'-3'-inverted abasic moiety, a 3'-2'-inverted nucleotide moiety, a 3'-2'-inverted abasic moiety, a 1,4-butanediol phosphate, a 3'-phosphoramidate, a hexylphosphate, an aminohexyl phosphate, a 3'-phosphate, a 3'-phosphorothioate, a phosphorodithioate, a bridging methylphosphonate moiety, and a non-bridging methylphosphonate moiety 5'-amino-alkyl phosphate, a 1,3-diamino-2-propyl phosphate,

3-aminopropyl phosphate, a 6-aminohexyl phosphate, a 1,2-aminododecyl phosphate, a hydroxypropyl phosphate, a 5'-5'-inverted nucleotide moiety, a 5'-5'-inverted abasic moiety, a 5'-phosphoramidate, a 5'-phosphorothioate, a 5'-amino, a bridging and/or non-bridging 5'-phosphoramidate, a phosphorothioate, and a 5'-mercapto moiety.

5 In certain embodiments, an oligonucleotide has a nucleobase sequence that is complementary to the nucleobase sequence of a miR-21 or a precursor thereof. Nucleobase sequences of mature miRNAs and their corresponding stem-loop sequences described herein are the sequences found in miRBase, an online searchable database of miRNA sequences and annotation (see above). Entries in the miRBase Sequence database represent a predicted hairpin
10 portion of a miRNA transcript (the stem-loop), with information on the location and sequence of the mature miRNA sequence. The miRNA stem-loop sequences in the database are not strictly precursor miRNAs (pre-miRNAs), and may in some instances include the pre-miRNA and some flanking sequence from the presumed primary transcript. The miRNA nucleobase sequences described herein encompass any version of the miRNA, including the sequences described in
15 Release 14.0 of the miRBase sequence database and sequences described in any earlier Release of the miRBase sequence database. A sequence database release may result in the re-naming of certain miRNAs. The compositions of the present invention encompass modified oligonucleotides that are complementary to any nucleobase sequence version of the miRNAs described herein.

20 In certain embodiments, an oligonucleotide has a nucleobase sequence that is complementary to miR-21 or a precursor thereof. Accordingly, in certain embodiments the nucleobase sequence of an oligonucleotide may have one or more mismatched basepairs with respect to its target miRNA or precursor sequence, and remains capable of hybridizing to its target sequence. In certain embodiments, an oligonucleotide has a nucleobase sequence that is
25 fully complementary to miR-21 or a precursor thereof.

In certain embodiments, an oligonucleotide has a sequence that is complementary to the nucleobase sequence of the miR-21 stem-loop: 5'-UGUCGGGUAGCUUAUCAGACUGAUGU
UGACUGUUGAAUCUCAUGGCAACACCAGUCGAUGGGCUGUCUGACA-3' (SEQ ID
NO: 2).

30 In certain embodiments, an oligonucleotide has a sequence that is complementary to a nucleobase sequence of miR-21, where the nucleobase sequence of miR-21 is UAGCUUAUCAGACUGAUGUUGA (SEQ ID NO: 1).

In certain embodiments, an oligonucleotide has a nucleobase sequence that is complementary to a region of the miR-21 stem-loop sequence (SEQ ID NO: 2). In certain

embodiments, an oligonucleotide has a nucleobase sequence that is complementary to the region of nucleobases 8-29 of SEQ ID NO: 2.

In certain embodiments, an oligonucleotide has a nucleobase sequence that is complementary to the nucleobase sequence of miR-21 (SEQ ID NO: 1). In certain embodiments, an oligonucleotide has a nucleobase sequence comprising the nucleobase sequence UCAACAU CAGUCUGAUAAGCUA (SEQ ID NO: 3). In certain embodiments, an oligonucleotide has a nucleobase sequence consisting of the nucleobase sequence UCAACAU CAGUCUGAUAAGC UA (SEQ ID NO: 3). In certain embodiments, an oligonucleotide has a nucleobase sequence comprising a nucleobase sequence selected from Table 1 below. In certain embodiments an oligonucleotide has a nucleobase sequence consisting of a nucleobase sequence selected from Table 1 below.

Table 1

Nucleobase sequence (5' to 3')	Length (in nucleobases)	SEQ ID NO:
UCAACAUCAGUCUGAUAAGCUA	22	3
CAACAUCAGUCUGAUAAGCUA	21	4
AACAUCAGUCUGAUAAGCUA	20	5
ACAUCAGUCUGAUAAGCUA	19	6
CAUCAGUCUGAUAAGCUA	18	7
AUCAGUCUGAUAAGCUA	17	8
UCAGUCUGAUAAGCUA	16	9
CAGUCUGAUAAGCUA	15	10
AGUCUGAUAAGCUA	14	11
GUCUGAUAAGCUA	13	12
UCUGAUAAGCUA	12	13
CUGAUAAGCUA	11	14
UGAUAAGCUA	10	15
GAUAAGCUA	9	
AUAAGCUA	8	
UAAGCUA	7	

In certain embodiments, an oligonucleotide comprises a nucleobase sequence that is complementary to a seed sequence of miR-21. In certain embodiments, an oligonucleotide

consists of a nucleobase sequence that is complementary to a seed sequence of miR-21. Modified oligonucleotides complementary to the seed sequence of a miRNA have been shown to inhibit activity of the miRNA. Such inhibitory activity is described in PCT Publication No.

WO/2009/043353, which is herein incorporated by reference in its entirety for its description of modified oligonucleotides targeting miRNA seed sequences.

In certain embodiments, a seed sequence is a hexamer seed sequence. In certain such embodiments, a seed sequence is nucleobases 1-6 of SEQ ID NO: 1 (UAGCUU). In certain such embodiments, a seed sequence is nucleobases 2-7 of SEQ ID NO: 1 (AGCUUA). In certain such embodiments, a seed sequence is nucleobases 3-8 of SEQ ID NO: 1 (GCUUAU).

In certain embodiments, a seed sequence is a heptamer seed sequence. In certain such embodiments, a heptamer seed sequence is nucleobases 1-7 of SEQ ID NO: 1 (UAGCUUA). In certain such embodiments, a heptamer seed sequence is nucleobases 2-8 of SEQ ID NO: 1 (AGCUUAU).

In certain embodiments, the seed sequence is an octamer seed sequence. In certain such embodiments, an octamer seed sequence is nucleobases 1-8 of SEQ ID NO: 1 (UAGCUUAU). In certain such embodiments, an octamer seed sequence is nucleobases 2-9 of SEQ ID NO: 1 (AGCUUAUC).

In certain embodiments, a modified oligonucleotide comprises a nucleobase sequence that is complementary to a seed sequence, i.e. a modified oligonucleotide comprises a seed-match sequence. In certain such embodiments, a modified oligonucleotide comprises the nucleobase sequence AAGCUA. In certain such embodiments, a modified oligonucleotide comprises the nucleobase sequence UAAGCU. In certain such embodiments, a modified oligonucleotide comprises the nucleobase sequence AUAAGC. In certain such embodiments, a modified oligonucleotide comprises the nucleobase sequence UAAGCUA. In certain such embodiments, a modified oligonucleotide comprises the nucleobase sequence of AUAAGCU. In certain such embodiments, a modified oligonucleotide comprises the nucleobase sequence AUAAGCUA. In certain such embodiments, a modified oligonucleotide comprises the nucleobase sequence GAUAAGCU.

Certain seed sequences and certain seed-match sequences are set forth in Table 2 below.

Table 2

Seed Sequence Type	Seed Sequence (5' to 3')	Seed Match Sequence (5' to 3')
Octamer 1-8	UAGCUUAU	AUAAGCUA

Heptamer 1-7	UAGCUUA	UAAGCUA
Heptamer 2-8	AGCUUAU	AUAAGCU
Hexamer 1-6	UAGCUU	AAGCUA
Hexamer 2-7	AGCUUA	UAAGCU
Hexamer 3-8	GCUUAU	AUAAGC
Octamer 2-9	AGCUUAUC	GAUAAGCU

In certain embodiments, a modified oligonucleotide consists of 6 linked nucleosides. In certain embodiments, a modified oligonucleotide consists of 7 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 8 linked nucleosides. In certain
5 embodiments, a modified oligonucleotide consists of 9 linked nucleosides. In certain embodiments, a modified oligonucleotide consists of 10 linked nucleosides. In certain embodiments, a modified oligonucleotide consists of 11 linked nucleosides. In certain embodiments, a modified oligonucleotide consists of 12 linked nucleosides.

An oligonucleotide may be cleaved by endonucleases or exonucleases, resulting in a
10 metabolite product that may have activity similar to the parent oligonucleotide. In certain embodiments, an oligonucleotide comprises a metabolite of any of the sequences in Table1. For example, a metabolite may have one less nucleoside on the 5' end, or the 3' end, or both.

In certain embodiments, an oligonucleotide has a nucleobase sequence that is complementary to a nucleobase sequence having at least 80% identity to a nucleobase sequence
15 of a miR-21 stem-loop sequence (SEQ ID NO: 2). In certain embodiments, an oligonucleotide has a nucleobase sequence that is complementary to a nucleobase sequence having at least 85%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98% identity, or 100% identity to a nucleobase sequence of a miR-21 stem-loop sequence (SEQ ID NO: 2).

In certain embodiments, an oligonucleotide has a nucleobase sequence that is
20 complementary to a nucleobase sequence having at least 80% identity to the nucleobase sequence of SEQ ID NO: 1. In certain embodiments, an oligonucleotide has a nucleobase sequence that is complementary to a nucleobase sequence having at least 85%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98% identity, or 100% identity to the nucleobase sequence of SEQ ID NO: 1.

25 In certain embodiments, a nucleobase sequence of an oligonucleotide is fully complementary to a miRNA nucleobase sequence listed herein, or a precursor thereof. In certain embodiments, an oligonucleotide has a nucleobase sequence having one mismatch with respect to the nucleobase sequence of the mature miRNA, or a precursor thereof. In certain

embodiments, an oligonucleotide has a nucleobase sequence having two mismatches with respect to the nucleobase sequence of the miRNA, or a precursor thereof. In certain such embodiments, an oligonucleotide has a nucleobase sequence having no more than two mismatches with respect to the nucleobase sequence of the mature miRNA, or a precursor thereof. In certain such embodiments, the mismatched nucleobases are contiguous. In certain such embodiments, the mismatched nucleobases are not contiguous.

In certain embodiments, an oligonucleotide consists of a number of linked nucleosides that is equal to the length of the mature miR to which it is complementary.

In certain embodiments, the number of linked nucleosides of an oligonucleotide is less than the length of the mature miRNA to which it is complementary. In certain such embodiments, the number of linked nucleosides of an oligonucleotide is one less than the length of the mature miR to which it is complementary. In certain such embodiments, an oligonucleotide has one less nucleoside at the 5' terminus. In certain such embodiments, an oligonucleotide has one less nucleoside at the 3' terminus. In certain such embodiments, an oligonucleotide has two fewer nucleosides at the 5' terminus. In certain such embodiments, an oligonucleotide has two fewer nucleosides at the 3' terminus. An oligonucleotide having a number of linked nucleosides that is less than the length of the miRNA, wherein each nucleobase of an oligonucleotide is complementary to each nucleobase at a corresponding position in a miRNA, is considered to be an oligonucleotide having a nucleobase sequence that is fully complementary to a portion of a miRNA sequence.

In certain embodiments, the number of linked nucleosides of an oligonucleotide is greater than the length of the miRNA to which it is complementary. In certain such embodiments, the nucleobase of an additional nucleoside is complementary to a nucleobase of a miRNA stem-loop sequence. In certain embodiments, the number of linked nucleosides of an oligonucleotide is one greater than the length of the miRNA to which it is complementary. In certain such embodiments, the additional nucleoside is at the 5' terminus of an oligonucleotide. In certain such embodiments, the additional nucleoside is at the 3' terminus of an oligonucleotide. In certain embodiments, the number of linked nucleosides of an oligonucleotide is two greater than the length of the miRNA to which it is complementary. In certain such embodiments, the two additional nucleosides are at the 5' terminus of an oligonucleotide. In certain such embodiments, the two additional nucleosides are at the 3' terminus of an oligonucleotide. In certain such embodiments, one additional nucleoside is located at the 5' terminus and one additional nucleoside is located at the 3' terminus of an oligonucleotide.

In certain embodiments, a portion of the nucleobase sequence of an oligonucleotide is fully complementary to the nucleobase sequence of the miRNA, but the entire modified oligonucleotide is not fully complementary to the miRNA. In certain such embodiments, the number of nucleosides of an oligonucleotide having a fully complementary portion is greater than the length of the miRNA. For example, an oligonucleotide consisting of 24 linked nucleosides, where the nucleobases of nucleosides 1 through 23 are each complementary to a corresponding position of a miRNA that is 23 nucleobases in length, has a 23 nucleoside portion that is fully complementary to the nucleobase sequence of the miRNA and approximately 96% overall complementarity to the nucleobase sequence of the miRNA.

In certain embodiments, the nucleobase sequence of an oligonucleotide is fully complementary to a portion of the nucleobase sequence of a miRNA. For example, an oligonucleotide consisting of 22 linked nucleosides, where the nucleobases of nucleosides 1 through 22 are each complementary to a corresponding position of a miRNA that is 23 nucleobases in length, is fully complementary to a 22 nucleobase portion of the nucleobase sequence of a miRNA. Such an oligonucleotide has approximately 96% overall complementarity to the nucleobase sequence of the entire miRNA, and has 100% complementarity to a 22 nucleobase portion of the miRNA.

In certain embodiments, a portion of the nucleobase sequence of an oligonucleotide is fully complementary to a portion of the nucleobase sequence of a miRNA, or a precursor thereof.

In certain such embodiments, 15 contiguous nucleobases of an oligonucleotide are each complementary to 15 contiguous nucleobases of a miRNA, or a precursor thereof. In certain such embodiments, 16 contiguous nucleobases of an oligonucleotide are each complementary to 16 contiguous nucleobases of a miRNA, or a precursor thereof. In certain such embodiments, 17 contiguous nucleobases of an oligonucleotide are each complementary to 17 contiguous nucleobases of a miRNA, or a precursor thereof. In certain such embodiments, 18 contiguous nucleobases of an oligonucleotide are each complementary to 18 contiguous nucleobases of a miRNA, or a precursor thereof. In certain such embodiments, 19 contiguous nucleobases of an oligonucleotide are each complementary to 19 contiguous nucleobases of a miRNA, or a precursor thereof. In certain such embodiments, 20 contiguous nucleobases of an oligonucleotide are each complementary to 20 contiguous nucleobases of a miRNA, or a precursor thereof. In certain such embodiments, 22 contiguous nucleobases of an oligonucleotide are each complementary to 22 contiguous nucleobases of a miRNA, or a precursor thereof. In certain such embodiments, 23 contiguous nucleobases of an oligonucleotide are each complementary to 23 contiguous nucleobases of a miRNA, or a precursor thereof. In certain such embodiments, 24

contiguous nucleobases of an oligonucleotide are each complementary to 24 contiguous nucleobases of a miRNA, or a precursor thereof.

The nucleobase sequences set forth herein, including but not limited to those found in the Examples and in the Sequence Listing, are independent of any modification to the nucleic acid. As such, nucleic acids defined by a SEQ ID NO may comprise, independently, one or more modifications to one or more sugar moieties, to one or more internucleoside linkages, and/or to one or more nucleobases.

Although the sequence listing accompanying this filing identifies each nucleobase sequence as either "RNA" or "DNA" as required, in practice, those sequences may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as "RNA" or "DNA" to describe modified oligonucleotides is somewhat arbitrary. For example, an oligonucleotide comprising a nucleoside comprising a 2'-OH sugar moiety and a thymine base could be described as a DNA having a modified sugar (2'-OH for the natural 2'-H of DNA) or as an RNA having a modified base (thymine (methylated uracil) for natural uracil of RNA).

Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of further example and without limitation, an oligomeric compound having the nucleobase sequence "ATCGATCG" encompasses any oligomeric compounds having such nucleobase sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence "AUCGAUCG" and those having some DNA bases and some RNA bases such as "AUCGATCG" and oligomeric compounds having other modified bases, such as "AT^{me}CGAUCG," wherein ^{me}C indicates a cytosine base comprising a methyl group at the 5-position.

In certain embodiments, an oligonucleotide consists of 8 to 25 linked nucleosides. In certain embodiments, an oligonucleotide consists of 15 to 23 linked nucleosides. In certain embodiments, an oligonucleotide consists of 15 to 30 linked nucleosides. In certain embodiments, an oligonucleotide consists of 19 to 24 linked nucleosides. In certain embodiments, an oligonucleotide consists of 21 to 24 linked nucleosides.

In certain embodiments, an oligonucleotide consists of 8 linked nucleosides. In certain embodiments, an oligonucleotide consists of 9 linked nucleosides. In certain embodiments, an oligonucleotide consists of 10 linked nucleosides. In certain embodiments, an oligonucleotide

consists of 11 linked nucleosides. In certain embodiments, an oligonucleotide consists of 12 linked nucleosides. In certain embodiments, an oligonucleotide consists of 13 linked nucleosides. In certain embodiments, an oligonucleotide consists of 14 linked nucleosides. In certain embodiments, an oligonucleotide consists of 15 linked nucleosides. In certain embodiments, an oligonucleotide consists of 16 linked nucleosides. In certain embodiments, an oligonucleotide consists of 17 linked nucleosides. In certain embodiments, an oligonucleotide consists of 18 linked nucleosides. In certain embodiments, an oligonucleotide consists of 19 linked nucleosides. In certain embodiments, an oligonucleotide consists of 20 linked nucleosides. In certain embodiments, an oligonucleotide consists of 21 linked nucleosides. In certain embodiments, an oligonucleotide consists of 22 linked nucleosides. In certain embodiments, an oligonucleotide consists of 23 linked nucleosides. In certain embodiments, an oligonucleotide consists of 24 linked nucleosides. In certain embodiments, an oligonucleotide consists of 25 linked nucleosides. In certain embodiments, an oligonucleotide consists of 26 linked nucleosides. In certain embodiments, an oligonucleotide consists of 27 linked nucleosides. In certain embodiments, an oligonucleotide consists of 28 linked nucleosides. In certain embodiments, an oligonucleotide consists of 29 linked nucleosides. In certain embodiments, an oligonucleotide consists of 30 linked nucleosides.

In certain embodiments, oligonucleotides provided herein may comprise one or more modifications to a nucleobase, sugar, and/or internucleoside linkage, and as such is a modified oligonucleotide. A modified nucleobase, sugar, and/or internucleoside linkage may be selected over an unmodified form because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for other oligonucleotides or nucleic acid targets and increased stability in the presence of nucleases.

In certain embodiments, a modified oligonucleotide comprises one or more modified nucleosides. In certain such embodiments, a modified nucleoside is a stabilizing nucleoside. An example of a stabilizing nucleoside is a sugar-modified nucleoside.

In certain embodiments, a modified nucleoside is a sugar-modified nucleoside. In certain such embodiments, the sugar-modified nucleosides can further comprise a natural or modified heterocyclic base moiety and/or a natural or modified internucleoside linkage and may include further modifications independent from the sugar modification. In certain embodiments, a sugar modified nucleoside is a 2'-modified nucleoside, wherein the sugar ring is modified at the 2' carbon from natural ribose or 2'-deoxy-ribose.

In certain embodiments, a 2'-modified nucleoside has a bicyclic sugar moiety. In certain embodiments, the bicyclic sugar moiety is a D sugar in the alpha configuration. In certain

embodiments, the bicyclic sugar moiety is a D sugar in the beta configuration. In certain embodiments, the bicyclic sugar moiety is an L sugar in the alpha configuration. In certain embodiments, the bicyclic sugar moiety is an L sugar in the beta configuration.

In certain embodiments, the bicyclic sugar moiety comprises a bridge group between the 2' and the 4'-carbon atoms. In certain such embodiments, the bridge group comprises from 1 to 8 linked biradical groups. In certain embodiments, the bicyclic sugar moiety comprises from 1 to 4 linked biradical groups. In certain embodiments, the bicyclic sugar moiety comprises 2 or 3 linked biradical groups. In certain embodiments, the bicyclic sugar moiety comprises 2 linked biradical groups. In certain embodiments, a linked biradical group is selected from -O-, -S-, -N(R₁)-, -C(R₁)(R₂)-, -C(R₁)=C(R₁)-, -C(R₁)=N-, -C(=NR₁)-, -Si(R₁)(R₂)-, -S(=O)₂-, -S(=O)-, -C(=O)- and -C(=S)-; where each R₁ and R₂ is, independently, H, hydroxyl, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₅-C₂₀ aryl, substituted C₅-C₂₀ aryl, a heterocycle radical, a substituted heterocycle radical, heteroaryl, substituted heteroaryl, C₅-C₇ alicyclic radical, substituted C₅-C₇ alicyclic radical, halogen, substituted oxy (-O-), amino, substituted amino, azido, carboxyl, substituted carboxyl, acyl, substituted acyl, CN, thiol, substituted thiol, sulfonyl (S(=O)₂-H), substituted sulfonyl, sulfoxyl (S(=O)-H) or substituted sulfoxyl; and each substituent group is, independently, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, amino, substituted amino, acyl, substituted acyl, C₁-C₁₂ aminoalkyl, C₁-C₁₂ aminoalkoxy, substituted C₁-C₁₂ aminoalkyl, substituted C₁-C₁₂ aminoalkoxy or a protecting group.

In some embodiments, the bicyclic sugar moiety is bridged between the 2' and 4' carbon atoms with a biradical group selected from -O-(CH₂)_p-, -O-CH₂-, -O-CH₂CH₂-, -O-CH(alkyl)-, -NH-(CH₂)_p-, -N(alkyl)-(CH₂)_p-, -O-CH(alkyl)-, -(CH(alkyl))-(CH₂)_p-, -NH-O-(CH₂)_p-, -N(alkyl)-O-(CH₂)_p-, or -O-N(alkyl)-(CH₂)_p-, wherein p is 1, 2, 3, 4 or 5 and each alkyl group can be further substituted. In certain embodiments, p is 1, 2 or 3.

In certain embodiments, a 2'-modified nucleoside comprises a 2'-substituent group selected from halo, allyl, amino, azido, SH, CN, OCN, CF₃, OCF₃, O-, S-, or N(R_m)-alkyl; O-, S-, or N(R_m)-alkenyl; O-, S- or N(R_m)-alkynyl; O-alkylenyl-O-alkyl, alkynyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, O(CH₂)₂SCH₃, O-(CH₂)₂-O-N(R_m)(R_n) or O-CH₂-C(=O)-N(R_m)(R_n), where each R_m and R_n is, independently, H, an amino protecting group or substituted or unsubstituted C₁-C₁₀ alkyl. These 2'-substituent groups can be further substituted with one or more substituent groups independently selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro (NO₂), thiol, thioalkoxy (S-alkyl), halogen, alkyl, aryl, alkenyl and alkynyl.

In certain embodiments, a 2'-modified nucleoside comprises a 2'-substituent group selected from F, NH₂, N₃, OCF₃, O-CH₃, O(CH₂)₃NH₂, CH₂-CH=CH₂, O-CH₂-CH=CH₂, OCH₂CH₂OCH₃, O(CH₂)₂SCH₃, O-(CH₂)₂-O-N(R_m)(R_n), -O(CH₂)₂O(CH₂)₂N(CH₃)₂, and N-substituted acetamide (O-CH₂-C(=O)-N(R_m)(R_n) where each R_m and R_n is, independently, H, an amino protecting group or substituted or unsubstituted C₁-C₁₀ alkyl.

In certain embodiments, a 2'-modified nucleoside comprises a 2'-substituent group selected from F, OCF₃, O-CH₃, OCH₂CH₂OCH₃, 2'-O(CH₂)₂SCH₃, O-(CH₂)₂-O-N(CH₃)₂, -O(CH₂)₂O(CH₂)₂N(CH₃)₂, and O-CH₂-C(=O)-N(H)CH₃.

In certain embodiments, a 2'-modified nucleoside comprises a 2'-substituent group selected from F, O-CH₃, and OCH₂CH₂OCH₃.

In certain embodiments, a sugar-modified nucleoside is a 4'-thio modified nucleoside. In certain embodiments, a sugar-modified nucleoside is a 4'-thio-2'-modified nucleoside. A 4'-thio modified nucleoside has a β-D-ribonucleoside where the 4'-O replaced with 4'-S. A 4'-thio-2'-modified nucleoside is a 4'-thio modified nucleoside having the 2'-OH replaced with a 2'-substituent group. Suitable 2'-substituent groups include 2'-OCH₃, 2'-O-(CH₂)₂-OCH₃, and 2'-F.

In certain embodiments, a modified oligonucleotide comprises one or more internucleoside modifications. In certain such embodiments, each internucleoside linkage of an oligonucleotide is a modified internucleoside linkage. In certain embodiments, a modified internucleoside linkage comprises a phosphorus atom.

In certain embodiments, a modified oligonucleotide comprises at least one phosphorothioate internucleoside linkage. In certain embodiments, each internucleoside linkage of a modified oligonucleotide is a phosphorothioate internucleoside linkage.

In certain embodiments, a modified internucleoside linkage does not comprise a phosphorus atom. In certain such embodiments, an internucleoside linkage is formed by a short chain alkyl internucleoside linkage. In certain such embodiments, an internucleoside linkage is formed by a cycloalkyl internucleoside linkages. In certain such embodiments, an internucleoside linkage is formed by a mixed heteroatom and alkyl internucleoside linkage. In certain such embodiments, an internucleoside linkage is formed by a mixed heteroatom and cycloalkyl internucleoside linkages. In certain such embodiments, an internucleoside linkage is formed by one or more short chain heteroatomic internucleoside linkages. In certain such embodiments, an internucleoside linkage is formed by one or more heterocyclic internucleoside linkages. In certain such embodiments, an internucleoside linkage has an amide backbone. In

certain such embodiments, an internucleoside linkage has mixed N, O, S and CH₂ component parts.

In certain embodiments, a modified oligonucleotide comprises one or more modified nucleobases. In certain embodiments, a modified oligonucleotide comprises one or more 5-methylcytosines. In certain embodiments, each cytosine of a modified oligonucleotide comprises a 5-methylcytosine.

In certain embodiments, a modified nucleobase is selected from 5-hydroxymethyl cytosine, 7-deazaguanine and 7-deazaadenine. In certain embodiments, a modified nucleobase is selected from 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. In certain 10 embodiments, a modified nucleobase is selected from 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2 aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

In certain embodiments, a modified nucleobase comprises a polycyclic heterocycle. In certain embodiments, a modified nucleobase comprises a tricyclic heterocycle. In certain 15 embodiments, a modified nucleobase comprises a phenoxazine derivative. In certain embodiments, the phenoxazine can be further modified to form a nucleobase known in the art as a G-clamp.

Suitable motifs for modified oligonucleotides of the present invention include, but are not limited to, fully modified, uniformly modified, positionally modified, and gapmer. Modified 20 oligonucleotides having a fully modified motif, including a uniformly modified motif, may be designed to target mature miRNAs. Alternatively, modified oligonucleotides having a fully modified motif, including a uniformly modified motif, may be designed to target certain sites of pri-miRNAs or pre-miRNAs, to block the processing of miRNA precursors into mature miRNAs. Modified oligonucleotides having a fully modified motif or uniformly modified motif 25 are effective inhibitors of miRNA activity.

In certain embodiments, a fully modified oligonucleotide comprises a sugar modification at each nucleoside. In certain such embodiments, pluralities of nucleosides are 2'-O-methoxyethyl nucleosides and the remaining nucleosides are 2'-fluoro nucleosides. In certain such embodiments, each of a plurality of nucleosides is a 2'-O-methoxyethyl nucleoside 30 and each of a plurality of nucleosides is a bicyclic nucleoside. In certain such embodiments, a fully modified oligonucleotide further comprises at least one modified internucleoside linkage. In certain such embodiments, each internucleoside linkage of a fully sugar-modified oligonucleotide is a modified internucleoside linkage. In certain embodiments, a fully sugar-modified oligonucleotide further comprises at least one phosphorothioate internucleoside

linkage. In certain such embodiments, each internucleoside linkage of a fully sugar-modified oligonucleotide is a phosphorothioate internucleoside linkage.

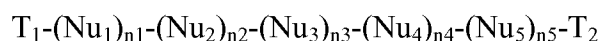
In certain embodiments, a fully modified oligonucleotide is modified at each internucleoside linkage. In certain such embodiments, each internucleoside linkage of a fully modified oligonucleotide is a phosphorothioate internucleoside linkage.

In certain embodiments, a uniformly modified oligonucleotide comprises the same sugar modification at each nucleoside. In certain such embodiments, each nucleoside of a modified oligonucleotide comprises a 2'-O-methoxyethyl sugar modification. In certain embodiments, each nucleoside of a modified oligonucleotide comprises a 2'-O-methyl sugar modification. In certain embodiments, each nucleoside of a modified oligonucleotide comprises a 2'-fluoro sugar modification. In certain such embodiments, a uniformly modified oligonucleotide further comprises at least one modified internucleoside linkage. In certain such embodiments, each internucleoside linkage of a uniformly sugar-modified oligonucleotide is a modified internucleoside linkage. In certain embodiments, a uniformly sugar-modified oligonucleotide further comprises at least one phosphorothioate internucleoside linkage. In certain such embodiments, each internucleoside linkage of a uniformly sugar-modified oligonucleotide is a phosphorothioate internucleoside linkage.

In certain embodiments, a uniformly modified oligonucleotide comprises the same internucleoside linkage modifications throughout. In certain such embodiments, each internucleoside linkage of a uniformly modified oligonucleotide is a phosphorothioate internucleoside linkage.

In certain embodiments, a positionally modified oligonucleotide comprises regions of linked nucleosides, where each nucleoside of each region comprises the same sugar moiety, and where each nucleoside of each region comprises a sugar moiety different from that of an adjacent region.

In certain embodiments, a modified oligonucleotide consisting of linked nucleosides is represented by Formula I:



wherein:

- Nu_1 and Nu_5 are, independently, 2' stabilizing nucleosides;
- Nu_2 and Nu_4 are 2'-fluoro nucleosides;
- Nu_3 is a 2'-modified nucleoside;
- each of n_1 and n_5 is, independently, from 0 to 3;
- the sum of n_2 plus n_4 is between 10 and 25 or from 10 to 25;

n_3 is from 0 to 5; and

each T_1 and T_2 is, independently, H, a hydroxyl protecting group, an optionally linked conjugate group or a capping group.

In certain embodiments, Nu_1 and Nu_5 are, independently, 2'-modified nucleosides.

5 In certain embodiments, Nu_1 is $O-(CH_2)_2-OCH_3$, Nu_3 is $O-(CH_2)_2-OCH_3$, and Nu_5 is $O-(CH_2)_2-OCH_3$.

In certain embodiments, each internucleoside linkage is a modified internucleoside linkage. In certain such embodiments, each internucleoside is a phosphorothioate linkage.

10 In certain embodiments, a nucleoside comprises a modified nucleobase. In certain embodiments, where a 2'-O-methoxyethyl nucleoside comprises cytosine, the cytosine is a 5-methylcytosine.

In certain embodiments, Nu_1 is $O-(CH_2)_2-OCH_3$, Nu_3 is $O-(CH_2)_2-OCH_3$, Nu_5 is $O-(CH_2)_2-OCH_3$, T_1 is H and T_2 is H.

15 In certain embodiments, T_1 and T_2 are each, independently, H or a hydroxyl protecting group. In certain embodiments, at least one of T_1 and T_2 is 4,4'-dimethoxytrityl. In certain embodiments, at least one of T_1 and T_2 is an optionally linked conjugate group. In certain embodiments, at least one of T_1 and T_2 is a capping group. In certain embodiments, the capping group is an inverted deoxy abasic group.

20 In certain embodiments, the sum of n_2 and n_4 is 13. In certain embodiments, the sum of n_2 and n_4 is 14. In certain embodiments, the sum of n_2 and n_4 is 15. In certain embodiments, the sum of n_2 and n_4 is 16. In certain embodiments, the sum of n_2 and n_4 is 17. In certain embodiments, the sum of n_2 and n_4 is 18.

25 In certain embodiments, n_1 , n_2 , and n_3 are each, independently, from 1 to 3. In certain embodiments, n_1 , n_2 , and n_3 are each, independently, 2 or 3. In certain embodiments, n_1 is 1 or 2; n_2 is 2 or 3; and n_3 is 1 or 2. In certain embodiments, n_1 is 2; n_3 is 2 or 3; and n_5 is 2. In certain embodiments, n_1 is 2; n_3 is 3; and n_5 is 2. In certain embodiments, n_1 is 2; n_3 is 2; and n_5 is 2. In certain embodiments, a modified oligonucleotide consists of 20 linked nucleosides. In certain such embodiments, the sum of n_2 and n_4 is 13; n_1 is 2; n_3 is 3; and n_5 is 2. In certain such embodiments, the sum of n_2 and n_4 is 14; n_1 is 2; n_3 is 2; and n_5 is 2.

30 In certain embodiments, a modified oligonucleotide consists of 21 linked nucleosides. In certain such embodiments, the sum of n_2 and n_4 is 14; n_1 is 2; n_3 is 3; and n_5 is 2. In certain such embodiments, the sum of n_2 and n_4 is 15; n_1 is 2; n_3 is 2; and n_5 is 2.

In certain embodiments, a modified oligonucleotide consists of 22 linked nucleosides. In certain such embodiments, the sum of n_2 and n_4 is 15; n_1 is 2; n_3 is 3; and n_5 is 2. In certain such embodiments, the sum of n_2 and n_4 is 16; n_1 is 2; n_3 is 2; and n_5 is 2.

In certain embodiments, a modified oligonucleotide consists of 23 linked nucleosides.

5 In certain such embodiments, the sum of n_2 and n_4 is 16; n_1 is 2; n_3 is 3; and n_5 is 2. In certain such embodiments, the sum of n_2 and n_4 is 17; n_1 is 2; n_3 is 2; and n_5 is 2.

In certain embodiments, a modified oligonucleotide consists of 24 linked nucleosides. In certain such embodiments, the sum of n_2 and n_4 is 17; n_1 is 2; n_3 is 3; and n_5 is 2. In certain such embodiments, the sum of n_2 and n_4 is 18; n_1 is 2; n_3 is 2; and n_5 is 2.

10 In certain embodiments, a modified oligonucleotide consists of 22 linked nucleosides; n_1 is 2; n_2 is 9; n_3 is 3; n_4 is 6; n_5 is 2; Nu_1 is $O-(CH_2)_2-OCH_3$; Nu_3 is $O-(CH_2)_2-OCH_3$; and Nu_5 is $O-(CH_2)_2-OCH_3$.

In certain embodiments, a modified oligonucleotide consists of 22 linked nucleosides; n_1 is 2; n_2 is 9; n_3 is 3; n_4 is 6; n_5 is 2; Nu_1 is $O-(CH_2)_2-OCH_3$; Nu_3 is $O-(CH_2)_2-OCH_3$; Nu_5 is

15 $O-(CH_2)_2-OCH_3$; and each internucleoside linkage is a phosphorothioate linkage.

In certain embodiments, a modified oligonucleotide consists of 22 linked nucleosides; n_1 is 2; n_2 is 9; n_3 is 3; n_4 is 6; n_5 is 2; Nu_1 is $O-(CH_2)_2-OCH_3$; Nu_3 is $O-(CH_2)_2-OCH_3$; Nu_5 is $O-(CH_2)_2$; internucleoside linkage is a phosphorothioate linkage.

In certain embodiments, a modified oligonucleotide consists of 22 linked nucleosides; has the nucleobase sequence of SEQ ID NO: 3; n_1 is 2; n_2 is 9; n_3 is 3; n_4 is 6; n_5 is 2; Nu_1 is $O-(CH_2)_2-OCH_3$; Nu_3 is $O-(CH_2)_2-OCH_3$; Nu_5 is $O-(CH_2)_2$; each internucleoside linkage is a phosphorothioate linkage; the cytosine at nucleobase 13 is a 5-methylcytosine; and the cytosine at nucleobase 21 is a 5-methylcytosine (referred to herein as anti-miR-21-1).

In certain embodiments, a modified oligonucleotide complementary to a miRNA and consisting of 22 linked nucleosides has a Formula I selected from Table 3, where each internucleoside linkage is a phosphorothioate internucleoside linkage. In certain embodiments, a modified oligonucleotide having a Formula I selected from Table 3 has the nucleobase sequence of SEQ ID NO: 3.

Table 3								
SEQ ID NO	n_1	n_2	n_3	n_4	n_5	Nu_1	Nu_3	Nu_5
3	2	2	3	13	2	2'-MOE	2'-MOE	2'-MOE
3	2	5	3	10	2	2'-MOE	2'-MOE	2'-MOE

3	2	8	3	7	2	2'-MOE	2'-MOE	2'-MOE
3	2	11	3	4	2	2'-MOE	2'-MOE	2'-MOE
3	2	9	3	6	2	2'-MOE	2'-MOE	2'-MOE
3	2	10	3	5	2	2'-MOE	2'-MOE	2'-MOE
3	2	12	3	3	2	2'-MOE	2'-MOE	2'-MOE
3	2	3	3	12	2	2'-MOE	2'-MOE	2'-MOE
3	2	4	3	11	2	2'-MOE	2'-MOE	2'-MOE
3	2	6	3	9	2	2'-MOE	2'-MOE	2'-MOE
3	2	7	3	8	2	2'-MOE	2'-MOE	2'-MOE
3	2	13	3	2	2	2'-MOE	2'-MOE	2'-MOE
3	2	8	6	4	2	2'-MOE	2'-MOE	2'-MOE
3	2	2	2	14	2	2'-MOE	2'-MOE	2'-MOE
3	2	3	2	13	2	2'-MOE	2'-MOE	2'-MOE
3	2	4	2	12	2	2'-MOE	2'-MOE	2'-MOE
3	2	5	2	11	2	2'-MOE	2'-MOE	2'-MOE
3	2	6	2	10	2	2'-MOE	2'-MOE	2'-MOE
3	2	7	2	9	2	2'-MOE	2'-MOE	2'-MOE
3	2	8	2	8	2	2'-MOE	2'-MOE	2'-MOE
3	2	9	2	7	2	2'-MOE	2'-MOE	2'-MOE
3	2	10	2	6	2	2'-MOE	2'-MOE	2'-MOE
3	2	11	2	5	2	2'-MOE	2'-MOE	2'-MOE
3	2	12	2	4	2	2'-MOE	2'-MOE	2'-MOE
3	2	13	2	3	2	2'-MOE	2'-MOE	2'-MOE
3	2	14	2	2	2	2'-MOE	2'-MOE	2'-MOE

In certain embodiments, a modified oligonucleotide complementary to a miRNA and consisting of 21 linked nucleosides has a Formula I selected from Table 4, where each internucleoside linkage is a phosphorothioate internucleoside linkage. In certain embodiments, a modified oligonucleotide having a Formula I selected from Table 4 has the nucleobase sequence of SEQ ID NO: 4.

Table 4								
SEQ ID NO	n ₁	n ₂	n ₃	n ₄	n ₅	Nu ₁	Nu ₃	Nu ₅
4	2	2	2	13	2	2'-MOE	2'-MOE	2'-MOE
4	2	3	2	12	2	2'-MOE	2'-MOE	2'-MOE
4	2	4	2	11	2	2'-MOE	2'-MOE	2'-MOE
4	2	5	2	10	2	2'-MOE	2'-MOE	2'-MOE
4	2	6	2	9	2	2'-MOE	2'-MOE	2'-MOE
4	2	7	2	8	2	2'-MOE	2'-MOE	2'-MOE
4	2	8	2	7	2	2'-MOE	2'-MOE	2'-MOE
4	2	9	2	6	2	2'-MOE	2'-MOE	2'-MOE
4	2	10	2	5	2	2'-MOE	2'-MOE	2'-MOE
4	2	11	2	4	2	2'-MOE	2'-MOE	2'-MOE
4	2	12	2	3	2	2'-MOE	2'-MOE	2'-MOE
4	2	13	2	2	2	2'-MOE	2'-MOE	2'-MOE
4	2	2	3	12	2	2'-MOE	2'-MOE	2'-MOE
4	2	3	3	11	2	2'-MOE	2'-MOE	2'-MOE
4	2	4	3	10	2	2'-MOE	2'-MOE	2'-MOE
4	2	5	3	9	2	2'-MOE	2'-MOE	2'-MOE
4	2	6	3	8	2	2'-MOE	2'-MOE	2'-MOE
4	2	7	3	7	2	2'-MOE	2'-MOE	2'-MOE
4	2	8	3	6	2	2'-MOE	2'-MOE	2'-MOE
4	2	9	3	5	2	2'-MOE	2'-MOE	2'-MOE
4	2	10	3	4	2	2'-MOE	2'-MOE	2'-MOE
4	2	11	3	3	2	2'-MOE	2'-MOE	2'-MOE
4	2	12	3	2	2	2'-MOE	2'-MOE	2'-MOE
4	2	8	6	3	2	2'-MOE	2'-MOE	2'-MOE

In certain embodiments, a modified oligonucleotide complementary to a miRNA and consisting of 20 linked nucleosides has a Formula I selected from Table 5, where each internucleoside linkage is a phosphorothioate internucleoside linkage. In such certain

5 embodiments, a modified oligonucleotide having a Formula I selected from Table 5 has the nucleobase sequence of SEQ ID NO: 5.

Table 5								
SEQ ID NO	n1	n2	n3	n4	n5	Nu1	Nu3	Nu5
5	2	2	3	11	2	2'-MOE	2'-MOE	2'-MOE
5	2	5	3	8	2	2'-MOE	2'-MOE	2'-MOE
5	2	8	3	5	2	2'-MOE	2'-MOE	2'-MOE
5	2	11	3	2	2	2'-MOE	2'-MOE	2'-MOE
5	2	9	3	4	2	2'-MOE	2'-MOE	2'-MOE
5	2	10	3	3	2	2'-MOE	2'-MOE	2'-MOE
5	2	3	3	10	2	2'-MOE	2'-MOE	2'-MOE
5	2	4	3	9	2	2'-MOE	2'-MOE	2'-MOE
5	2	6	3	7	2	2'-MOE	2'-MOE	2'-MOE
5	2	7	3	6	2	2'-MOE	2'-MOE	2'-MOE
5	2	8	6	2	2	2'-MOE	2'-MOE	2'-MOE
5	2	2	2	12	2	2'-MOE	2'-MOE	2'-MOE
5	2	3	2	11	2	2'-MOE	2'-MOE	2'-MOE
5	2	4	2	10	2	2'-MOE	2'-MOE	2'-MOE
5	2	5	2	9	2	2'-MOE	2'-MOE	2'-MOE
5	2	6	2	8	2	2'-MOE	2'-MOE	2'-MOE
5	2	7	2	7	2	2'-MOE	2'-MOE	2'-MOE
5	2	8	2	6	2	2'-MOE	2'-MOE	2'-MOE
5	2	9	2	5	2	2'-MOE	2'-MOE	2'-MOE
5	2	10	2	4	2	2'-MOE	2'-MOE	2'-MOE
5	2	11	2	3	2	2'-MOE	2'-MOE	2'-MOE
5	2	12	2	2	2	2'-MOE	2'-MOE	2'-MOE

In certain embodiments, a modified oligonucleotide complementary to a miRNA and consisting of 22 linked nucleosides has a Formula I selected from Table 6, where each internucleoside linkage is a phosphorothioate internucleoside linkage. In certain embodiments, a modified

5 oligonucleotide having a Formula I selected from Table 6 comprises a nucleobase sequence selected from among the nucleobase sequences SEQ ID NOs 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, GAUAAGCUA, AUAAGCUA, UAAGCUA, AUAAGCUA, UAAGCUA, AUAAGCU, AAGCUA, UAAGCU, AUAAGC, and GAUAAGCU.

Table 6

n ₁	n ₂	n ₃	n ₄	n ₅	Nu ₁	Nu ₃	Nu ₅
2	2	3	13	2	2'-MOE	2'-MOE	2'-MOE
2	5	3	10	2	2'-MOE	2'-MOE	2'-MOE
2	8	3	7	2	2'-MOE	2'-MOE	2'-MOE
2	11	3	4	2	2'-MOE	2'-MOE	2'-MOE
2	9	3	6	2	2'-MOE	2'-MOE	2'-MOE
2	10	3	5	2	2'-MOE	2'-MOE	2'-MOE
2	12	3	3	2	2'-MOE	2'-MOE	2'-MOE
2	3	3	12	2	2'-MOE	2'-MOE	2'-MOE
2	4	3	11	2	2'-MOE	2'-MOE	2'-MOE
2	6	3	9	2	2'-MOE	2'-MOE	2'-MOE
2	7	3	8	2	2'-MOE	2'-MOE	2'-MOE
2	13	3	2	2	2'-MOE	2'-MOE	2'-MOE
2	8	6	4	2	2'-MOE	2'-MOE	2'-MOE
2	2	2	14	2	2'-MOE	2'-MOE	2'-MOE
2	3	2	13	2	2'-MOE	2'-MOE	2'-MOE
2	4	2	12	2	2'-MOE	2'-MOE	2'-MOE
2	5	2	11	2	2'-MOE	2'-MOE	2'-MOE
2	6	2	10	2	2'-MOE	2'-MOE	2'-MOE
2	7	2	9	2	2'-MOE	2'-MOE	2'-MOE
2	8	2	8	2	2'-MOE	2'-MOE	2'-MOE
2	9	2	7	2	2'-MOE	2'-MOE	2'-MOE
2	10	2	6	2	2'-MOE	2'-MOE	2'-MOE
2	11	2	5	2	2'-MOE	2'-MOE	2'-MOE
2	12	2	4	2	2'-MOE	2'-MOE	2'-MOE
2	13	2	3	2	2'-MOE	2'-MOE	2'-MOE
2	14	2	2	2	2'-MOE	2'-MOE	2'-MOE

In certain embodiments, a modified oligonucleotide complementary to a miRNA and consisting of 23 linked nucleosides has a Formula I selected from Table 7, where each

5 internucleoside linkage is a phosphorothioate internucleoside linkage. In certain embodiments, a modified oligonucleotide having a Formula I selected from Table 7 comprises a nucleobase

sequence selected from among SEQ ID NOs 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, GAUAAGCUA, AUAAGCUA, UAAGCUA, AUAAGCUA, UAAGCUA, AUAAGCU, AAGCUA, UAAGCU, AUAAGC, and GAUAAGCU.

Table 7

n1	n2	n3	n4	n5	Nu1	Nu3	Nu5
2	2	3	14	2	2'-MOE	2'-MOE	2'-MOE
2	5	3	11	2	2'-MOE	2'-MOE	2'-MOE
2	8	3	8	2	2'-MOE	2'-MOE	2'-MOE
2	11	3	5	2	2'-MOE	2'-MOE	2'-MOE
2	14	3	2	2	2'-MOE	2'-MOE	2'-MOE
2	9	3	7	2	2'-MOE	2'-MOE	2'-MOE
2	10	3	6	2	2'-MOE	2'-MOE	2'-MOE
2	12	3	4	2	2'-MOE	2'-MOE	2'-MOE
2	3	3	13	2	2'-MOE	2'-MOE	2'-MOE
2	4	3	12	2	2'-MOE	2'-MOE	2'-MOE
2	6	3	10	2	2'-MOE	2'-MOE	2'-MOE
2	7	3	9	2	2'-MOE	2'-MOE	2'-MOE
2	13	3	3	2	2'-MOE	2'-MOE	2'-MOE
2	8	6	5	2	2'-MOE	2'-MOE	2'-MOE
2	2	2	15	2	2'-MOE	2'-MOE	2'-MOE
2	3	2	14	2	2'-MOE	2'-MOE	2'-MOE
2	4	2	13	2	2'-MOE	2'-MOE	2'-MOE
2	5	2	12	2	2'-MOE	2'-MOE	2'-MOE
2	6	2	11	2	2'-MOE	2'-MOE	2'-MOE
2	7	2	10	2	2'-MOE	2'-MOE	2'-MOE
2	8	2	9	2	2'-MOE	2'-MOE	2'-MOE
2	9	2	8	2	2'-MOE	2'-MOE	2'-MOE
2	10	2	7	2	2'-MOE	2'-MOE	2'-MOE
2	11	2	6	2	2'-MOE	2'-MOE	2'-MOE
2	12	2	5	2	2'-MOE	2'-MOE	2'-MOE
2	13	2	4	2	2'-MOE	2'-MOE	2'-MOE

In certain embodiments, a modified oligonucleotide complementary to a miRNA and consisting of 24 linked nucleosides has a Formula I selected from Table 8, where each internucleoside linkage is a phosphorothioate internucleoside linkage. In certain embodiments, a modified oligonucleotide having a Formula I selected from Table 8 comprises a nucleobase sequence selected from among SEQ ID NOs 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, GAUAAGCUA, AUAAGCUA, UAAGCUA, AUAAGCUA, UAAGCUA, AUAAGCU, AAGCUA, UAAGCU, AUAAGC, and GAUAAGCU..

Table 8

n ₁	n ₂	n ₃	n ₄	n ₅	Nu ₁	Nu ₃	Nu ₅
2	2	2	16	2	2'-MOE	2'-MOE	2'-MOE
2	3	2	15	2	2'-MOE	2'-MOE	2'-MOE
2	4	2	14	2	2'-MOE	2'-MOE	2'-MOE
2	5	2	13	2	2'-MOE	2'-MOE	2'-MOE
2	6	2	12	2	2'-MOE	2'-MOE	2'-MOE
2	7	2	11	2	2'-MOE	2'-MOE	2'-MOE
2	8	2	10	2	2'-MOE	2'-MOE	2'-MOE
2	9	2	9	2	2'-MOE	2'-MOE	2'-MOE
2	10	2	8	2	2'-MOE	2'-MOE	2'-MOE
2	11	2	7	2	2'-MOE	2'-MOE	2'-MOE
2	12	2	6	2	2'-MOE	2'-MOE	2'-MOE
2	13	2	5	2	2'-MOE	2'-MOE	2'-MOE
2	14	2	4	2	2'-MOE	2'-MOE	2'-MOE
2	15	2	3	2	2'-MOE	2'-MOE	2'-MOE
2	16	2	2	2	2'-MOE	2'-MOE	2'-MOE
2	2	3	15	2	2'-MOE	2'-MOE	2'-MOE
2	3	3	14	2	2'-MOE	2'-MOE	2'-MOE
2	4	3	13	2	2'-MOE	2'-MOE	2'-MOE
2	5	3	12	2	2'-MOE	2'-MOE	2'-MOE
2	6	3	11	2	2'-MOE	2'-MOE	2'-MOE
2	7	3	10	2	2'-MOE	2'-MOE	2'-MOE
2	8	3	9	2	2'-MOE	2'-MOE	2'-MOE
2	9	3	8	2	2'-MOE	2'-MOE	2'-MOE
2	10	3	7	2	2'-MOE	2'-MOE	2'-MOE

2	11	3	6	2	2'-MOE	2'-MOE	2'-MOE
2	12	3	5	2	2'-MOE	2'-MOE	2'-MOE
2	13	3	4	2	2'-MOE	2'-MOE	2'-MOE
2	14	3	3	2	2'-MOE	2'-MOE	2'-MOE
2	15	3	2	2	2'-MOE	2'-MOE	2'-MOE
2	8	6	6	2	2'-MOE	2'-MOE	2'-MOE

In certain embodiments, a modified oligonucleotide having a positionally modified motif comprises LNA. In certain embodiments, a modified oligonucleotide has a motif selected from among one of the motifs listed below, wherein L = an LNA nucleoside, d = a DNA nucleoside, M = a 2'-MOE nucleoside, and F = a 2'-Fluoro nucleoside. In certain embodiments, nucleosides in parentheses are optionally included in the modified oligonucleotide, in other words, the motif encompasses modified oligonucleotides of varying lengths depending upon how many nucleosides in parentheses are included.

LdLddLLddLdLdLL

10 Ld Ld LLLd d LLLd LL

LMLMMLLMMLMLMML

LMLMLLLMMMLLLMML

LFLFFLLFFLFLFLL

LFLFLLLFFLLLFL

15 LddLddLddL(d)(d)(L)(d)(d)(L)(d)

dLddLddLdd(L)(d)(d)(L)(d)(d)(L)

ddLddLddLd(d)(L)(d)(d)(L)(d)(d)

LMMLMMLMML(M)(M)(L)(M)(M)(L)(M)

MLMMLMMLMM(L)(M)(M)(L)(M)(M)(L)

20 MMLMMLMMLM(M)(L)(M)(M)(L)(M)(M)

LFFLFFLFFL(F)(F)(L)(F)(F)(L)(F)

FLFFLFFLFF(L)(F)(F)(L)(F)(F)(L)

FFLFFLFFLF(F)(L)(F)(F)(L)(F)(F)

dLdLdLdLdL(d)(L)(d)(L)(d)(L)(d)

25 LdLdLdLdL(d)(L)(d)(L)(d)(L)(d)(L)

MLMLMLMLML(M)(L)(M)(L)(M)(L)(M)

LMLMLMLML(M)(L)(M)(L)(M)(L)(M)(L)

FLFLFLFLFL(F)(L)(F)(L)(F)(L)(F)

LFLFLFLFL(F)(L)(F)(L)(F)(L)(F)(L)

Additional motifs are disclosed in PCT Publication No. WO/2007/112754, which is herein incorporated by reference in its entirety for the description of oligonucleotide modifications and patterns of oligonucleotide modifications, including but not limited to the description of LNA-containing oligonucleotides.

A modified oligonucleotide consisting of 6 to 12 linked nucleosides may comprise LNA modifications at certain positions. In certain embodiments, a modified oligonucleotide consisting of 6 to 12 linked nucleosides comprises a LNA modification at each nucleoside. In certain embodiments, at least 50% of the nucleosides of a modified oligonucleotide of 6 to 12 nucleosides in length comprise an LNA modification. In certain embodiments, at least 60% of the nucleosides of a modified oligonucleotide of 6 to 12 nucleosides in length comprise an LNA modification. In certain embodiments, at least 70% of the nucleosides of a modified oligonucleotide of 6 to 12 nucleosides in length comprise an LNA modification. In certain embodiments, at least 80% of the nucleosides of a modified oligonucleotide of 6 to 12 nucleosides in length comprise an LNA modification. In certain embodiments, at least 90% of the nucleosides of a modified oligonucleotide of 6 to 12 nucleosides in length comprise an LNA modification. In certain embodiments, a modified oligonucleotide complementary to the seed sequence of miR-21 comprises an LNA modification at each nucleoside of the seed match sequence. Additional motifs are described in PCT Publication No. WO 2009/043353, which is herein incorporated by reference in its entirety for the description of modified oligonucleotides complementary to microRNA, that are 6 to 12 nucleosides in length.

A modified oligonucleotide having a gapmer motif may have an internal region consisting of linked 2'-deoxynucleotides, and external regions consisting of linked 2'-modified nucleosides. Such a gapmer may be designed to elicit RNase H cleavage of a miRNA precursor. The internal 2'-deoxynucleoside region serves as a substrate for RNase H, allowing the cleavage of the miRNA precursor to which a modified oligonucleotide is targeted. In certain embodiments, each nucleoside of each external region comprises the same 2'-modified nucleoside. In certain embodiments, one external region is uniformly comprised of a first 2'-modified nucleoside and the other external region is uniformly comprised of a second 2'-modified nucleoside.

A modified oligonucleotide having a gapmer motif may have a sugar modification at each nucleoside. In certain embodiments, the internal region is uniformly comprised of a first

2'-modified nucleoside and each of the wings is uniformly comprised of a second 2'-modified nucleoside. In certain such embodiments, the internal region is uniformly comprised of 2'-fluoro nucleosides and each external region is uniformly comprised of 2'-O-methoxyethyl nucleosides.

In certain embodiments, each external region of a gapmer consists of linked
5 2'-O-methoxyethyl nucleosides. In certain embodiments, each external region of a gapmer consists of linked 2'-O-methyl nucleosides. In certain embodiments, each external region of a gapmer consists of 2'-fluoro nucleosides. In certain embodiments, each external region of a gapmer consists of linked bicyclic nucleosides.

In certain embodiments, each nucleoside of one external region of a gapmer comprises
10 2'-O-methoxyethyl nucleosides and each nucleoside of the other external region comprises a different 2'-modification. In certain such embodiments, each nucleoside of one external region of a gapmer comprises 2'-O-methoxyethyl nucleosides and each nucleoside of the other external region comprises 2'-O-methyl nucleosides. In certain such embodiments, each nucleoside of one external region of a gapmer comprises 2'-O-methoxyethyl nucleosides and each nucleoside of
15 the other external region comprises 2'-fluoro nucleosides. In certain such embodiments, each nucleoside of one external region of a gapmer comprises 2'-O-methyl nucleosides and each nucleoside of the other external region comprises 2'-fluoro nucleosides. In certain such embodiments, each nucleoside of one external region of a gapmer comprises 2'-O-methoxyethyl nucleosides and each nucleoside of the other external region comprises bicyclic nucleosides. In
20 certain such embodiments, each nucleoside of one external region of a gapmer comprises 2'-O-methyl nucleosides and each nucleoside of the other external region comprises bicyclic nucleosides.

In certain embodiments, nucleosides of one external region comprise two or more sugar modifications. In certain embodiments, nucleosides of each external region comprise two or
25 more sugar modifications. In certain embodiments, at least one nucleoside of an external region comprises a 2'-O-methoxyethyl sugar and at least one nucleoside of the same external region comprises a 2'-fluoro sugar. In certain embodiments, at least one nucleoside of an external region comprises a 2'-O-methoxyethyl sugar and at least one nucleoside of the same external region comprises a bicyclic sugar moiety. In certain embodiments, at least one nucleoside of an
30 external region comprises a 2'-O-methyl sugar and at least one nucleoside of the same external region comprises a bicyclic sugar moiety. In certain embodiments at least one nucleoside of an external region comprises a 2'-O-methyl sugar and at least one nucleoside of the same external region comprises a 2'-fluoro sugar. In certain embodiments, at least one nucleoside of an

external region comprises a 2'-fluoro sugar and at least one nucleoside of the same external region comprises a bicyclic sugar moiety.

In certain embodiments, each external region of a gapmer consists of the same number of linked nucleosides. In certain embodiments, one external region of a gapmer consists a
5 number of linked nucleosides different than that of the other external region.

In certain embodiments, the external regions comprise, independently, from 1 to 6 nucleosides. In certain embodiments, an external region comprises 1 nucleoside. In certain embodiments, an external region comprises 2 nucleosides. In certain embodiments, an external region comprises 3 nucleosides. In certain embodiments, an external region comprises 4
10 nucleosides. In certain embodiments, an external region comprises 5 nucleosides. In certain embodiments, an external region comprises 6 nucleosides. In certain embodiments, the internal region consists of 17 to 28 linked nucleosides. In certain embodiments, an internal region consists of 17 to 21 linked nucleosides. In certain embodiments, an internal region consists of 17 linked nucleosides. In certain embodiments, an internal region consists of 18 linked nucleosides.
15 In certain embodiments, an internal region consists of 19 linked nucleosides. In certain embodiments, an internal region consists of 20 linked nucleosides. In certain embodiments, an internal region consists of 21 linked nucleosides. In certain embodiments, an internal region consists of 22 linked nucleosides. In certain embodiments, an internal region consists of 23 linked nucleosides. In certain embodiments, an internal region consists of 24 linked nucleosides.
20 In certain embodiments, an internal region consists of 25 linked nucleosides. In certain embodiments, an internal region consists of 26 linked nucleosides. In certain embodiments, an internal region consists of 27 linked nucleosides. In certain embodiments, an internal region consists of 28 linked nucleosides.

Provided herein are pharmaceutical compositions comprising oligonucleotides. In
25 certain embodiments, such pharmaceutical compositions are used for the treatment of metabolic disorders, and associated conditions. In certain embodiments, a pharmaceutical composition provided herein comprises a compound comprising a modified oligonucleotide consisting of 8 to 30 linked nucleosides and having a nucleobase sequence complementary to miR-21, or a precursor thereof. In certain embodiments, a pharmaceutical composition provided herein
30 comprises a compound consisting of a modified oligonucleotide consisting of 8 to 30 linked nucleosides and having a nucleobase sequence complementary to miR-21, or a precursor thereof.

Suitable administration routes include, but are not limited to, oral, rectal, transmucosal, intestinal, enteral, topical, suppository, through inhalation, intrathecal, intraventricular, intraperitoneal, intranasal, intraocular, intratumoral, and parenteral (e.g., intravenous,

intramuscular, intramedullary, and subcutaneous). In certain embodiments, pharmaceutical intrathecal are administered to achieve local rather than systemic exposures. For example, pharmaceutical compositions may be injected directly in the area of desired effect (e.g., into the liver).

5 In certain embodiments, a pharmaceutical composition is administered in the form of a dosage unit (e.g., tablet, capsule, bolus, etc.). In certain embodiments, such pharmaceutical compositions comprise an oligonucleotide in a dose selected from 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 105 mg, 110 mg, 115 mg, 120 mg, 125 mg, 130 mg, 135 mg, 140 mg, 145 mg, 150 mg, 155 mg, 160
10 mg, 165 mg, 170 mg, 175 mg, 180 mg, 185 mg, 190 mg, 195 mg, 200 mg, 205 mg, 210 mg, 215 mg, 220 mg, 225 mg, 230 mg, 235 mg, 240 mg, 245 mg, 250 mg, 255 mg, 260 mg, 265 mg, 270 mg, 275 mg, 280 mg, 285 mg, 290 mg, 295 mg, 300 mg, 305 mg, 310 mg, 315 mg, 320 mg, 325 mg, 330 mg, 335 mg, 340 mg, 345 mg, 350 mg, 355 mg, 360 mg, 365 mg, 370 mg, 375 mg, 380 mg, 385 mg, 390 mg, 395 mg, 400 mg, 405 mg, 410 mg, 415 mg, 420 mg, 425 mg, 430 mg, 435
15 mg, 440 mg, 445 mg, 450 mg, 455 mg, 460 mg, 465 mg, 470 mg, 475 mg, 480 mg, 485 mg, 490 mg, 495 mg, 500 mg, 505 mg, 510 mg, 515 mg, 520 mg, 525 mg, 530 mg, 535 mg, 540 mg, 545 mg, 550 mg, 555 mg, 560 mg, 565 mg, 570 mg, 575 mg, 580 mg, 585 mg, 590 mg, 595 mg, 600 mg, 605 mg, 610 mg, 615 mg, 620 mg, 625 mg, 630 mg, 635 mg, 640 mg, 645 mg, 650 mg, 655 mg, 660 mg, 665 mg, 670 mg, 675 mg, 680 mg, 685 mg, 690 mg, 695 mg, 700 mg, 705 mg, 710
20 mg, 715 mg, 720 mg, 725 mg, 730 mg, 735 mg, 740 mg, 745 mg, 750 mg, 755 mg, 760 mg, 765 mg, 770 mg, 775 mg, 780 mg, 785 mg, 790 mg, 795 mg, and 800 mg. In certain such embodiments, a pharmaceutical composition of the comprises a dose of modified oligonucleotide selected from 25 mg, 50 mg, 75 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 500 mg, 600 mg, 700 mg, and 800 mg.

25 In certain embodiments, a pharmaceutical agent is sterile lyophilized modified oligonucleotide that is reconstituted with a suitable diluent, e.g., sterile water for injection or sterile saline for injection. The reconstituted product is administered as a subcutaneous injection or as an intravenous infusion after dilution into saline. The lyophilized drug product consists of an oligonucleotide which has been prepared in water for injection, or in saline for injection,
30 adjusted to pH 7.0-9.0 with acid or base during preparation, and then lyophilized. The lyophilized modified oligonucleotide may be 25-800 mg of an oligonucleotide. It is understood that this encompasses 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, and 800 mg of modified lyophilized oligonucleotide. The lyophilized drug product may be packaged in a 2 mL

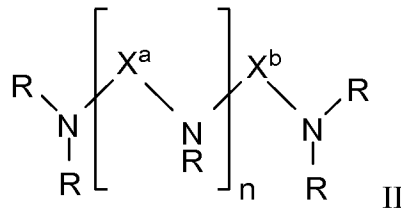
Type I, clear glass vial (ammonium sulfate-treated), stoppered with a bromobutyl rubber closure and sealed with an aluminum FLIP-OFF® overseal.

In certain embodiments, the pharmaceutical compositions provided herein may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the oligonucleotide(s) of the formulation.

Lipid moieties have been used in nucleic acid therapies in a variety of methods. In one method, the nucleic acid is introduced into preformed liposomes or lipoplexes made of mixtures of cationic lipids and neutral lipids. In another method, DNA complexes with mono- or polycationic lipids are formed without the presence of a neutral lipid. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to a particular cell or tissue. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to fat tissue. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to muscle tissue.

In certain embodiments, INTRALIPID® (30% i.v. Fat Emulsion) is used to prepare a pharmaceutical composition comprising an oligonucleotide. It is a fat emulsion prepared for intravenous administration. It is made up of 10% soybean oil, 1.2% egg yolk phospholipids, 2.25% glycerin, and water for injection. In addition, sodium hydroxide has been added to adjust the pH so that the final product pH range is 6 to 8.9.

In certain embodiments, a pharmaceutical composition provided herein comprises a polyamine compound or a lipid moiety complexed with a nucleic acid. In certain embodiments, such preparations comprise one or more compounds each individually having a structure defined by Formula II or a pharmaceutically acceptable salt thereof,

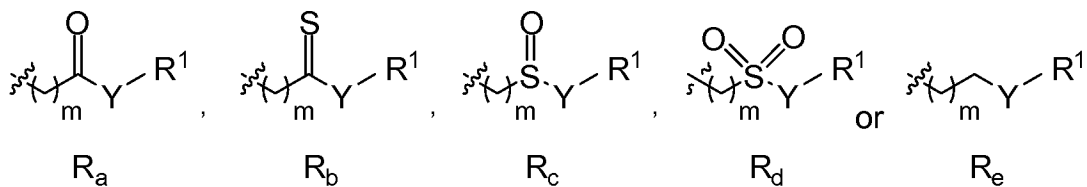


wherein:

each X^a and X^b, for each occurrence, is independently C₁₋₆ alkylene

n is 0, 1, 2, 3, 4, or 5;

5 each R is, independently H,



wherein at least $n + 2$ of the R moieties in at least about 80% of the molecules of the compound of Formula II in the preparation are not H;

m is 1, 2, 3 or 4;

10 Y is O, NR², or S;

R¹ is alkyl, alkenyl, or alkynyl, each of which is optionally substituted with one or more substituents; and

R² is H, alkyl, alkenyl, or alkynyl, each of which is optionally substituted with one or more substituents;

provided that, if $n = 0$, then at least $n + 3$ of the R moieties are not H. Such preparations are described in PCT publication WO/2008/042973, which is herein incorporated by reference in its entirety for the disclosure of lipid preparations. Certain additional preparations are described in Akinc et al., *Nature Biotechnology* 26, 561 - 569 (01 May 2008), which is herein incorporated by reference in its entirety for the disclosure of lipid preparations.

20 In certain embodiments, pharmaceutical compositions provided herein comprise one or more modified oligonucleotides and one or more excipients. In certain such embodiments, excipients are selected from water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylase, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose and polyvinylpyrrolidone.

25 In certain embodiments, a pharmaceutical composition provided herein is prepared using known techniques, including, but not limited to mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or tableting processes.

In certain embodiments, a pharmaceutical composition provided herein is a liquid (e.g., a suspension, elixir and/or solution). In certain of such embodiments, a liquid pharmaceutical composition is prepared using ingredients known in the art, including, but not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents.

5 In certain embodiments, a pharmaceutical composition provided herein is a solid (e.g., a powder, tablet, and/or capsule). In certain of such embodiments, a solid pharmaceutical composition comprising one or more oligonucleotides is prepared using ingredients known in the art, including, but not limited to, starches, sugars, diluents, granulating agents, lubricants, binders, and disintegrating agents.

10 In certain embodiments, a pharmaceutical composition provided herein is formulated as a depot preparation. Certain such depot preparations are typically longer acting than non-depot preparations. In certain embodiments, such preparations are administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. In certain embodiments, depot preparations are prepared using suitable polymeric or hydrophobic materials
15 (for example an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

In certain embodiments, a pharmaceutical composition provided herein comprises a delivery system. Examples of delivery systems include, but are not limited to, liposomes and emulsions. Certain delivery systems are useful for preparing certain pharmaceutical
20 compositions including those comprising hydrophobic compounds. In certain embodiments, certain organic solvents such as dimethylsulfoxide are used.

In certain embodiments, a pharmaceutical composition provided herein comprises one or more tissue-specific delivery molecules designed to deliver the one or more pharmaceutical agents of the present invention to specific tissues or cell types. For example, in certain
25 embodiments, pharmaceutical compositions include liposomes coated with a tissue-specific antibody.

In certain embodiments, a pharmaceutical composition provided herein comprises a co-solvent system. Certain of such co-solvent systems comprise, for example, benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. In certain
30 embodiments, such co-solvent systems are used for hydrophobic compounds. A non-limiting example of such a co-solvent system is the VPD co-solvent system, which is a solution of absolute ethanol comprising 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant POLYSORBATE 80™ and 65% w/v polyethylene glycol 300. The proportions of such

co-solvent systems may be varied considerably without significantly altering their solubility and toxicity characteristics. Furthermore, the identity of co-solvent components may be varied: for example, other surfactants may be used instead of POLYSORBATE 80™; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g., polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.

In certain embodiments, a pharmaceutical composition provided herein comprises a sustained-release system. A non-limiting example of such a sustained-release system is a semi-permeable matrix of solid hydrophobic polymers. In certain embodiments, sustained-release systems may, depending on their chemical nature, release pharmaceutical agents over a period of hours, days, weeks or months.

In certain embodiments, a pharmaceutical composition provided herein is prepared for oral administration. In certain of such embodiments, a pharmaceutical composition is formulated by combining one or more compounds comprising an oligonucleotide with one or more pharmaceutically acceptable carriers. Certain of such carriers enable pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject. In certain embodiments, pharmaceutical compositions for oral use are obtained by mixing oligonucleotide and one or more solid excipient. Suitable excipients include, but are not limited to, fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). In certain embodiments, such a mixture is optionally ground and auxiliaries are optionally added. In certain embodiments, pharmaceutical compositions are formed to obtain tablets or dragee cores. In certain embodiments, disintegrating agents (e.g., cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate) are added.

In certain embodiments, dragee cores are provided with coatings. In certain such embodiments, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to tablets or dragee coatings.

In certain embodiments, pharmaceutical compositions for oral administration are push-fit capsules made of gelatin. Certain of such push-fit capsules comprise one or more pharmaceutical agents of the present invention in admixture with one or more filler such as

lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In certain embodiments, pharmaceutical compositions for oral administration are soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. In certain soft capsules, one or more pharmaceutical agents of the present invention are
5 be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

In certain embodiments, pharmaceutical compositions are prepared for buccal administration. Certain of such pharmaceutical compositions are tablets or lozenges formulated in conventional manner.

10 In certain embodiments, a pharmaceutical composition is prepared for administration by injection (e.g., intravenous, subcutaneous, intramuscular, etc.). In certain of such embodiments, a pharmaceutical composition comprises a carrier and is formulated in aqueous solution, such as water or physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. In certain embodiments, other ingredients are included (e.g.,
15 ingredients that aid in solubility or serve as preservatives). In certain embodiments, injectable suspensions are prepared using appropriate liquid carriers, suspending agents and the like. Certain pharmaceutical compositions for injection are presented in unit dosage form, e.g., in ampoules or in multi-dose containers. Certain pharmaceutical compositions for injection are suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory
20 agents such as suspending, stabilizing and/or dispersing agents. Certain solvents suitable for use in pharmaceutical compositions for injection include, but are not limited to, lipophilic solvents and fatty oils, such as sesame oil, synthetic fatty acid esters, such as ethyl oleate or triglycerides, and liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, such
25 suspensions may also contain suitable stabilizers or agents that increase the solubility of the pharmaceutical agents to allow for the preparation of highly concentrated solutions.

In certain embodiments, a pharmaceutical composition is prepared for transmucosal administration. In certain of such embodiments penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

30 In certain embodiments, a pharmaceutical composition is prepared for administration by inhalation. Certain of such pharmaceutical compositions for inhalation are prepared in the form of an aerosol spray in a pressurized pack or a nebulizer. Certain of such pharmaceutical compositions comprise a propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In certain embodiments using a

pressurized aerosol, the dosage unit may be determined with a valve that delivers a metered amount. In certain embodiments, capsules and cartridges for use in an inhaler or insufflator may be formulated. Certain of such formulations comprise a powder mixture of a pharmaceutical agent of the invention and a suitable powder base such as lactose or starch.

5 In certain embodiments, a pharmaceutical composition is prepared for rectal administration, such as a suppositories or retention enema. Certain of such pharmaceutical compositions comprise known ingredients, such as cocoa butter and/or other glycerides.

In certain embodiments, a pharmaceutical composition is prepared for topical administration. Certain of such pharmaceutical compositions comprise bland moisturizing bases, 10 such as ointments or creams. Exemplary suitable ointment bases include, but are not limited to, petrolatum, petrolatum plus volatile silicones, and lanolin and water in oil emulsions. Exemplary suitable cream bases include, but are not limited to, cold cream and hydrophilic ointment.

In certain embodiments, a pharmaceutical composition provided herein comprises an oligonucleotide in a therapeutically effective amount. In certain embodiments, the 15 therapeutically effective amount is sufficient to prevent, alleviate or ameliorate symptoms of a disease or to prolong the survival of the subject being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art.

In certain embodiments, one or more modified oligonucleotides provided herein is formulated as a prodrug. In certain embodiments, upon *in vivo* administration, a prodrug is 20 chemically converted to the biologically, pharmaceutically or therapeutically more active form of an oligonucleotide. In certain embodiments, prodrugs are useful because they are easier to administer than the corresponding active form. For example, in certain instances, a prodrug may be more bioavailable (e.g., through oral administration) than is the corresponding active form. In certain instances, a prodrug may have improved solubility compared to the corresponding active 25 form. In certain embodiments, prodrugs are less water soluble than the corresponding active form. In certain instances, such prodrugs possess superior transmittal across cell membranes, where water solubility is detrimental to mobility. In certain embodiments, a prodrug is an ester. In certain such embodiments, the ester is metabolically hydrolyzed to carboxylic acid upon administration. In certain instances the carboxylic acid containing compound is the 30 corresponding active form. In certain embodiments, a prodrug comprises a short peptide (polyaminoacid) bound to an acid group. In certain of such embodiments, the peptide is cleaved upon administration to form the corresponding active form.

In certain embodiments, a prodrug is produced by modifying a pharmaceutically active compound such that the active compound will be regenerated upon *in vivo* administration. The

prodrug can be designed to alter the metabolic stability or the transport characteristics of a drug, to mask side effects or toxicity, to improve the flavor of a drug or to alter other characteristics or properties of a drug. By virtue of knowledge of pharmacodynamic processes and drug metabolism *in vivo*, those of skill in this art, once a pharmaceutically active compound is known, can design prodrugs of the compound (see, e.g., Nogrady (1985) Medicinal Chemistry A Biochemical Approach, Oxford University Press, New York, pages 388-392).

The present invention also provides kits. In some embodiments, the kits comprise one or more compounds of the invention comprising a modified oligonucleotide, wherein the nucleobase sequence of the oligonucleotide is complementary to miR-21. The compounds complementary to miR-21 can be any of the compounds described herein, and can have any of the modifications described herein. In some embodiments, the compounds complementary to miR-21 can be present within a vial. A plurality of vials, such as 10, can be present in, for example, dispensing packs. In some embodiments, the vial is manufactured so as to be accessible with a syringe. The kit can also contain instructions for using the compounds complementary to miR-21.

In some embodiments, the kits may be used for administration of the compound complementary to miR-21 to a subject. In such instances, in addition to compounds complementary to miR-21, the kit can further comprise one or more of the following: syringe, alcohol swab, cotton ball, and/or gauze pad. In some embodiments, the compounds complementary to miR-21 can be present in a pre-filled syringe (such as a single-dose syringes with, for example, a 27 gauge, ½ inch needle with a needle guard), rather than in a vial. A plurality of pre-filled syringes, such as 10, can be present in, for example, dispensing packs. The kit can also contain instructions for administering the compounds complementary to miR-21.

In certain embodiments, the present invention provides methods of using and/or testing modified oligonucleotides of the present invention in an experimental model. Those having skill in the art are able to select and modify the protocols for such experimental models to evaluate a pharmaceutical agent of the invention.

Generally, modified oligonucleotides are first tested in cultured cells. Suitable cell types include those that are related to the cell type to which delivery of an oligonucleotide is desired *in vivo*. For example, suitable cell types for the study of the methods described herein include primary or cultured cardiac fibroblasts and cardiomyocytes.

In certain embodiments, the extent to which an oligonucleotide interferes with the activity of a miRNA is assessed in cultured cells. In certain embodiments, inhibition of miRNA activity may be assessed by measuring the levels of the miRNA. Alternatively, the level of a

predicted or validated miRNA target may be measured. An inhibition of miRNA activity may result in the increase in the mRNA and/or protein of a miRNA target. Further, in certain embodiments, certain phenotypic outcomes may be measured.

Suitable experimental animal models for the testing of the methods described herein include but are not limited to a pressure overload-induced hypertrophy, an isoproterenol-induced cardiac hypertrophy model, an exercise-induced cardiac hypertrophy model, a high-salt diet-induced cardiac hypertrophy model, and a hormone-induced cardiac hypertrophy model.

Pressure Overload-Induced Cardiac Hypertrophy

A pressure overload-induced hypertrophy mouse model may be used as a model for human heart failure. In this model a reproducible cardiac stress response is achieved through transverse aortic constriction (TAC), also known as transverse aortic banding (TAB). This model highly resembles the failing human heart due to its matching pattern of both microRNA and mRNA changes in global expression profiles. Test compounds are administered following induction of the transortic banding. This model is used to test the ability of compounds to prevent or treat cardiac hypertrophy and fibrosis. Endpoints measured include heart weight, collagen content of the myocardium, interstitial fibrosis, and genes relevant to cardiac fibrosis. Cardiac function, including left ventricular end-diastolic diameters and fractional shortening, is assessed by echocardiography.

Isoproterenol-Induced Cardiac Hypertrophy

An isoproterenol-induced mouse model of cardiac hypertrophy may be used as a model for human heart failure. In this model, isoproterenol is continuously delivered via a surgically implanted osmotic pump. Isoproterenol is delivered a doses ranging from 30 to 40 mg/kg/day. Test compounds are administered following implantation of the osmotic pump. This model may be used to test the ability of compounds to prevent or treat cardiac hypertrophy and fibrosis. Endpoints measured include heart weight, collagen content of the myocardium, interstitial fibrosis, and genes relevant to cardiac fibrosis. Cardiac function, including left ventricular end-diastolic diameters and fractional shortening, is assessed by echocardiography. Fibrosis may be measured by in situ analysis, using a marker for collagen, such as Sirius red.

Certain Quantitation Assays

The effects of antisense inhibition of a miRNA following the administration of modified oligonucleotides may be assessed by a variety of methods known in the art. In certain

embodiments, these methods are be used to quantitate miRNA levels in cells or tissues *in vitro* or *in vivo*. In certain embodiments, changes in miRNA levels are measured by microarray analysis. In certain embodiments, changes in miRNA levels are measured by one of several commercially available PCR assays, such as the TAQMAN® MicroRNA Assay (Applied Biosystems). In certain embodiments, antisense inhibition of a miRNA is assessed by measuring the mRNA and/or protein level of a target of a miRNA. Antisense inhibition of a miRNA generally results in the increase in the level of mRNA and/or protein of a target of the miRNA.

Target Engagement Assay

Modulation of microRNA activity with an anti-miR or microRNA mimic may be assessed by measuring target engagement. In certain embodiments, target engagement is measured by microarray profiling of mRNAs. The sequences of the mRNAs that are modulated (either increased or decreased) by the anti-miR or microRNA mimic are searched for microRNA seed sequences, to compare modulation of mRNAs that are targets of the miRNA to modulation of mRNAs that are not targets of the miRNA. In this manner, the interaction of the anti-miR with a miRNA, or a miRNA mimic with its targets, can be evaluated. In the case of an anti-miR, mRNAs whose expression levels are increased are screened for the mRNA sequences that comprise a seed match to the miRNA to which the anti-miR is complementary.

The following examples are presented in order to more fully illustrate some embodiments of the invention. They should, in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLES

Example 1: Delivery of anti-miRs to heart tissue

To evaluate methods of anti-miR delivery, anti-miR without cholesterol-conjugate was measured in heart and liver following intravenous delivery (via tail vein) or intrajugular delivery.

Eight-week old male C57BL/6 mice were purchased from the Jackson Laboratory. Anti-miR-21-1, modified with 2'-fluoro and 2'-MOE modifications as described herein, was administered to a first group of mice via tail vein injection, and to a second group of mice via intra-jugular injection. Anti-miR-21-1 was administered at a dose of 20 mg/kg, daily, for three days. 24 hours following the third and final dose, animals were sacrificed, and tissues were collected for analysis. Anti-miR was measured by mass spectrometry.

Intravenous injection delivered anti-miR to both heart and liver at amounts comparable to intrajugular injection (see Table 9).

Table 9

Tissue	Route of administration	Tissue Concentration (µg/g)
Heart	Intra-jugular	14
Heart	Intravenous	12
Liver	Intrajugular	172
Liver	Intravenous	236

5 An additional study was performed, to evaluate the relationship between tissue concentration and dose of anti-miR. Eight-week old male C57BL/6 mice were purchased from the Jackson Laboratory. Mice were administered one of three doses: 9 mg/kg, 27 mg/kg, or 80 mg/kg, intravenously (via tail vein injection), once daily for three days. 24 hours following the third and final dose, mice were sacrificed and tissues were collected. Anti-miR-21-1 was
10 efficiently delivered to heart in a dose-dependent manner (Table 10).

Table 10

Treatment Group	Dose of anti-miR-21-1	Heart tissue Concentration µg/g
Group 1	9 mg/kg	25
Group 2	27 mg/kg	23
Group 3	80 mg/kg	10

Accordingly, it is demonstrated herein that intravenous injection of an anti-miR without a cholesterol moiety delivers anti-miR to heart and liver tissue as efficiently as intra-jugular
15 injection of anti-miR. Furthermore, anti-miR without cholesterol conjugate is delivered to the heart in a dose-dependent manner.

Example 2: Inhibition of miR-21 in model of human cardiac disease

Isoproterenol-induced cardiac hypertrophy in mice is used to model human cardiac
20 disease. Isoproterenol induces heart weight increases and plasma MMP-9 increases, among other phenotypes. Accordingly, modified oligonucleotides complementary to miR-21 were evaluated in a mouse model of isoproterenol-induced cardiac hypertrophy.

Eight-week old male C57BL/6 mice were purchased from the Jackson Laboratory. Under anesthesia, osmotic mini-pumps were implanted into the infrascapular subcutaneous tissue, for delivery of a continuous infusion of isoproterenol at a dose of 30 µg/g body weight/day, for the duration of the study. The day of pump implantation was considered Day 0 of the study. Anti-miR-21-1, having the nucleobase sequence of SEQ ID NO: 3 and comprising 2'-MOE and 2'-fluoro modifications as described herein, was injected on Days 1, 2, and 3 of the study, at a dose of 20 mg/kg or 60 mg/kg. Control groups were (1) animals with isoproterenol pumps injected with saline and (2) normal mice, without isoproterenol pumps, injected with saline. A summary of the treatment groups is shown in the following table (Table 11). Each treatment group contained 10 to 12 mice.

Table 11

Treatment Group	Osmotic Pump	Injections on Days 1, 2, and 3
Group 1	None	Saline
Group 2	Isoproterenol	Saline
Group 3	Isoproterenol	F/MOE anti-miR-21-1, 20 mg/kg
Group 4	Isoproterenol	F/MOE anti-miR-21-1, 60 mg/kg

On Day 10 of the study, animals were sacrificed and hearts were isolated. Serum was also isolated.

Heart weight was recorded, and normalized to body weight. A comparison of anti-miR-21 treated mice to saline-treated mice revealed a statistically significant, dose-dependent reduction in heart weight in animals treated with anti-miR-21-1.

An additional study was performed in the model of isoproterenol-induced cardiac hypertrophy, and included the treatment groups as detailed in the following table (Table 12).

Table 12

Treatment Group	Osmotic Pump	Injections on Days 1, 2, and 3
Group 1	None	Saline
Group 2	Isoproterenol	Control anti-miR
Group 3	Isoproterenol	F/MOE anti-miR-21-1, 80 mg/kg

Treatment of mice with anti-miR-21-1 reduced the heart weight/body weight ratio in a statistically significant manner, compared to either the saline or control anti-miR treatment groups.

These results demonstrate that inhibition of miR-21 in the heart can be achieved using systemically administered miR-21 that is not cholesterol-conjugated, and further that this inhibition results in beneficial effects in a model of human cardiac hypertrophy.

5 *Example 3: Inhibition of miR-21 in a pressure overload-induced cardiac hypertrophy*

Pressure overload-induced cardiac hypertrophy in mice (Buitrago et al., Nature Medicine 11, 837-844 (2005)) is used to model human cardiac disease. In this model a reproducible cardiac stress response is achieved through transverse aortic constriction (TAC), which causes heart enlargement and fibrosis, among other phenotypes. Accordingly, modified
10 oligonucleotides complementary to miR-21 were evaluated in a mouse model of pressure overload-induced cardiac hypertrophy.

The oligonucleotides tested included (1) the F/MOE modified anti-miR-21-1 having the nucleobase sequence of SEQ ID NO: 3 and described herein, (2) anti-miR-21-2, having the nucleobase sequence of SEQ ID NO: 3, where each nucleoside is a 2'-O-methyl nucleoside, the
15 first two internucleoside linkages at the 5' end are phosphorothioate, the last four internucleoside linkages at the 3' end are phosphorothioate, all other linkages are phosphodiester, and a cholesterol moiety is conjugated at the 3' terminus; and (3) an oligonucleotide 8 nucleobases in length, consisting of the nucleobase sequence GAUAAGCU, where each nucleoside comprises a bicyclic sugar moiety bridged between the 2' and 4' carbon atoms with a -O-CH₂- biradical
20 group (an LNA sugar moiety). Aortic constriction was performed on Day 0 of the study, and oligonucleotides were injected intravenously on Day 1 and Day 2 of the study. Doses of 10 mg/kg or 80 mg/kg were administered. A saline-treated group served as a control group. Tissue harvesting and echocardiography was performed on Day 21 of the study.

Treatment with either the cholesterol-conjugated anti-miR-21-2 or F/MOE anti-miR-21-
25 1 was able to attenuate the increase in interstitial fibrosis and cardiac mass observed in the control mice subjected to aortic constriction. These two oligonucleotides also prevented a decline in cardiac function, as determined by echocardiography.

In contrast, the 8-nucleobase oligonucleotide having the sequence GAUAAGCU was not effective in attenuating the increased fibrosis, increased cardiac mass or decline in cardiac
30 function. In a separate *in vitro* study, cells were treated with 4 nM or 100 nM of the 8-nucleobase oligonucleotide, and the regulation of transcripts containing miR-21 seed matches was measured. It was demonstrated that the 8-nucleobase oligonucleotide was able to regulate transcripts containing miR-21 seed matches at the higher dose of 100 nM, but not at the lower dose of 4 nM. Thus, the 8-nucleobase oligonucleotide complementary to the seed region of miR-21 (at

nucleobases 2-9) is able to modulate the activity of miR-21 *in vitro*, this shorter oligonucleotide has lower potency *in vivo*, relative to the longer oligonucleotides complementary to miR-21.

These results further demonstrate, in an additional model of cardiac disease, that inhibition of miR-21 in the heart can be achieved using systemically administered miR-21 that is not cholesterol-conjugated, and further that this inhibition results in beneficial effects in a model of human cardiac hypertrophy.

Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method comprising administering to a subject having a cardiac disease a compound comprising a modified oligonucleotide consisting of 8 to 25 linked nucleosides, wherein the nucleobase sequence of the modified oligonucleotide is complementary to the nucleobase sequence of miR-21, wherein the modified oligonucleotide is not conjugated to a cholesterol moiety.
2. A method of preventing or delaying the onset of a cardiac disease comprising administering to a subject having a cardiac disease a compound comprising a modified oligonucleotide consisting of 8 to 25 linked nucleosides, wherein the nucleobase sequence of the modified oligonucleotide is complementary to the nucleobase sequence of miR-21, wherein the modified oligonucleotide is not conjugated to a cholesterol moiety, thereby preventing or delaying the onset of the cardiac disease.
3. A method of treating a cardiac disease comprising administering to a subject having a cardiac disease a compound comprising a modified oligonucleotide consisting of 8 to 25 linked nucleosides, wherein the nucleobase sequence of the modified oligonucleotide is complementary to the nucleobase sequence of miR-21, wherein the modified oligonucleotide is not conjugated to a cholesterol moiety, thereby treating the cardiac disease.
4. The method of any of claims 1 to 3 comprising selecting a subject having a cardiac disease.
5. The method any of claims 1 to 4, wherein the cardiac disease is cardiac fibrosis, cardiac enlargement, heart failure, post-myocardial infarction remodeling, cardiomyopathy, or myocardial infarction.
6. The method of claim 5, wherein the administering delays the transition from cardiac enlargement to cardiac failure.
7. The method of claim 5 or claim 6, wherein the cardiac enlargement is cardiac hypertrophy or cardiac dilation.

8. The method of claim 7, wherein the cardiac hypertrophy is hypertrophic cardiomyopathy.

9. The method of any one of claims 1-8 comprising improving one or more symptoms of cardiac disease.

10. The method of any one of claims 1-9 wherein the administering increases exercise tolerance, increases cardiac ejection volume, increases cardiac output, reduces ventricular end diastolic blood pressure, increases ventricular compliance, and/or reduces cardiac fibrosis.

11. The method of any one of claims 1-10 comprising selecting a subject having elevated serum troponin levels and/or elevated serum natriuretic peptide levels.

12. The method of any one of claims 1-11 comprising administering at least one additional therapeutic agent selected from an ACE inhibitor, an aldosterone antagonist, an angiotensin II receptor blocker, a calcium channel blocker, a sodium channel blocker, an antiarrhythmic agent, an antihypertensive agent, an alpha blocker, a beta blocker, and a diuretic.

13. A method comprising contacting a cardiac cell with a compound comprising a modified oligonucleotide consisting of 8 to 25 linked nucleosides, wherein the nucleobase sequence of the modified oligonucleotide is complementary to the nucleobase sequence of miR-21, provided that the modified oligonucleotide is not conjugated to a cholesterol moiety.

14. The method of claim 13 wherein the cardiac cell is a cardiac fibroblast or a cardiac myocyte, wherein the cardiac cell is optionally *in vivo*.

15. The method of any one of claims 1-14, wherein the modified oligonucleotide is not an antagomir.

16. The method of any one of claims 1-15, wherein the compound consists of the modified oligonucleotide.

17. The method of any one of claims 1-16, wherein the nucleobase sequence of the modified oligonucleotide is fully complementary to a nucleobase sequence selected from SEQ ID NO: 1 and 2.
- 5 18. The method of any one of claims 1-17, wherein the nucleobase sequence of the modified oligonucleotide consists of a nucleobase sequence selected from SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, GAUAAGCUA, AUAAGCUA, UAAGCUA, and GAUAAGCU.
- 10 19. The method of any one of claims 1-18, wherein the modified oligonucleotide consists of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 linked nucleosides.
20. The method of any one of claims 1-19, wherein each internucleoside linkage of the modified oligonucleotide is a modified internucleoside linkage.
- 15 21. The method of any one of claims 1-20, wherein each nucleoside of the modified oligonucleotide comprises a modified sugar.
22. The method of claim 21 wherein each modified sugar is independently selected from a 20 2'-O-methoxyethyl sugar, a 2'-fluoro sugar, 2'-O-methyl sugar, and a bicyclic sugar moiety.
23. The method of claim 22 wherein the bicyclic sugar moiety is bridged between the 2' and 4' carbon atoms with a -O-CH₂- biradical group.
- 25 24. The method of any one of claims 1 to 16 wherein:
the compound consists of the modified oligonucleotide;
the modified oligonucleotide has the entire nucleobase sequence of SEQ ID NO: 3;
each internucleoside linkage of the modified oligonucleotide is a phosphorothioate
internucleoside linkage;
30 nucleosides 1, 2, 12, 13, 14, 21, and 22 are 2'-O-methoxyethyl nucleosides;
nucleosides 3, 4, 5, 6, 7, 8, 9, 10, 11, 15, 16, 17, 18, 19, and 20 are 2'-fluoro
nucleosides; and
nucleosides 13 and 21 comprise 5-methylcytosines.

25. The method of any one of claims 1-24, wherein the administering comprises peripheral intravenous administration or intracardiac administration.