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(54) **ANTISENSE MODULATION OF PTP1B EXPRESSION**

(71) Applicant: **Ionis Pharmaceuticals, Inc.**, Carlsbad, CA (US)

(72) Inventors: **Sanjay Bhanot**, Carlsbad, CA (US); **Susan M. Freier**, San Diego, CA (US)

(73) Assignee: **Ionis Pharmaceuticals, Inc.**, Carlsbad, CA (US)

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(58) **Field of Classification Search**

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See application file for complete search history.

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Primary Examiner — Sharon Turner

(74) *Attorney, Agent, or Firm* — Knobbe, Martens, Olson & Bear, LLP

(57) **ABSTRACT**

Provided herein are methods, compounds, and compositions for reducing expression of PTP1B mRNA and protein in an animal. Such methods, compounds, and compositions are useful to treat, prevent, delay, or ameliorate metabolic disease, for example, diabetes, or a symptom thereof.

37 Claims, 3 Drawing Sheets

Specification includes a Sequence Listing.

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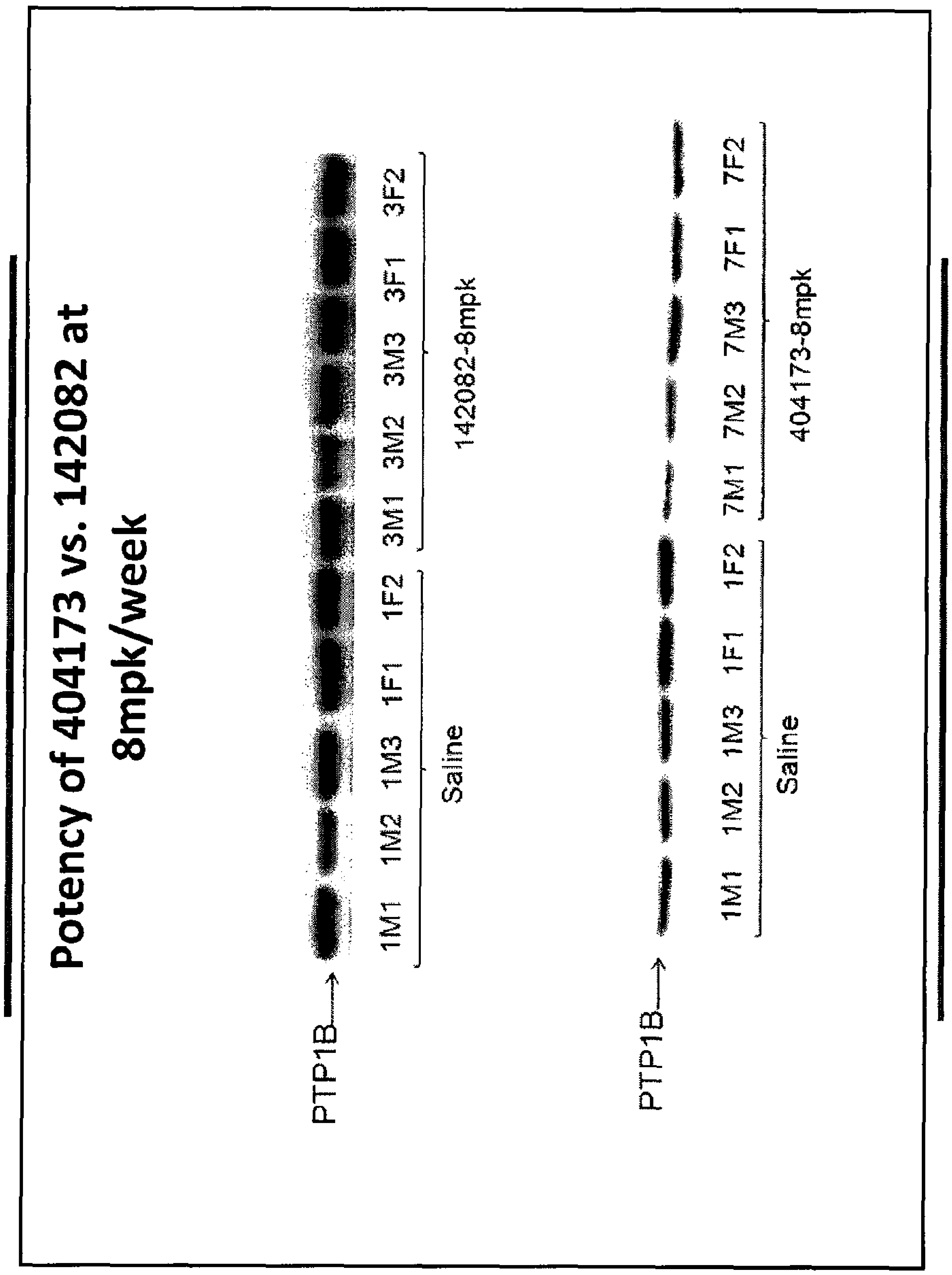
Potency of 404173 vs. 142082 at 8mpk/week
PTP1B
1M1 1M2 1M3 1F1 1F2 3M1 3M2 3M3 3F1 3F2
Saline
142082-8mpk
PTP1B
1M1 1M2 1M3 1F1 1F2 7M1 7M2 7M3 7F1 7F2
Saline
404173-8mpk

Figure 1

Comparison of Key Tolerability Parameters for 409826, 404173 and 142082

40 mpk/week			Fold over Saline		
ISIS #	Chemistry	Walk Position	Spleen Wt	Kidney Wt	Liver Wt
409826	5-10-5	4518	2.5	1.4	1.7
142082	5-10-5	4522	3.0	2.1	1.7
404173	5-10-5	4521	1.6	1.5	1.5

40 mpk/week	Fold Change vs Baseline			
ISIS #	C3	CRP	MCP1	IL-1
Saline	-0.05	0.23	-0.29	-0.44
409826	-0.30	3.90	2.31	2.37
142082	-0.16	14.52	5.36	1.64
404173	-0.18	-0.48	0.82	0.09

* 1 animal very high at day 93; otherwise fold-increase was ~3-4 fold

Figure 2

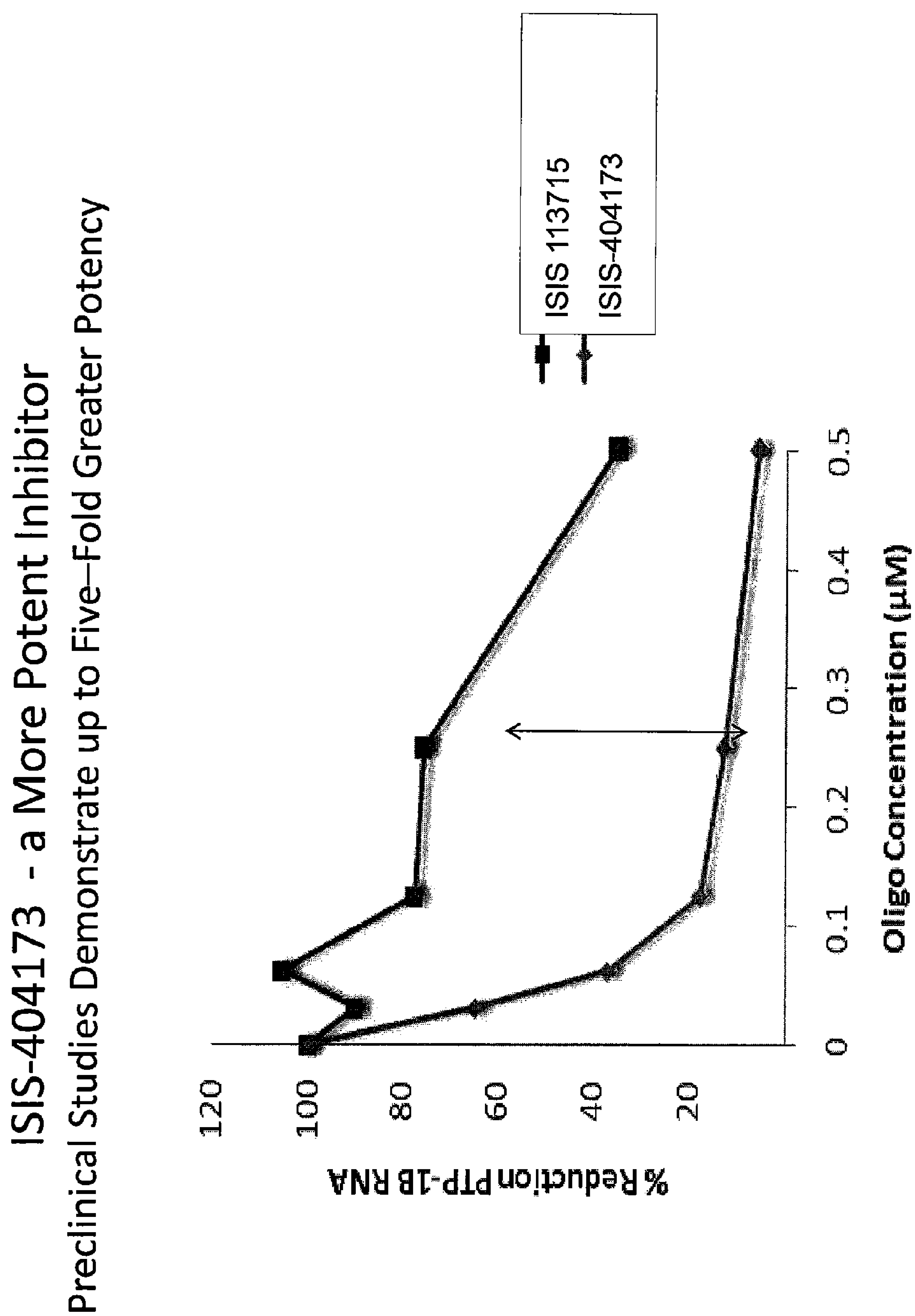
ISIS-404173 - a More Potent Inhibitor
Preclinical Studies Demonstrate up to Five–Fold Greater Potency
% Reduction PTP-1B RNA
120
100
80
60
40
20
0
0.1
0.2
0.3
0.4
0.5
Oligo Concentration (µM)
ISIS 113715
ISIS-404173

Figure 3

ANTISENSE MODULATION OF PTP1B EXPRESSION

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue; a claim printed with strikethrough indicates that the claim was canceled, disclaimed, or held invalid by a prior post-patent action or proceeding.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 13/446,763, filed Apr. 13, 2012, which claims priority under 35 U.S.C. §119(e) to U.S. Provisional Application No. 61/474,981, filed Apr. 13, 2011, each of which are herein incorporated by reference in their entirety.

SEQUENCE LISTING

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0149USD1SEQ.txt created Jan. 10, 2014, which is 112 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD

Provided herein are methods, compounds, and compositions for reducing expression of PTP1B mRNA and protein in an animal. Such methods, compounds, and compositions are useful, for example, to treat, prevent, delay or ameliorate diseases associated with metabolic disorders, particularly disorders associated with diabetes.

BACKGROUND

Protein tyrosine phosphatase 1B (PTP1B) is a member of a family of PTPs (Barford, et al., Science 1994. 263: 1397-1404) and is a cytosolic enzyme (Neel and Tonks, Curr. Opin. Cell Biol. 1997. 9: 193-204). PTP1B is expressed ubiquitously including tissues that are key regulators of insulin metabolism such as liver, muscle and fat (Goldstein, Receptor 1993. 3: 1-15), where it is the main PTP enzyme.

PTP1B is considered to be a negative regulator of insulin signaling. PTP1B interacts with and dephosphorylates the insulin receptor, thus attenuating and potentially terminating the insulin signaling transduction (Goldstein et al., J. Biol. Chem. 2000. 275: 4383-4389). The physiological role of PTP1B in insulin signaling has been demonstrated in knockout mice models. Mice lacking the PTP1B gene were protected against insulin resistance and obesity (Elchebly et al., Science 1999. 283: 1544-1548). PTP1B-deficient mice had low adiposity, increased basal metabolic rate as well as total energy expenditure and were protected from diet-induced obesity. Insulin-stimulated glucose uptake was elevated in skeletal muscle, whereas adipose tissue was unaffected providing evidence that increased insulin sensitivity in PTP1B-deficient mice was tissue-specific (Klaman et al., Mol. Cell. Biol. 2000. 20: 5479-5489). These mice were phenotypically normal and were also resistant to diet-induced obesity, insulin resistance and had significantly lower triglyceride levels on a high-fat diet. Therefore, inhibition of PTP1B in patients suffering from Type II diabetes, metabolic syndrome, diabetic dyslipidemia, or related metabolic diseases would be beneficial.

Antisense inhibition of PTP1B provides a unique advantage over traditional small molecule inhibitors in that antisense inhibitors do not rely on competitive binding of the compound to the protein and inhibit activity directly by reducing the expression of PTP1B. Antisense technology is emerging as an effective means for reducing the expression of certain gene products and may therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of PTP1B.

There is a currently a lack of acceptable options for treating metabolic disorders. It is therefore an object herein to provide compounds and methods for the treatment of such diseases and disorder.

All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, and treatises, are hereby expressly incorporated-by-reference for the portions of the document discussed herein, as well as in their entirety.

SUMMARY

Provided herein are methods, compounds, and compositions for modulating expression of PTP1B and treating, preventing, delaying or ameliorating diseases associated with metabolic disorders, particularly disorders associated with diabetes and/or a symptom thereof.

BRIEF DESCRIPTION OF THE FIGURES

The numerous objects and advantages of the present invention can be better understood by those skilled in the art by reference to the accompanying figures, in which:

FIG. **1** shows a western blot of PTP1B antisense oligonucleotides, ISIS 404173 and ISIS 142082, decreasing PTP1B protein expression at 8 mgk/week demonstrating potency of the compounds. See Table 47.

FIG. **2** is a summary table of key tolerability studies in cynomolgus monkeys (see Example 17).

FIG. **3** is a graphical representation reduction of human PTP1B mRNA in a dose response preclinical study. Treatment with ISIS 404173 was compared with that of ISIS 113715, the previous clinical candidate. As shown here, dosing with ISIS 404173 was more potent and caused significant reduction in PTP1B mRNA levels compared to dosing with ISIS 113715. Particularly, at 0.3 μM dose, there was a five-fold decrease in PTP1B mRNA levels with ISIS 404173 compared to ISIS 113715.

DETAILED DESCRIPTION

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including" as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, and treatises, are hereby expressly incorporated-by-reference for the portions of the document discussed herein, as well as in their entirety.

Definitions

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. Standard techniques can be used for chemical synthesis, and chemical analysis. Where permitted, all documents, or portions of documents, cited in this application, including, but not limited to, all patents, applications, published applications and other journal publications, GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout in the disclosure herein are incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

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Unless otherwise indicated, the following terms have the following meanings:

"2'-O-methoxyethyl" (also 2'-MOE and 2'-O$(CH_2)_2$—OCH_3) refers to an O-methoxy-ethyl modification of the 2' position of a furosyl ring. A 2'-O-methoxyethyl modified sugar is a modified sugar.

"2'-O-methoxyethyl nucleotide" means a nucleotide comprising a 2'-O-methoxyethyl modified sugar moiety.

"3' target site" refers to the nucleotide of a target nucleic acid which is complementary to the 3'-most nucleotide of a particular antisense compound.

"5' target site" refers to the nucleotide of a target nucleic acid which is complementary to the 5'-most nucleotide of a particular antisense compound.

"5-methylcytosine" means a cytosine modified with a methyl group attached to the 5' position. A 5-methylcytosine is a modified nucleobase.

"About" means within ±10% of a value. For example, if it is stated, "a marker may be increased by about 50%", it is implied that the marker may be increased between 45%-55%.

"Active pharmaceutical agent" means the substance or substances in a pharmaceutical composition that provide a therapeutic benefit when administered to an individual. For example, in certain embodiments an antisense oligonucleotide targeted to PTP1B is an active pharmaceutical agent.

"Active target region" or "target region" means a region to which one or more active antisense compounds is targeted. "Active antisense compounds" means antisense compounds that reduce target nucleic acid levels or protein levels.

"Adipogenesis" means the development of fat cells from preadipocytes. "Lipogenesis" means the production or formation of fat, either fatty degeneration or fatty infiltration.

"Adiposity" or "Obesity" refers to the state of being obese or an excessively high amount of body fat or adipose tissue in relation to lean body mass. The amount of body fat includes concern for both the distribution of fat throughout the body and the size and mass of the adipose tissue deposits. Body fat distribution can be estimated by skin-fold measures, waist-to-hip circumference ratios, or techniques such as ultrasound, computed tomography, or magnetic resonance imaging. According to the Center for Disease Control and Prevention, individuals with a body mass index (BMI) of 30 or more are considered obese. The term "Obesity" as used herein includes conditions where there is an increase in body fat beyond the physical requirement as a result of excess accumulation of adipose tissue in the body. The term "obesity" includes, but is not limited to, the following conditions: adult-onset obesity; alimentary obesity; endogenous or inflammatory obesity; endocrine obesity; familial obesity; hyperinsulinar obesity; hyperplastic-hypertrophic obesity; hypogonadal obesity; hypothyroid obesity; lifelong obesity; morbid obesity and exogenous obesity.

"Administered concomitantly" refers to the co-administration of 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 effects of both agents need not manifest themselves at the same time. The effects need only be overlapping for a period of time and need not be coextensive.

"Administering" means providing an agent to an animal, and includes, but is not limited to, administering by a medical professional and self-administering.

"Agent" means an active substance that can provide a therapeutic benefit when administered to an animal. "First Agent" means a therapeutic compound provided herein. For example, a first agent can be an antisense oligonucleotide targeting PTP1B. "Second agent" means a second therapeutic compound of the invention (e.g. a second antisense oligonucleotide targeting PTP1B) and/or a non-PTP1B therapeutic compound.

"Amelioration" refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. The severity of indicators can be determined by subjective or objective measures, which are known to those skilled in the art.

"Animal" refers to a human or non-human animal, including, but not limited to, mice, rats, rabbits, dogs, cats, pigs, and non-human primates, including, but not limited to, monkeys and chimpanzees.

"Antisense activity" means any detectable or measurable activity attributable to the hybridization of an antisense compound to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid.

"Antisense compound" means an oligomeric compound that is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

"Antisense inhibition" means reduction of target nucleic acid levels or target protein levels in the presence of an antisense compound complementary to a target nucleic acid compared to target nucleic acid levels or target protein levels in the absence of the antisense compound.

"Antisense oligonucleotide" means a single-stranded oligonucleotide having a nucleobase sequence that permits hybridization to a corresponding region or segment of a target nucleic acid.

"Bicyclic sugar" means a furosyl ring modified by the bridging of two non-geminal ring atoms. A bicyclic sugar is a modified sugar.

"Bicyclic nucleic acid" or "BNA" refers to a nucleoside or nucleotide wherein the furanose portion of the nucleoside or nucleotide includes a bridge connecting two carbon atoms on the furanose ring, thereby forming a bicyclic ring system.

"Cap structure" or "terminal cap moiety" means chemical modifications, which have been incorporated at either terminus of an antisense compound.

"Chemically distinct region" refers to a region of an antisense compound that is in some way chemically different than another region of the same antisense compound. For example, a region having 2'-O-methoxyethyl nucleotides is chemically distinct from a region having nucleotides without 2'-O-methoxyethyl modifications.

"Chimeric antisense compound" means an antisense compound that has at least two chemically distinct regions.

"Co-administration" means administration of two or more agents to an individual. The two or more agents can be in a single pharmaceutical composition, or can be in separate pharmaceutical compositions. Each of the two or more agents can be administered through the same or different routes of administration. Co-administration encompasses parallel or sequential administration.

"Cholesterol" is a sterol molecule found in the cell membranes of all animal tissues. Cholesterol must be transported in an animal's blood plasma by lipoproteins including very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). "Plasma cholesterol" refers to the sum of all lipoproteins (VDL, IDL, LDL, HDL) esterified and/or non-esterified cholesterol present in the plasma or serum.

"Cholesterol absorption inhibitor" means an agent that inhibits the absorption of exogenous cholesterol obtained from diet.

"Complementarity" means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

"Contiguous nucleobases" means nucleobases immediately adjacent to each other.

"Deoxyribonucleotide" means a nucleotide having a hydrogen at the 2' position of the sugar portion of the nucleotide. Deoxyribonucleotides may be modified with any of a variety of substituents.

"Diabetes mellitus" or "diabetes" is a syndrome characterized by disordered metabolism and abnormally high blood sugar (hyperglycemia) resulting from insufficient levels of insulin or reduced insulin sensitivity. The characteristic symptoms are excessive urine production (polyuria) due to high blood glucose levels, excessive thirst and increased fluid intake (polydipsia) attempting to compensate for increased urination, blurred vision due to high blood glucose effects on the eye's optics, unexplained weight loss, and lethargy.

"Diabetic dyslipidemia" or "type 2 diabetes with dyslipidemia" means a condition characterized by Type 2 diabetes, reduced HDL-C, elevated triglycerides, and elevated small, dense LDL particles.

"Diluent" means an ingredient in a composition that lacks pharmacological activity, but is pharmaceutically necessary or desirable. For example, the diluent in an injected composition can be a liquid, e.g. saline solution.

"Dyslipidemia" refers to a disorder of lipid and/or lipoprotein metabolism, including lipid and/or lipoprotein overproduction or deficiency. Dyslipidemias may be manifested by elevation of lipids such as cholesterol and triglycerides as well as lipoproteins such as low-density lipoprotein (LDL) cholesterol.

"Dosage unit" means a form in which a pharmaceutical agent is provided, e.g. pill, tablet, or other dosage unit known in the art. In certain embodiments, a dosage unit is a vial containing lyophilized antisense oligonucleotide. In certain embodiments, a dosage unit is a vial containing reconstituted antisense oligonucleotide.

"Dose" means a specified quantity of a pharmaceutical agent provided in a single administration, or in a specified time period. In certain embodiments, a dose can be administered in one, 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, therefore, two or more injections can be used to achieve the desired dose. In certain embodiments, the pharmaceutical agent is administered by infusion over an extended period of time or continuously. Doses can be stated as the amount of pharmaceutical agent per hour, day, week, or month.

"Effective amount" or "therapeutically effective amount" means the amount of active pharmaceutical agent sufficient to effectuate a desired physiological outcome in an individual in need of the agent. The effective amount can vary among individuals depending on the health and physical condition of the individual to be treated, the taxonomic group of the individuals to be treated, the formulation of the composition, assessment of the individual's medical condition, and other relevant factors.

"Fully complementary" or "100% complementary" means each nucleobase of a nucleobase sequence of a first nucleic acid has a complementary nucleobase in a second nucleobase sequence of a second nucleic acid. In certain embodiments, a first nucleic acid is an antisense compound and a target nucleic acid is a second nucleic acid.

"Gapmer" means a chimeric antisense compound in which an internal region having a plurality of nucleosides that support RNase H cleavage is positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region can be referred to as a "gap segment" and the external regions can be referred to as "wing segments."

"Gap-widened" means a chimeric antisense compound having a gap segment of 12 or more contiguous 2'-deoxyribonucleosides positioned between and immediately adjacent to 5' and 3' wing segments having from one to six nucleosides.

"Glucose" is a monosaccharide used by cells as a source of energy and inflammatory intermediate. "Plasma glucose" refers to glucose present in the plasma.

"HMG-CoA reductase inhibitor" means an agent that acts through the inhibition of the enzyme HMG-CoA reductase, such as atorvastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin.

"Hybridization" means the annealing of complementary nucleic acid molecules. In certain embodiments, complementary nucleic acid molecules include an antisense compound and a target nucleic acid.

"Hyperlipidemia" or "hyperlipemia" is a condition characterized by elevated serum lipids or circulating (plasma) lipids. This condition manifests an abnormally high concentration of fats. The lipid fractions in the circulating blood are cholesterol, low density lipoproteins, very low density lipoproteins and triglycerides.

"Hypertriglyceridemia" means a condition characterized by elevated triglyceride levels.

"Identifying" or "selecting an animal with metabolic" means identifying or selecting a subject having been diagnosed with a metabolic disease, or a metabolic disorder; or, identifying or selecting a subject having any symptom of a metabolic disease, including, but not limited to, metabolic syndrome, hyperglycemia, hypertriglyceridemia, hypertension increased insulin resistance, decreased insulin sensitivity, above normal body weight, and/or above normal body fat or any combination thereof. Such identification may be accomplished by any method, including but not limited to, standard clinical tests or assessments, such as measuring serum or circulating (plasma) blood-glucose, measuring serum or circulating (plasma) triglycerides, measuring blood-pressure, measuring body fat, measuring body weight, and the like.

"Immediately adjacent" means there are no intervening elements between the immediately adjacent elements.

"Individual" or "subject" or "animal" means a human or non-human animal selected for treatment or therapy.

"Inhibiting the expression or activity" refers to a reduction or blockade of the expression or activity of a RNA or protein and does not necessarily indicate a total elimination of expression or activity.

"Insulin resistance" is defined as the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells. Insulin resistance in fat cells results in hydrolysis of stored triglycerides, which elevates free fatty acids in the blood plasma. Insulin resistance in muscle reduces glucose uptake whereas insulin resistance in liver reduces glucose storage, with both effects serving to elevate blood glucose. High plasma levels of insulin and glucose due to insulin resistance often leads to metabolic syndrome and type 2 diabetes.

"Insulin sensitivity" is a measure of how effectively an individual processes glucose. An individual having high insulin sensitivity effectively processes glucose whereas an individual with low insulin sensitivity does not effectively process glucose.

"Internucleoside linkage" refers to the chemical bond between nucleosides.

"Intravenous administration" means administration into a vein.

"Linked nucleosides" means adjacent nucleosides which are bonded together.

"Lipid-lowering therapy" or "lipid lowering agent" means a therapeutic regimen provided to a subject to reduce one or more lipids in a subject. In certain embodiments, a lipid-lowering therapy is provided to reduce one or more of ApoB, total cholesterol, LDL-C, VLDL-C, IDL-C, non-HDL-C, triglycerides, small dense LDL particles, and Lp(a) in a subject. Examples of lipid-lowering therapy include statins, fibrates, and MTP inhibitors.

"Major risk factors" refers to factors that contribute to a high risk for a particular disease or condition. In certain embodiments, major risk factors for coronary heart disease include, without limitation, cigarette smoking, hypertension, low HDL-C, family history of coronary heart disease, age, and other factors disclosed herein.

"Metabolic disease" or "metabolic disorder" refers to a condition characterized by an alteration or disturbance in metabolic function. "Metabolic" and "metabolism" are terms well known in the art and generally include the whole range of biochemical processes that occur within a living organism. Metabolic diseases or disorders include, but are not limited to, obesity, diabetes, hyperglycemia, prediabetes, non-alcoholic fatty liver disease (NAFLD), metabolic syndrome, insulin resistance, diabetic dyslipidemia, or hypertriglyceridemia or a combination thereof.

"Metabolic syndrome" means a condition characterized by a clustering of lipid and non-lipid cardiovascular risk factors of metabolic origin. In certain embodiments, metabolic syndrome is identified by the presence of any 3 of the following factors: waist circumference of greater than 102 cm in men or greater than 88 cm in women; serum triglyceride of at least 150 mg/dL; HDL-C less than 40 mg/dL in men or less than 50 mg/dL in women; blood pressure of at least 130/85 mmHg; and fasting glucose of at least 110 mg/dL. These determinants can be readily measured in clinical practice (JAMA, 2001, 285: 2486-2497).

"Mismatch" or "non-complementary nucleobase" refers to the case when a nucleobase of a first nucleic acid is not capable of pairing with the corresponding nucleobase of a second or target nucleic acid.

"Mixed dyslipidemia" means a condition characterized by elevated cholesterol and elevated triglycerides.

"Modified internucleoside linkage" refers to a substitution or any change from a naturally occurring internucleoside bond (i.e. a phosphodiester internucleoside bond).

"Modified nucleobase" refers to any nucleobase other than adenine, cytosine, guanine, thymidine, or uracil. An "unmodified nucleobase" means the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U).

"Modified nucleoside" means a nucleoside having, independently, a modified sugar moiety or modified nucleobase.

"Modified nucleotide" means a nucleotide having, independently, a modified sugar moiety, modified internucleoside linkage, or modified nucleobase. A "modified nucleoside" means a nucleoside having, independently, a modified sugar moiety or modified nucleobase.

"Modified oligonucleotide" means an oligonucleotide comprising at least one modified nucleotide.

"Modified sugar" refers to a substitution or change from a natural sugar.

"Motif" means the pattern of chemically distinct regions in an antisense compound.

"MTP inhibitor" means an agent inhibits the enzyme, microsomal triglyceride transfer protein.

"Naturally occurring internucleoside linkage" means a 3' to 5' phosphodiester linkage.

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

"Non-alcoholic fatty liver disease" or "NAFLD" means a condition characterized by fatty inflammation of the liver that is not due to excessive alcohol use (for example, alcohol consumption of over 20 g/day). In certain embodiments, NAFLD is related to insulin resistance and the metabolic syndrome. NAFLD encompasses a disease spectrum ranging from simple triglyceride accumulation in hepatocytes (hepatic steatosis) to hepatic steatosis with inflammation (steatohepatitis), fibrosis, and cirrhosis.

"Nonalcoholic steatohepatitis" (NASH) occurs from progression of NAFLD beyond deposition of triglycerides. A "second hit" capable of inducing necrosis, inflammation, and fibrosis is required for development of NASH. Candidates for the second-hit can be grouped into broad categories: factors causing an increase in oxidative stress and factors promoting expression of proinflammatory cytokines

"Nucleic acid" refers to molecules composed of monomeric nucleotides. A nucleic acid includes ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, double-stranded nucleic acids, small interfering ribonucleic acids (siRNA), and microRNAs (miRNA). A nucleic acid can also comprise a combination of these elements in a single molecule.

"Nucleobase" means a heterocyclic moiety capable of pairing with a base of another nucleic acid.

"Nucleobase sequence" means the order of contiguous nucleobases independent of any sugar, linkage, or nucleobase modification.

"Nucleoside" means a nucleobase linked to a sugar.

"Nucleoside mimetic" includes those structures used to replace the sugar or the sugar and the base and not necessarily the linkage at one or more positions of an oligomeric compound such as for example nucleoside mimetics having morpholino, cyclohexenyl, cyclohexyl, tetrahydropyranyl, bicyclo or tricyclo sugar mimetics e.g. non furanose sugar units.

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

"Nucleotide mimetic" includes those structures used to replace the nucleoside and the linkage at one or more positions of an oligomeric compound such as for example peptide nucleic acids or morpholinos (morpholinos linked by —N(H)—C(═O)—O— or other non-phosphodiester linkage).

"Oligomeric compound" or "oligomer" refers to a polymeric structure comprising two or more sub-structures and capable of hybridizing to a region of a nucleic acid molecule. In certain embodiments, oligomeric compounds are oligonucleosides. In certain embodiments, oligomeric compounds are oligonucleotides. In certain embodiments, oligomeric compounds are antisense compounds. In certain embodiments, oligomeric compounds are antisense oligonucleotides. In certain embodiments, oligomeric compounds are chimeric oligonucleotides.

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

"Parenteral administration" means administration through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g. intrathecal or intracerebroventricular administration. Administration can be continuous, or chronic, or short or intermittent.

"Peptide" means a molecule formed by linking at least two amino acids by amide bonds. Peptide refers to polypeptides and proteins.

"Pharmaceutical agent" means a substance that provides a therapeutic benefit when administered to an individual. For example, in certain embodiments, an antisense oligonucleotide targeted to PTP1B is pharmaceutical agent.

"Pharmaceutical composition" means a mixture of substances suitable for administering to an individual. For example, a pharmaceutical composition can comprise one or more active agents and a sterile aqueous solution.

"Pharmaceutically acceptable carrier" means a medium or diluent that does not interfere with the structure of the oligonucleotide. Certain, of such carries enable pharmaceutical compositions to be formulated as, for example, tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspension and lozenges for the oral ingestion by a subject. For example, a pharmaceutically acceptable carrier can be a sterile aqueous solution.

"Pharmaceutically acceptable derivative" encompasses pharmaceutically acceptable salts, conjugates, prodrugs or isomers of the compounds described herein.

"Pharmaceutically acceptable salts" means physiologically and pharmaceutically acceptable salts of antisense compounds, i.e., salts that retain the desired biological activity of the parent oligonucleotide and do not impart undesired toxicological effects thereto.

"Phosphorothioate linkage" means a linkage between nucleosides where the phosphodiester bond is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom. A phosphorothioate linkage is a modified internucleoside linkage.

"Portion" means a defined number of contiguous (i.e. linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an antisense compound.

"Prevent" refers to delaying or forestalling the onset or development of a disease, disorder, or condition for a period of time from minutes to indefinitely. Prevent also means reducing risk of developing a disease, disorder, or condition.

"Prodrug" means a therapeutic agent that is prepared in an inactive form that is converted to an active form within the body or cells thereof by the action of endogenous enzymes or other chemicals or conditions.

"Protein tyrosine phosphatase 1B" or "PTP1B" (also known as PTPN1; protein tyrosine phosphatase, non-receptor type 1; PTP-1B; RKPTP) means any nucleic acid or protein of PTP1B.

"PTP1B expression" means the level of mRNA transcribed from the gene encoding PTP1B or the level of protein translated from the mRNA. PTP1B expression can be determined by art known methods such as a Northern or Western blot.

"PTP1B nucleic acid" means any nucleic acid encoding PTP1B. For example, in certain embodiments, a PTP1B nucleic acid includes a DNA sequence encoding PTP1B, a RNA sequence transcribed from DNA encoding PTP1B (including genomic DNA comprising introns and exons), and a mRNA sequence encoding PTP1B. "PTP1B mRNA" means a mRNA encoding a PTP1B protein.

"Side effects" means physiological responses attributable to a treatment other than the desired effects. In certain embodiments, side effects include injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, myopathies, and malaise. For example, increased aminotransferase levels in serum can indicate liver toxicity or liver function abnormality. For example, increased bilirubin can indicate liver toxicity or liver function abnormality.

"Single-stranded oligonucleotide" means an oligonucleotide which is not hybridized to a complementary strand.

"Specifically hybridizable" refers to an antisense compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, i.e. under physiological conditions in the case of in vivo assays and therapeutic treatments.

"Statin" means an agent that inhibits the activity of HMG-CoA reductase.

"Subcutaneous administration" means administration just below the skin.

"Targeting" or "targeted" means the process of design and selection of an antisense compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

"Target nucleic acid," "target RNA," and "target RNA transcript" all refer to a nucleic acid capable of being targeted by antisense compounds.

"Target segment" means the sequence of nucleotides of a target nucleic acid to which an antisense compound is targeted. "5' target site" refers to the 5'-most nucleotide of a target segment. "3' target site" refers to the 3'-most nucleotide of a target segment.

"Therapeutically effective amount" means an amount of an agent that provides a therapeutic benefit to an individual.

"Therapeutic lifestyle change" means dietary and lifestyle changes intended to lower fat/adipose tissue mass and/or cholesterol. Such change can reduce the risk of developing heart disease, and may includes recommendations for dietary intake of total daily calories, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, carbohydrate, protein, cholesterol, insoluble fiber, as well as recommendations for physical activity.

"Triglyceride" or "TG" means a lipid or neutral fat consisting of glycerol combined with three fatty acid molecules.

"Type 2 diabetes," (also known as "type 2 diabetes mellitus" or "diabetes mellitus, type 2", and formerly called "diabetes mellitus type 2", "non-insulin-dependent diabetes (NIDDM)", "obesity related diabetes", or "adult-onset diabetes") is a metabolic disorder that is primarily characterized by insulin resistance, relative insulin deficiency, and hyperglycemia.

"Treat" refers to administering a pharmaceutical composition to an animal to effect an alteration or improvement of a disease, disorder, or condition.

"Unmodified nucleotide" means a nucleotide composed of naturally occurring nucleobases, sugar moieties, and internucleoside linkages. In certain embodiments, an unmodified nucleotide is an RNA nucleotide (i.e. β-D-ribonucleosides) or a DNA nucleotide (i.e. β-D-deoxyribonucleoside).

Certain Embodiments

Certain embodiments provide methods, compounds, and compositions for inhibiting PTP1B expression.

Certain embodiments provide antisense compounds targeted to a PTP1B nucleic acid. In certain embodiments, the PTP1B nucleic acid is any of the sequences set forth in GENBANK Accession No. NM_002827.2 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NT_011362.9 truncated from nucleotides 14178000 to 14256000 (incorporated herein as SEQ ID NO: 2); and a concatenation of sequences from exons 1-9, intron 9 and exon 10 of the rhesus monkey PTP1B scaffold (incorporated herein as SEQ ID NO: 3).

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 10 to 30 nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein can consist of 10 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 4-32 or 100-111.

In certain embodiments, the compounds or compositions provided herein can consist of 10 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 26 or 44.

In certain embodiments, the compounds or compositions provided herein can consist of 10 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of ISIS NOs: 404173, 410002, 438373, 438383, 438445, 438454, 438463, or 438472.

In certain embodiments, the compounds or compositions provided herein can consist of 10 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of ISIS NO: 404173

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 15 to 30 nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 15 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein can consist of 15 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 4-32 or 100-111.

In certain embodiments, the compounds or compositions provided herein can consist of 15 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 26 or 44.

In certain embodiments, the compounds or compositions provided herein can consist of 15 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of ISIS NOs: 404173, 410002, 438373, 438383, 438445, 438454, 438463, or 438472.

In certain embodiments, the compounds or compositions provided herein can consist of 15 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of ISIS NO: 404173

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 18 to 21 nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 18 to 21 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein consist of 18 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of any of SEQ ID NOs: 4-32 or 39-49.

In certain embodiments, the compounds or compositions provided herein consist of 18 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of any of SEQ ID NOs: 26 or 44.

In certain embodiments, the compounds or compositions provided herein consist of 18 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of any of ISIS NOs: 404173, 410002, 438373, 438383, 438445, 438454, 438463, or 438472.

In certain embodiments, the compounds or compositions provided herein consist of 18 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of ISIS NO: 404173.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 35 nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 35 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 35 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 4-32 or 50.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 35 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NO: 26.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 35 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of any of ISIS NOs: 404173, 410002, 438373, 438383, 438445, 438454, 438463, or 438472.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 35 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of ISIS NO: 404173.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 30 nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 4-32 or 50.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NO: 26.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of any of ISIS NOs: 404173, 410002, 438383, 438445, 438454, 438463, or 438472,

In certain embodiments, the compounds or compositions provided herein consist of 20 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of ISIS NO: 404173.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 25 nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 25 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 25 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NOs: 4-32.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 25 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NO: 26.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 25 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of any of ISIS NOs: 404173, 410002, 438383, 438445, 438454, 438463, or 438472,

In certain embodiments, the compounds or compositions provided herein consist of 20 to 25 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of ISIS NO: 404173.

In certain embodiments, the compounds or compositions of the invention comprise a modified oligonucleotide consisting of 20 to 24 nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 24 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 24 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NOs: 4-32.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 24 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NO: 26.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 24 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of any of ISIS NOs: 404173, 410002, 438373, 438383, 438445, 438454, 438463, or 438472.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 24 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of ISIS NO: 404173

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 23 nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 23 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 23 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NOs: 4-32.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 23 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NO: 26.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 23 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of any of ISIS NOs: 404173, 410002, 438373, 438383, 438445, 438454, 438463, or 438472.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 23 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of ISIS NO: 404173.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 22 nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 22 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 22 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NOs: 4-32.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 22 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NO: 26.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 22 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of any of ISIS NOs: 404173, 410002, 438373, 438383, 438445, 438454, 438463, or 438472.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 22 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of ISIS NO: 404173.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 21 nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 to 21 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NOs: 4-32.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NO: 26.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of any of ISIS NOs: 404173, 410002, 438373, 438383, 438445, 438454, 438463, or 438472.

In certain embodiments, the compounds or compositions provided herein consist of 20 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of ISIS NO: 404173.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3.

In certain embodiments, the compounds or compositions provided herein consist of 20 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NOs: 4-32.

In certain embodiments, the compounds or compositions provided herein consist of 20 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or contiguous nucleobases of SEQ ID NO: 26.

In certain embodiments, the compounds or compositions provided herein consist of any of ISIS NOs: 404173, 410002, 438373, 438383, 438445, 438454, 438463, or 438472.

In certain embodiments, the compounds or compositions provided herein consist of ISIS NO: 404173.

In certain embodiments, the compounds or compositions provided herein consist of SEQ ID NO: 26.

In certain embodiments, the compounds or compositions provided herein comprise a salt of the modified oligonucleotide.

In certain embodiments, the compounds or compositions provided herein further comprise a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complementary to any one of SEQ ID NOs: 1-3 as measured over the entirety of the modified oligonucleotide.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of SEQ ID NO: 4-32 or 39-50. as measured over the entirety of the modified oligonucleotide.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of SEQ ID NO: 26 or 44. as measured over the entirety of the modified oligonucleotide.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one ISIS NOs: 404173, 410002, 438373, 438383, 438445, 438454, 438463, or 438472 as measured over the entirety of the modified oligonucleotide.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to ISIS NO: 404173 as measured over the entirety of the modified oligonucleotide.

In certain embodiments, the compound provided herein consists of a single-stranded modified oligonucleotide.

In certain embodiments, the modified oligonucleotide consists of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 18 linked nucleosides.

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

In certain embodiments, at least one nucleoside of said modified oligonucleotide comprises a modified nucleobase. In certain embodiments, the modified nucleobase is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide comprises: a) a gap segment consisting of linked deoxynucleosides; b) a 5' wing segment consisting of linked nucleosides; and c) a 3' wing segment consisting of linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment and each nucleoside of each wing segment comprises a modified sugar.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of eight linked deoxynucleosides, the 5' wing segment consisting of six linked nucleosides, the 3' wing segment consisting of six linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of thirteen linked deoxynucleosides, the 5' wing segment consisting of two linked nucleosides, the 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 18 linked nucleosides, the gap segment consisting of eight linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 18 linked nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1-3, wherein the modified oligonucleotide comprises: a) a gap segment consisting of eight linked deoxynucleosides; b) a 5' wing segment consisting of six linked nucleosides; and c) a 3' wing segment consisting of six linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 19 contiguous nucleobases of SEQ ID NOs: 4-32, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 19 contiguous nucleobases of SEQ ID NO: 26, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having the nucleobase sequence of SEQ ID NO: 26, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 19 contiguous nucleobases of SEQ ID NO: 26 or 44, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine. In certain embodiments, the compound or composition comprises the compound of any of ISIS NOs: 404173, 410002, 438383, 438445, 438454, 438463, or 438472.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 20 contiguous nucleobases of SEQ ID NOs: 4-32, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 20 contiguous nucleobases of SEQ ID NO: 26, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 20 contiguous nucleobases of SEQ ID NO: 26, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine. In certain embodiments, the compound or composition comprises the compound ISIS NOs: 404173.

Certain embodiments provide methods, compounds, and compositions for inhibiting PTP1B expression.

Certain embodiments provide a method of reducing PTP1B expression in an animal comprising administering to the animal a compound as described herein. In certain embodiments, the compound comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to PTP1B. In certain embodiments, the compound comprises a modified oligonucleotide 20 to 35 linked nucleosides in length targeted to PTP1B. In certain embodiments, the compound comprises a modified oligonucleotide 20 to 25 linked nucleosides in length targeted to PTP1B. In certain embodiments, the compound comprises a modified oligonucleotide 20 to 24 linked nucleosides in length targeted to PTP1B. In certain embodiments, the compound comprises a modified oligonucleotide 20 to 23 linked nucleosides in length targeted to PTP1B. In certain embodiments, the compound comprises a modified oligonucleotide 20 to 22 linked nucleosides in length targeted to PTP1B. In certain embodiments, the compound comprises a modified oligonucleotide 20 to 21 linked nucleosides in length targeted to PTP1B. In certain embodiments, the compound comprises a modified oligonucleotide 20 linked nucleosides in length targeted to PTP1B.

Certain embodiments provide a method of preventing, ameliorating or treating an metabolic disease in an animal comprising administering to the animal a compound as described herein. In certain embodiments, the compound comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to PTP1B. In certain embodiments, the compound comprises a modified oligonucleotide 20 linked nucleosides in length targeted to PTP1B. Examples of metabolic diseases or disorders include, but are not limited to obesity, diabetes, hyperglycemia, prediabetes, non-alcoholic fatty liver disease (NAFLD), metabolic syndrome, insulin resistance, diabetic dyslipidemia, or hypertriglyceridemia or a combination thereof.

Certain embodiments provide a method of reducing glucose levels in an animal comprising administering to the animal a compound as described herein. In certain embodiments, the compound comprises a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to PTP1B. In certain embodiments, the compound comprises a modified oligonucleotide 20 linked nucleosides in length targeted to PTP1B. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats a metabolic disease. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats diabetes. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats obesity. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats metabolic syndrome. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats insulin resistance. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats hyperglycemia. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats NAFLD. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats diabetic dyslipidemia. In certain embodiments, the glucose level is reduced by at least 5%, 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

In certain embodiments, PTP1B has the sequence as set forth in any of the GenBank Accession Numbers GENBANK Accession No. NM_002827.2 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NT_011362.9 truncated from nucleotides 14178000 to 14256000 (incorporated herein as SEQ ID NO: 2); and a concatenation of sequences from exons 1-9, intron 9 and exon 10 of the rhesus monkey PTP1B scaffold (incorporated herein as SEQ ID NO: 3). In certain embodiments, PTP1B has the human sequence as set forth in SEQ ID NOs: 1-2. In certain embodiments, PTP1B has the rhesus monkey sequence as set forth in SEQ ID NOs: 3).

In certain embodiments, the compounds or compositions provided herein comprise a salt thereof, and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NOs: 4-32, 50 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 to 25 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NOs: 4-32, 50 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 4-32, 50 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the compounds or compositions provided herein comprise a salt thereof, and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 26 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 to 25 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 26 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 26 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the compounds or compositions of the invention comprise a salt thereof, and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in ISIS NOs: 404173, 410002, 438383, 438445, 438454, 438463, or 438472 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 to 25 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in ISIS NOs: 404173, 410002, 438383, 438445, 438454, 438463, or 438472 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in ISIS NOs: 404173, 410002, 438383, 438445, 438454, 438463, or 438472 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the compounds or compositions of the invention comprise a salt thereof, and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in ISIS NO: 404173 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 to 25 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in ISIS NO: 404173 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in ISIS NO: 404173 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

Certain embodiments provide a method for treating an animal with a PTP1B related disease or condition comprising: a) identifying said animal with the PTP1B related disease or condition, and b) administering to said animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence at least 90% complementary to any of SEQ ID NOs: 1-3 as measured over the entirety of said modified oligonucleotide. In certain embodiments, the therapeutically effective amount of the compound administered to the animal treats or reduces the PTP1B related disease or condition, or a symptom thereof, in the animal. In certain embodiments, the PTP1B related disease or condition is diabetes.

Certain embodiments provide a method for treating an animal with a PTP1B related disease or condition comprising: a) identifying said animal with the PTP1B related disease or condition, and b) administering to said animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence at least 100% complementary to any of SEQ ID NOs: 1-3 as measured over the entirety of said modified oligonucleotide. In certain embodiments, the therapeutically effective amount of the compound administered to the animal treats or reduces the PTP1B related disease or condition, or a symptom thereof, in the animal. In certain embodiments, the PTP1B related disease or condition is diabetes.

Certain embodiments provide methods of treating, preventing, or ameliorating a metabolic disease. In certain embodiments the metabolic disease is obesity, diabetes, hyperglycemia, prediabetes, non-alcoholic fatty liver disease (NAFLD), metabolic syndrome, insulin resistance, diabetic dyslipidemia, or hypertriglyceridemia or a combination thereof.

Certain embodiments provide methods of treating, preventing, or ameliorating a hyperproliferative disorder.

Certain embodiments provide methods comprising administering to an animal a compound as described herein to an animal. In certain embodiments, the method comprises administering to an animal a modified oligonucleotide consisting of 20 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NOs: 4-32 or 50 Certain embodiments provide methods comprising administering to an animal a compound as described herein to an animal. In certain embodiments, the method comprises administering to an animal a modified oligonucleotide consisting of 20 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 26.

Certain embodiments provide methods comprising administering to an animal a compound as described herein to an animal. In certain embodiments, the method comprises administering to an animal a modified oligonucleotide consisting of 20 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in ISIS NOs: 404173, 410002, 438383, 438445, 438454, 438463, or 438472.

Certain embodiments provide methods comprising administering to an animal a compound as described herein to an animal. In certain embodiments, the method comprises administering to an animal a modified oligonucleotide consisting of 20 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in ISIS NO: 404173.

In certain embodiments, the animal is a human.

In certain embodiments, the administering prevents, treats, ameliorates, or slows progression of a metabolic disease as described herein.

In certain embodiments, the administering prevents, treats, ameliorates, or slows progression of diabetes as described herein.

In certain embodiments, the compound is co-administered with a second agent.

In certain embodiments, the compound and the second agent are administered concomitantly.

In certain embodiments, the administering is parenteral administration.

Certain embodiments further provide a method to reduce PTP1B mRNA or protein expression in an animal comprising administering to the animal a compound or composition as described herein to reduce PTP1B mRNA or protein expression in the animal. In certain embodiments, the animal is a human. In certain embodiments, reducing PTP1B mRNA or protein expression prevents, treats, ameliorates, or slows progression of metabolic disease. In certain embodiments, the metabolic disease or condition is diabetes.

Certain embodiments provide a method for treating a human with a metabolic disease comprising identifying the human with the disease and administering to the human a therapeutically effective amount of a compound or composition as described herein. In certain embodiments, the treatment reduces a symptom selected from the group consisting of metabolic syndrome, hyperglycemia, hypertriglyceridemia, hypertension, increased glucose levels, increased insulin resistance, decreased insulin sensitivity, above normal body weight, and/or above normal body fat or any combination thereof.

Certain embodiments provide a method for treating a human with diabetes comprising identifying the human with the disease and administering to the human a therapeutically effective amount of a compound or composition as described herein. In certain embodiments, the treatment reduces a symptom selected from the group consisting of metabolic syndrome, hyperglycemia, hypertriglyceridemia, hypertension, increased glucose levels, increased insulin resistance, decreased insulin sensitivity, above normal body weight, and/or above normal body fat or any combination thereof.

Further provided is a method for reducing or preventing metabolic disease comprising administering to a human a therapeutically effective amount compound or composition as described herein, thereby reducing or preventing metabolic disease.

Further provided is a method for reducing or preventing diabetes comprising administering to a human a therapeutically effective amount compound or composition as described herein, thereby reducing or preventing diabetes.

Further provided is a method for ameliorating a symptom of metabolic disease, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 20 to 35 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1, 2, or 3, thereby ameliorating a symptom of metabolic disease in the human.

Further provided is a method for ameliorating a symptom of diabetes, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1, 2, or 3, thereby ameliorating a symptom of diabetes in the human.

Further provided is a method for ameliorating a symptom of diabetes, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1, 2, or 3, thereby ameliorating a symptom of diabetes in the human.

Further provided is a method for reducing the rate of progression of a symptom associated with metabolic disease, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1, 2, or 3, thereby reducing the rate of progression a symptom of metabolic disease in the human.

Further provided is a method for reducing the rate of progression of a symptom associated with diabetes, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 20 to 35 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1, 2, or 3, thereby reducing the rate of progression a symptom of diabetes in the human.

Further provided is a method for reducing the rate of progression of a symptom associated with diabetes, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1, 2, or 3, thereby reducing the rate of progression a symptom of diabetes in the human.

Also provided are methods and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of metabolic disease.

Also provided are methods and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of diabetes.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, ameliorating, or preventing metabolic disease.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, ameliorating, or preventing diabetes.

Certain embodiments provide a compound as described herein for use in treating, preventing, or ameliorating metabolic disease as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Certain embodiments provide a compound as described herein for use in treating, preventing, or ameliorating diabetes as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating metabolic disease as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating diabetes as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating metabolic disease as described herein in a patient who is subsequently administered an additional agent or therapy as described herein.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating diabetes as described herein in a patient who is subsequently administered an additional agent or therapy as described herein.

Certain embodiments provide a kit for treating, preventing, or ameliorating metabolic disease as described herein wherein the kit comprises:

(i) a compound as described herein; and alternatively

(ii) an additional agent or therapy as described herein.

Certain embodiments provide a kit for treating, preventing, or ameliorating diabetes as described herein wherein the kit comprises:

(i) a compound as described herein; and alternatively

(ii) an additional agent or therapy as described herein.

A kit as described herein may further include instructions for using the kit to treat, prevent, or ameliorate metabolic disease as described herein by combination therapy as described herein. In certain embodiments, the metabolic disease is diabetes.

Antisense Compounds

Oligomeric compounds include, but are not limited to, oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics, antisense compounds, antisense oligonucleotides, and siRNAs. An oligomeric compound may be "antisense" to a target nucleic acid, meaning that is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

In certain embodiments, an antisense compound has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted. In certain such embodiments, an antisense oligonucleotide has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted.

In certain embodiments, an antisense compound targeted to a PTP1B nucleic acid is 10 to 30 nucleotides in length. In other words, antisense compounds are from 10 to 30 linked nucleobases. In other embodiments, the antisense compound comprises a modified oligonucleotide consisting of 8 to 80, 10 to 50, 15 to 30, 18 to 21, 20 to 80, 20 to 35, 20 to 30, 20 to 29, 20 to 28, 20 to 27, 20 to 26, 20 to 25, 20 to 24, 20 to 23, 20 to 22, 20 to 21 or 20 linked nucleobases. In certain such embodiments, the antisense compound comprises a modified oligonucleotide consisting of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked nucleobases in length, or a range defined by any two of the above values.

In certain embodiments, the antisense compound comprises a shortened or truncated modified oligonucleotide. The shortened or truncated modified oligonucleotide can have a single nucleoside deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated oligonucleotide may have two nucleosides deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end. Alternatively, the deleted nucleosides may be dispersed throughout the modified oligonucleotide, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

When a single additional nucleoside is present in a lengthened oligonucleotide, the additional nucleoside may be located at the 5' or 3' end of the oligonucleotide. When two or more additional nucleosides are present, the added nucleosides may be adjacent to each other, for example, in an oligonucleotide having two nucleosides added to the 5' end (5' addition), or alternatively to the 3' end (3' addition), of the oligonucleotide. Alternatively, the added nucleoside may be dispersed throughout the antisense compound, for example, in an oligonucleotide having one nucleoside added to the 5' end and one subunit added to the 3' end.

It is possible to increase or decrease the length of an antisense compound, such as an antisense oligonucleotide, and/or introduce mismatch bases without eliminating activity. For example, in Woolf et al. (Proc. Natl. Acad. Sci. USA 89:7305-7309, 1992), a series of antisense oligonucleotides 13-25 nucleobases in length were tested for their ability to induce cleavage of a target RNA in an oocyte injection model. Antisense oligonucleotides 25 nucleobases in length with 8 or 11 mismatch bases near the ends of the antisense oligonucleotides were able to direct specific cleavage of the target mRNA, albeit to a lesser extent than the antisense oligonucleotides that contained no mismatches. Similarly, target specific cleavage was achieved using 13 nucleobase antisense oligonucleotides, including those with 1 or 3 mismatches.

Gautschi et al (J. Natl. Cancer Inst. 93:463-471, March 2001) demonstrated the ability of an oligonucleotide having 100% complementarity to the bcl-2 mRNA and having 3 mismatches to the bcl-xL mRNA to reduce the expression of both bcl-2 and bcl-xL in vitro and in vivo. Furthermore, this oligonucleotide demonstrated potent anti-tumor activity in vivo.

Maher and Dolnick (Nuc. Acid. Res. 16:3341-3358, 1988) tested a series of tandem 14 nucleobase antisense oligonucleotides, and a 28 and 42 nucleobase antisense oligonucleotides comprised of the sequence of two or three of the tandem antisense oligonucleotides, respectively, for their ability to arrest translation of human DHFR in a rabbit reticulocyte assay. Each of the three 14 nucleobase antisense oligonucleotides alone was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase antisense oligonucleotides.

Antisense Compound Motifs

In certain embodiments, antisense compounds targeted to a PTP1B nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by in vivo nucleases.

Chimeric antisense compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding affinity for the target nucleic acid, and/or increased inhibitory activity. A second region of a chimeric antisense compound may optionally serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β-D-ribonucleosides, β-D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE and 2'-O—CH_3, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a constrained ethyl). In certain embodiments, wings may include several modified sugar moieties, including, for example 2'-MOE and constrained ethyl. In certain embodiments, wings may include several modified and unmodified sugar moieties. In certain embodiments, wings may include various combinations of 2'-MOE nucleosides, constrained ethyl nucleosides, and 2'-deoxynucleosides.

Each distinct region may comprise uniform sugar moieties, variant, or alternating sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5'-wing, "Y" represents the length of the gap, and "Z" represents the length of the 3'-wing. "X" and "Z" may comprise uniform, variant, or alternating sugar moieties. In certain embodiments, "X" and "Y" may include one or more 2'-deoxynucleosides. "Y" may comprise 2'-deoxynucleosides. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap is positioned immediately adjacent to each of the 5'-wing and the 3' wing. Thus, no intervening nucleotides exist between the 5'-wing and gap, or the gap and the 3'-wing. Any of the antisense compounds described herein can have a gapmer motif. In certain embodiments, "X" and "Z" are the same, in other embodiments they are different. In certain embodiments, "Y" is between 8 and 15 nucleosides. X, Y, or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more nucleosides.

In certain embodiments, antisense compounds targeted to a PTP1B nucleic acid possess a 5-10-5 gapmer motif.

In certain embodiments, antisense compounds targeted to a PTP1B nucleic acid possess a 6-8-6 gapmer motif.

In certain embodiments, antisense compounds targeted to a PTP1B nucleic acid possess a 5-8-5 gapmer motif.

In certain embodiments, an antisense compound targeted to a PTP1B nucleic acid has a gap-widened motif.

In certain embodiments, antisense compounds targeted to a PTP1B nucleic acid possess a 2-13-5 gap-widened motif

Target Nucleic Acids, Target Regions and Nucleotide Sequences

In certain embodiments, the PTP1B nucleic acid is any of the sequences set forth in GENBANK Accession No. NM_002827.2 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NT_011362.9 truncated from nucleotides 14178000 to 14256000 (incorporated herein as SEQ ID NO: 2); and a concatenation of sequences from exons 1-9, intron 9 and exon 10 of the rhesus monkey PTP1B scaffold (incorporated herein as SEQ ID NO: 3).

It is understood that the sequence set forth in each SEQ ID NO in the Examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, antisense compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Antisense compounds described by Isis Number (Isis No) indicate a combination of nucleobase sequence and motif.

In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for PTP1B can be obtained by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In certain embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the same target region.

Targeting includes determination of at least one target segment to which an antisense compound hybridizes, such that a desired effect occurs. In certain embodiments, the desired effect is a reduction in mRNA target nucleic acid levels. In certain embodiments, the desired effect is reduction of levels of protein encoded by the target nucleic acid or a phenotypic change associated with the target nucleic acid.

A target region may contain one or more target segments. Multiple target segments within a target region may be overlapping. Alternatively, they may be non-overlapping. In certain embodiments, target segments within a target region are separated by no more than about 300 nucleotides. In certain emodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more than, is no more than about, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid, or is a range defined by any two of the preceeding values. In certain embodiments, target segments within a target region are separated by no more than, or no more than about, 5 nucleotides on the target nucleic acid. In certain embodiments, target segments are contiguous. Contemplated are target regions defined by a range having a starting nucleic acid that is any of the 5' target sites or 3' target sites listed herein.

Suitable target segments may be found within a 5' UTR, a coding region, a 3' UTR, an intron, an exon, or an exon/intron junction. Target segments containing a start codon or a stop codon are also suitable target segments. A suitable target segment may specifcally exclude a certain structurally defined region such as the start codon or stop codon.

The determination of suitable target segments may include a comparison of the sequence of a target nucleic acid to other sequences throughout the genome. For example, the BLAST algorithm may be used to identify regions of similarity amongst different nucleic acids. This comparison can prevent the selection of antisense compound sequences that may hybridize in a non-specific manner to sequences other than a selected target nucleic acid (i.e., non-target or off-target sequences).

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within an active target region. In certain embodiments, reductions in PTP1B mRNA levels are indicative of inhibition of PTP1B expression. Reductions in levels of a PTP1B protein are also indicative of inhibition of target mRNA expression. Further, phenotypic changes are indicative of inhibition of PTP1B expression. In certain embodiments, reduced glucose levels, reduced lipid levels, and reduced body weight can be indicative of inhibition of PTP1B expression. In certain embodiments, amelioration of symptoms associated with metabolic disease can be indicative of inhibition of PTP1B expression. In certain embodiments, amelioration of symptoms associated with diabetes can be indicative of inhibition of PTP1B expression. In certain embodiments, reduction of insulin resistance is indicative of inhibition of PTP1B expression. In certain embodiments, reduction of diabetes biomarkers can be indicative of inhibition of PTP1B expression.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a PTP1B nucleic acid. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

Hybridization can occur under varying conditions. Stringent conditions are sequence-dependent and are determined by the nature and composition of the nucleic acid molecules to be hybridized.

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the antisense compounds provided herein are specifically hybridizable with a PTP1B nucleic acid.

Complementarity

An antisense compound and a target nucleic acid are complementary to each other when a sufficient number of nucleobases of the antisense compound can hydrogen bond with the corresponding nucleobases of the target nucleic acid, such that a desired effect will occur (e.g., antisense inhibition of a target nucleic acid, such as a PTP1B nucleic acid).

An antisense compound may hybridize over one or more segments of a PTP1B nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a PTP1B nucleic acid, a target region, target segment, or specified portion thereof. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods.

For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having 4 (four) noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul et al., J. Mol. Biol., 1990, 215, 403 410; Zhang and Madden, Genome Res., 1997, 7, 649 656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482 489).

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, antisense compound may be fully complementary to a PTP1B nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and/or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

The location of a non-complementary nucleobase may be at the 5' end or 3' end of the antisense compound. Alternatively, the non-complementary nucleobase or nucleobases may be at an internal position of the antisense compound. When two or more non-complementary nucleobases are present, they may be contiguous (i.e. linked) or non-contiguous. In one embodiment, a non-complementary nucleobase is located in the wing segment of a gapmer antisense oligonucleotide.

In certain embodiments, antisense compounds that are, or are up to 12, 13, 14, 15, 16, 17, 18, 19, or nucleobases in length comprise no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a PTP1B nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a PTP1B nucleic acid, or specified portion thereof.

The antisense compounds provided herein also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A "portion" can also refer to a defined number of contiguous nucleobases of an antisense compound. In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 13 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 14 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 15 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 16 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 17 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 18 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 19 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 20 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least a 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

The antisense compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific Isis number, or portion thereof. As used herein, an antisense compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the antisense compounds described herein as well as compounds having non-identical bases relative to the antisense compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

In certain embodiments, the antisense compounds, or portions thereof, are at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

Modifications

A nucleoside is a base-sugar combination. The nucleobase (also known as base) portion of the nucleoside is normally a heterocyclic base moiety. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar. Oligonucleotides are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

Modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified antisense compounds are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases, or increased inhibitory activity.

Chemically modified nucleosides may also be employed to increase the binding affinity of a shortened or truncated antisense oligonucleotide for its target nucleic acid. Consequently, comparable results can often be obtained with shorter antisense compounds that have such chemically modified nucleosides.

Modified Internucleoside Linkages

The naturally occurring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having naturally occurring internucleoside linkages because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

In certain embodiments, antisense compounds targeted to a PTP1B nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

Modified Sugar Moieties

Antisense compounds provided herein can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise a chemically modified ribofuranose ring moiety. Examples of chemically modified ribofuranose rings include, without limitation, addition of substitutent groups (including 5' and 2' substituent groups); bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA); replacement of the ribosyl ring oxygen atom with S, N(R), or C(R1)(R)2 (R═H, C_1-C_{12} alkyl or a protecting group); and combinations thereof. Examples of chemically modified sugars include, 2'-F-5'-methyl substituted nucleoside (see, PCT International Application WO 2008/101157, published on Aug. 21, 2008 for other disclosed 5',2'-bis substituted nucleosides), replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (see, published U.S. Patent Application US2005/0130923, published on Jun. 16, 2005), or, alternatively, 5'-substitution of a BNA (see, PCT International Application WO 2007/134181, published on Nov. 22, 2007, wherein LNA is substituted with, for example, a 5'-methyl or a 5'-vinyl group).

Examples of nucleosides having modified sugar moieties include, without limitation, nucleosides comprising 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH_3, and 2'—$O(CH_2)2OCH_3$ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O—C_1-C_{10} alkyl, OCF_3, $O(CH_2)2SCH_3$, $O(CH_2)2$—O—N(Rm)(Rn) and O—CH_2—C(═O)—N(Rm)(Rn), where each Rm and Rn is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl.

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include, without limitation, nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides wherein the bridge comprises a 4' to 2' bicyclic nucleoside. Examples of such 4' to 2' bicyclic nucleosides, include, but are not limited to, one of the formulae: 4'-(CH_2)—O-2' (LNA); 4'-(CH_2)—S-2; 4'-$(CH_2)_2$—O-2' (ENA); 4'-CH(CH_3)—O-2' and 4'-CH(CH_2OCH_3)—O-2', and analogs thereof (see, U.S. Pat. No. 7,399,845, issued on Jul. 15, 2008); 4'-C(CH_3)(CH_3)—O-2', and analogs thereof (see, published PCT International Application WO2009/006478, published Jan. 8, 2009); 4'-CH_2—N(OCH_3)-2', and analogs thereof (see, published PCT International Application WO2008/150729, published Dec. 11, 2008); 4'-CH_2—O—N(CH_3)-2' (see, published U.S. Patent Application US2004/0171570, published Sep. 2, 2004); 4'-CH_2—N(R)—O-2', wherein R is H, C_1-C_{12} alkyl, or a protecting group (see, U.S. Pat. No. 7,427,672, issued on Sep. 23, 2008); 4'-CH_2—C(H)(CH_3)-2' (see, Chattopadhyaya, et al., J. Org. Chem., 2009, 74, 118-134); and 4'-CH_2—C(═CH_2)-2', and analogs thereof (see, published PCT International Application WO 2008/154401, published on Dec. 8, 2008). Also see, for example: Singh et al., Chem. Commun., 1998, 4, 455-456; Koshkin et al., Tetrahedron, 1998, 54, 3607-3630; Wahlestedt et al., Proc. Natl. Acad. Sci. U.S. A., 2000, 97, 5633-5638; Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222; Singh et al., J. Org. Chem., 1998, 63, 10035-10039; Srivastava et al., J. Am. Chem. Soc., 129(26) 8362-8379 (Jul. 4, 2007); Elayadi et al., Curr. Opinion Invens. Drugs, 2001, 2, 558-561; Braasch et al., Chem. Biol., 2001, 8, 1-7; Orum et al., Curr. Opinion Mol. Ther., 2001, 3, 239-243; U.S. Pat. Nos. 6,670,461, 7,053,207, 6,268,490, 6,770,748, 6,794,499, 7,034,133, 6,525,191, 7,399,845; published PCT International applications WO 2004/106356, WO 94/14226, WO 2005/021570, and WO 2007/134181; U.S. Patent Publication Nos. US2004/0171570, US2007/0287831, and US2008/0039618; and U.S. patent Ser. Nos. 12/129,154, 60/989,574, 61/026,995, 61/026,998, 61/056,564, 61/086,231, 61/097,787, and 61/099,844; and PCT International Application Nos. PCT/US2008/064591, PCT/US2008/066154, and PCT/US2008/068922. Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see PCT international application PCT/DK98/00393, published on Mar. 25, 1999 as WO 99/14226).

In certain embodiments, bicyclic sugar moieties of BNA nucleosides include, but are not limited to, compounds having at least one bridge between the 4' and the 2' position of the pentofuranosyl sugar moiety wherein such bridges independently comprises 1 or from 2 to 4 linked groups independently selected from —$[C(R_a)(R_b)]_n$—, —C(R_a)═C(R_b)—, —C(R_a)═N—, —C(═NR_a)—, —C(═O)—, —C(═S)—, —O—, —Si$(R_a)_2$—, —S(═O)$_x$—, and —N(R_a)—;

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

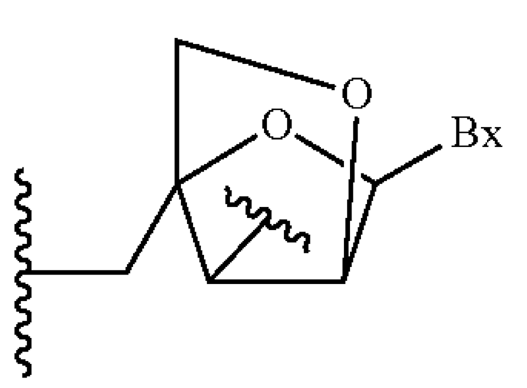
each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(═O)—H), substituted acyl, CN, sulfonyl (S(═O)$_2$—J_1), or sulfoxyl (S(═O)—J_1); and each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)—H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl, or a protecting group.

In certain embodiments, the bridge of a bicyclic sugar moiety is, —[C(R_a)(R_b)]$_n$—, —[C(R_a)(R_b)]$_n$—O—, —C(R_aR_b)—N(R)—O— or, —C(R_aR_b)—O—N(R)—. In certain embodiments, the bridge is 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2—O-2', 4'-$(CH_2)_2$—O-2', 4'-CH_2—O—N(R)-2', and 4'-CH_2—N(R)—O-2'-, wherein each R is, independently, H, a protecting group, or C_1-C_{12} alkyl.

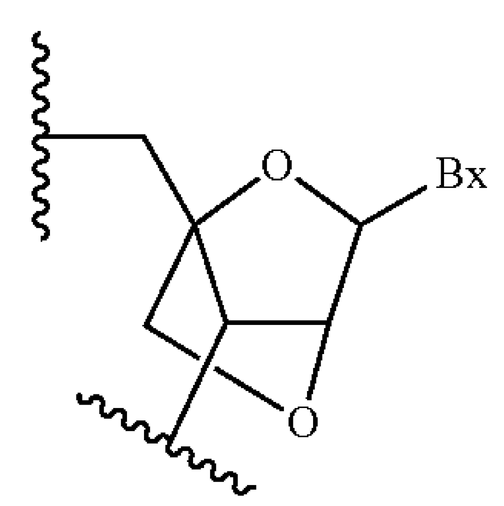
In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-2' methylene-oxy bridge, may be in the α-L configuration or in the β-D configuration. Previously, α-L-methyleneoxy (4'-CH_2—O-2') BNA's have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden et al., Nucleic Acids Research, 2003, 21, 6365-6372).

In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α-L-Methyleneoxy (4'-CH_2—O-2') BNA, (B) β-D-Methyleneoxy (4'-CH_2—O-2') BNA, (C) Ethyleneoxy (4'-$(CH_2)_2$—O-2') BNA, (D) Aminooxy (4'-CH_2—O—N(R)-2') BNA, (E) Oxyamino (4'-CH_2—N(R)—O-2') BNA, (F) Methyl(methyleneoxy) (4'-CH(CH_3)—O-2') BNA, (G) methylene-thio (4'-CH_2—S-2') BNA, (H) methylene-amino (4'-CH2—N(R)-2') BNA, (I) methyl carbocyclic (4'-CH_2—CH(CH_3)-2') BNA, and (J) propylene carbocyclic (4'-$(CH_2)_3$-2') BNA as depicted below.

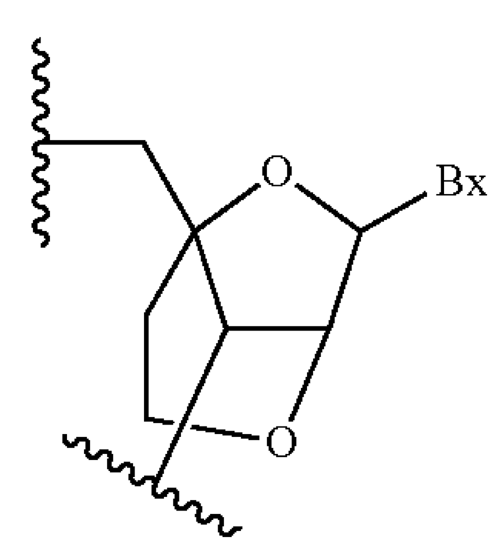
(A)



(B)



(C)



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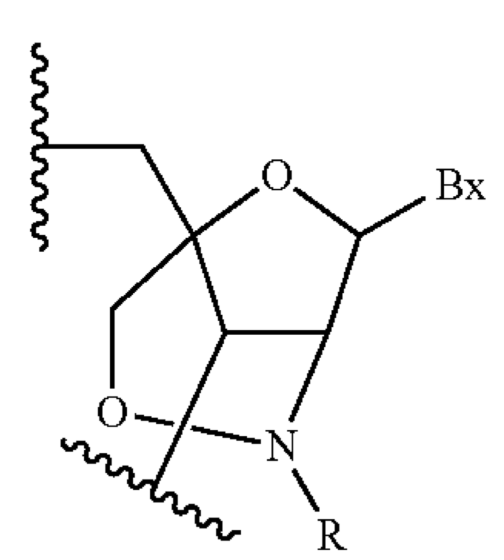

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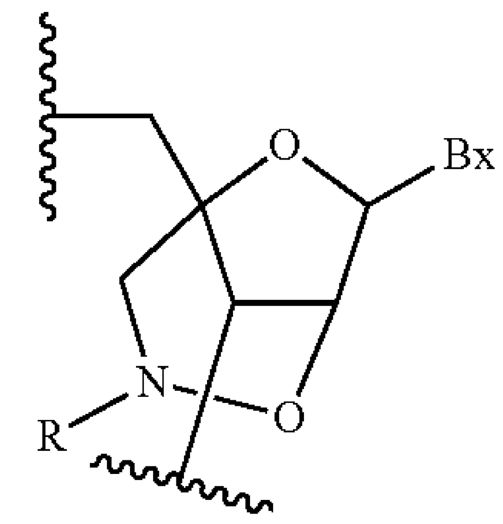
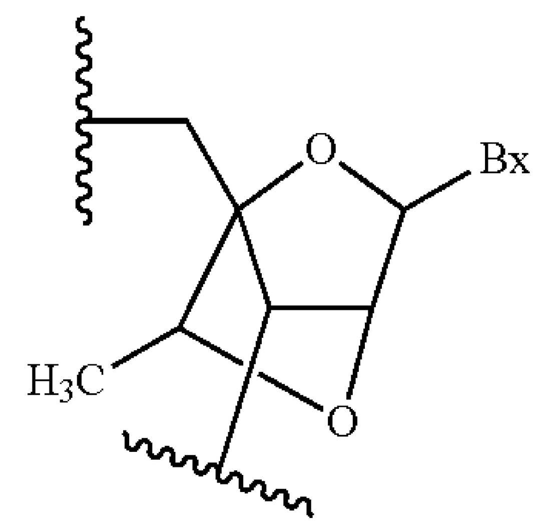
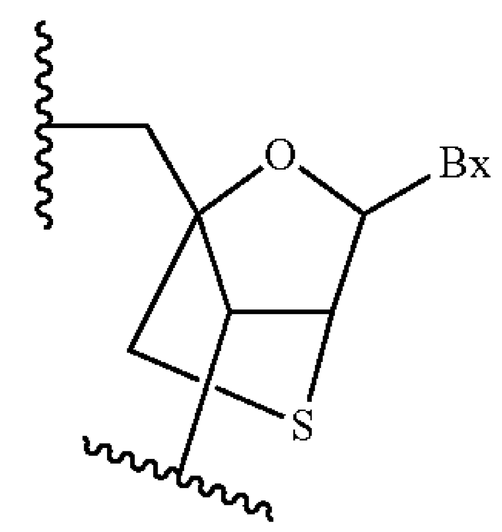
(E)



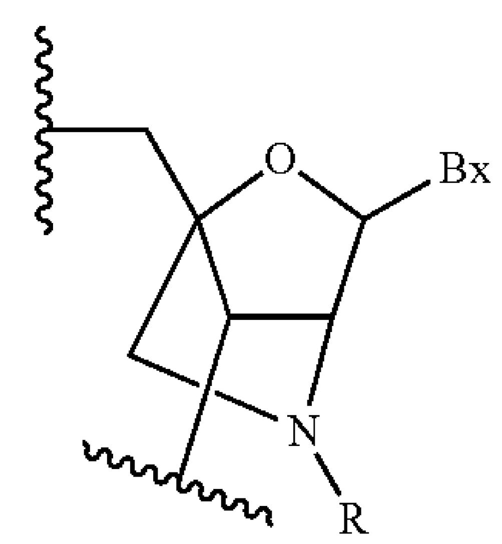
(F)



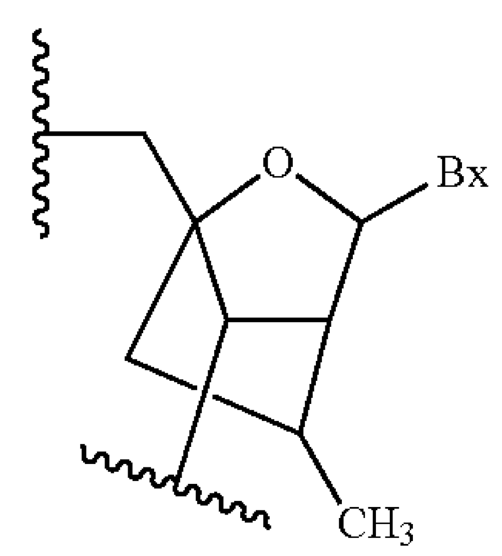
(G)



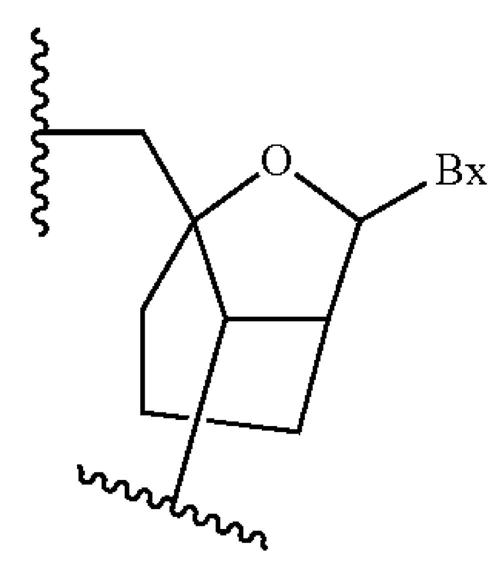
(H)



(I)



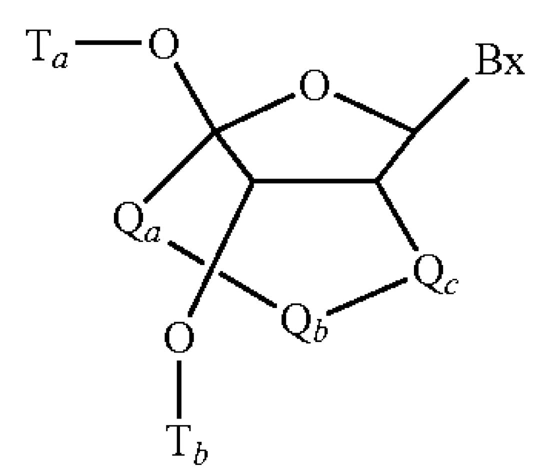
(J)



wherein Bx is the base moiety and R is, independently, H, a protecting group or C_1-C_{12} alkyl.

In certain embodiments, bicyclic nucleoside having Formula I:

I



wherein:

Bx is a heterocyclic base moiety;

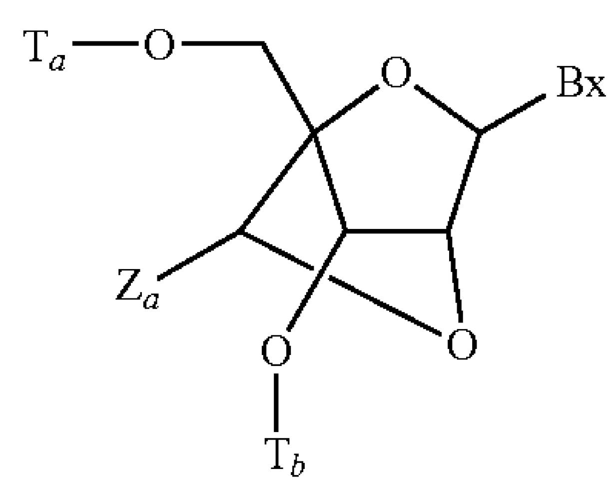
$-Q_a-Q_b-Q_c-$ is $—CH_2—N(R_c)—CH_2—$, $—C(=O)—N(R_c)—CH_2—$, $—CH_2—O—N(R_c)—$, $—CH_2—N(R_c)—O—$, or $—N(R_c)—O—CH_2$;

R_c is C_1-C_{12} alkyl or an amino protecting group; and

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium.

In certain embodiments, bicyclic nucleoside having Formula II:

II



wherein:

Bx is a heterocyclic base moiety;

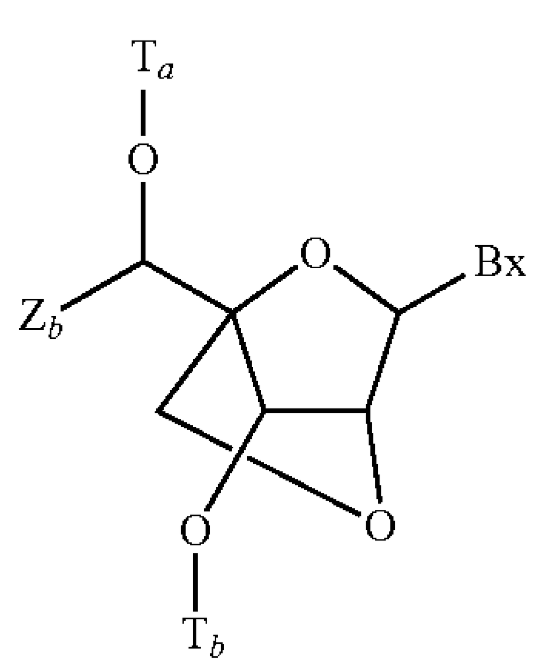
T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

Z_a is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl, acyl, substituted acyl, substituted amide, thiol, or substituted thio.

In one embodiment, each of the substituted groups is, independently, mono or poly substituted with substituent groups independently selected from halogen, oxo, hydroxyl, OJ_c, NJ_cJ_d, SJ_c, N_3, $OC(=X)J_c$, and $NJ_eC(=X)NJ_cJ_d$, wherein each J_c, J_d, and J_e is, independently, H, C_1-C_6 alkyl, or substituted C_1-C_6 alkyl and X is O or NJ_c.

In certain embodiments, bicyclic nucleoside having Formula III:

III



wherein:

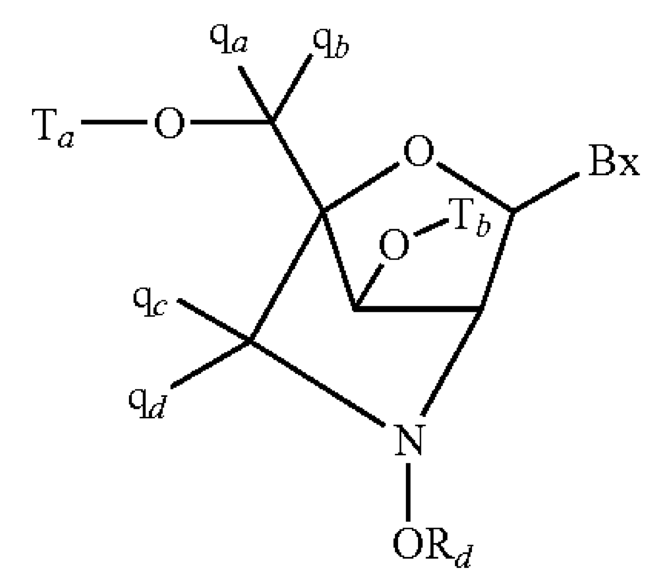
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

Z_b is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl, or substituted acyl (C(=O)—).

In certain embodiments, bicyclic nucleoside having Formula IV:

IV



wherein:

Bx is a heterocyclic base moiety;

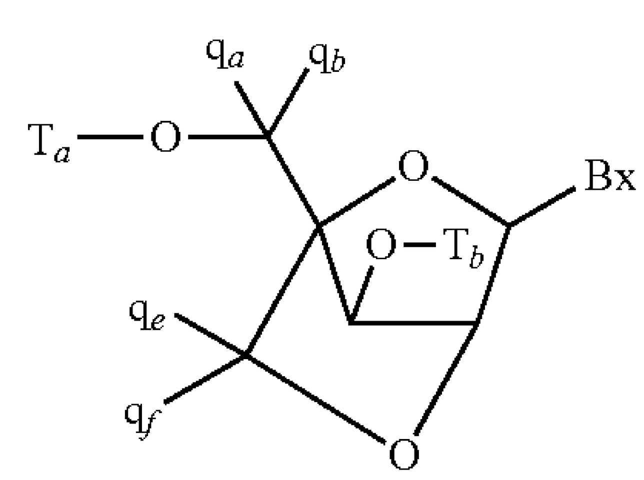
T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

R_d is C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, or substituted C_2-C_6 alkynyl;

each q_a, q_b, q_c and q_d is, independently, H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, or substituted C_2-C_6 alkynyl, C_1-C_6 alkoxyl, substituted C_1-C_6 alkoxyl, acyl, substituted acyl, C_1-C_6 aminoalkyl, or substituted C_1-C_6 aminoalkyl;

In certain embodiments, bicyclic nucleoside having Formula V:

V



wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

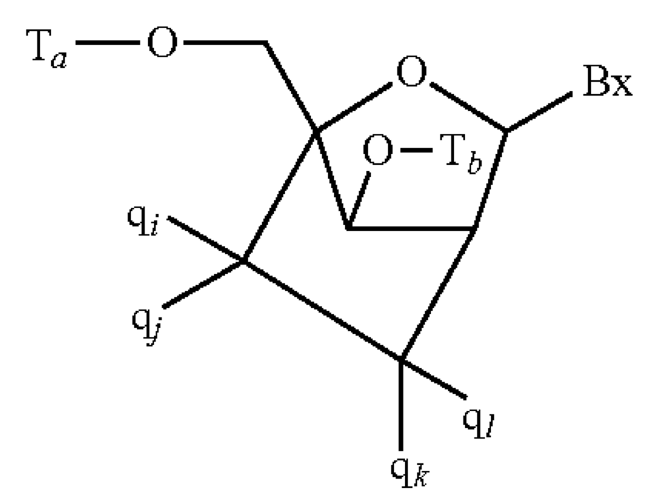
q_a, q_b, q_e and q_f are each, independently, hydrogen, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, substituted C_1-C_{12} alkoxy, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O—C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$;

or q_e and q_f together are $=C(q_g)(q_h)$;

q_g and q_h are each, independently, H, halogen, C_1-C_{12} alkyl, or substituted C_1-C_{12} alkyl.

The synthesis and preparation of the methyleneoxy (4'-CH_2—O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine, and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (see, e.g., Koshkin et al., Tetrahedron, 1998, 54, 3607-3630). BNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

Analogs of methyleneoxy (4'-CH_2—O-2') BNA, methyleneoxy (4'-CH_2—O-2') BNA, and 2'-thio-BNAs, have also been prepared (see, e.g., Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (see, e.g., Wengel et al., WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel comformationally restricted high-affinity oligonucleotide analog, has been described in the art (see, e.g., Singh et al., J. Org. Chem., 1998, 63, 10035-10039). In addition, 2'-amino- and 2'-methylamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported. In certain embodiments, bicyclic nucleoside having Formula VI:



VI

wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety, or a covalent attachment to a support medium;

each q_i, q_j, q_k and q_l is, independently, H, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxyl, substituted C_1-C_{12} alkoxyl, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O-C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$, or $N(H)C(=S)NJ_jJ_k$; and

q_i and q_j or q_l and q_k together are $=C(q_g)(q_h)$, wherein q_g and q_h are each, independently, H, halogen, C_1-C_{12} alkyl, or substituted C_1-C_{12} alkyl.

One carbocyclic bicyclic nucleoside having a 4'-$(CH_2)_3$-2' bridge and the alkenyl analog, bridge 4'-CH=CH—CH_2-2', have been described (see, e.g., Freier et al., Nucleic Acids Research, 1997, 25(22), 4429-4443 and Albaek et al., J. Org. Chem., 2006, 71, 7731-7740). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (see, e.g., Srivastava et al., J. Am. Chem. Soc. 2007, 129(26), 8362-8379).

As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting the 2' carbon atom and the 4' carbon atom.

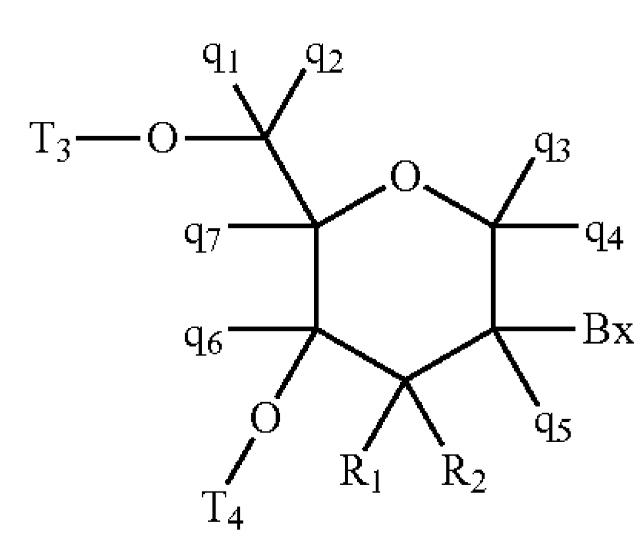
As used herein, "monocylic nucleosides" refer to nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, of a nucleoside may be modified or substituted at any position.

As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2'-substituent groups can also be selected from: C_1-C_{12} alkyl; substituted alkyl; alkenyl; alkynyl; alkaryl; aralkyl; O-alkaryl or O-aralkyl; SH; SCH_3; OCN; Cl; Br; CN; CF_3; OCF_3; $SOCH_3$; SO_2CH_3; ONO_2; NO_2; N_3; NH_2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving pharmacokinetic properties; and a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modified nucleosides comprise a 2'-MOE side chain (see, e.g., Baker et al., J. Biol. Chem., 1997, 272, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'-O-methyl, O-propyl, and O-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for in vivo use (see, e.g., Martin, P., Helv. Chim. Acta, 1995, 78, 486-504; Altmann et al., Chimia, 1996, 50, 168-176; Altmann et al., Biochem. Soc. Trans., 1996, 24, 630-637; and Altmann et al., Nucleosides Nucleotides, 1997, 16, 917-926).

As used herein, a "modified tetrahydropyran nucleoside" or "modified THP nucleoside" means a nucleoside having a six-membered tetrahydropyran "sugar" substituted in for the pentofuranosyl residue in normal nucleosides (a sugar surrogate). Modified THP nucleosides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (see Leumann, C J. Bioorg. & Med. Chem. (2002) 10:841-854), fluoro HNA (F—HNA), or those compounds having Formula X:

Formula X:

X



wherein independently for each of said at least one tetrahydropyran nucleoside analog of Formula X:

Bx is a heterocyclic base moiety;

T_3 and T_4 are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound or one of T_3 and T_4 is an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound and the other of T_3 and T_4 is H, a hydroxyl protecting group, a linked conjugate group, or a 5' or 3'-terminal group;

q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each, independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, or substituted C_2-C_6 alkynyl; and

one of R_1 and R_2 is hydrogen and the other is selected from halogen, substituted or unsubstituted alkoxy, NJ_1J_2, SJ_1, N_3, $OC(=X)NJ_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$, and CN, wherein X is O, S, or NJ_1, and each J_1, J_2, and J_3 is, independently, H or C_1-C_6 alkyl.

In certain embodiments, the modified THP nucleosides of Formula X are provided wherein q_m, q_n, q_p, q_r, q_s, q_t, and q_u are each H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t, and q_u is other than H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t and q_u is methyl. In certain embodiments, THP nucleosides of Formula X are provided wherein one of R_1 and R_2 is F. In certain embodiments, R_1 is fluoro and R_2 is H, R_1 is methoxy and R_2 is H, and R_1 is methoxyethoxy and R_2 is H.

As used herein, "2'-modified" or "2'-substituted" refers to a nucleoside comprising a sugar comprising a substituent at the 2' position other than H or OH. 2'-modified nucleosides, include, but are not limited to, bicyclic nucleosides wherein the bridge connecting two carbon atoms of the sugar ring connects the 2' carbon and another carbon of the sugar ring and nucleosides with non-bridging 2' substituents, such as allyl, amino, azido, thio, O-allyl, O—C_1-C_{10} alkyl, —OCF_3, O—$(CH_2)_2$—O—CH_3, 2'-$O(CH_2)_2SCH_3$, O—$(CH_2)_2$—O—$N(R_m)(R_n)$, or O—CH_2—C(=O)—$N(R_m)(R_n)$, where each R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl. 2'-modified nucleosides may further comprise other modifications, for example, at other positions of the sugar and/or at the nucleobase.

As used herein, "2'-F" refers to a sugar comprising a fluoro group at the 2' position.

As used herein, "2'-OMe" or "2'-OCH_3" or "2'-O-methyl" each refers to a sugar comprising an —OCH_3 group at the 2' position of the sugar ring.

As used herein, "oligonucleotide" refers to a compound comprising a plurality of linked nucleosides. In certain embodiments, one or more of the plurality of nucleosides is modified. In certain embodiments, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or deoxyribonucleosides (DNA).

Many other bicyclo and tricyclo sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds (see, e.g., review article: Leumann, J. C, Bioorganic & Medicinal Chemistry, 2002, 10, 841-854). Such ring systems can undergo various additional substitutions to enhance activity.

Methods for the preparations of modified sugars are well known to those skilled in the art.

In nucleotides having modified sugar moieties, the nucleobase moieties (natural, modified, or a combination thereof) are maintained for hybridization with an appropriate nucleic acid target.

In certain embodiments, antisense compounds comprise one or more nucleotides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleotides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a cEt. In certain embodiments, the cEt modified nucleotides are arranged throughout the wings of a gapmer motif.

Modified Nucleobases

Nucleobase (or base) modifications or substitutions are structurally distinguishable from, yet functionally interchangeable with, naturally occurring or synthetic unmodified nucleobases. Both natural and modified nucleobases are capable of participating in hydrogen bonding. Such nucleobase modifications may impart nuclease stability, binding affinity or some other beneficial biological property to antisense compounds. Modified nucleobases include synthetic and natural nucleobases such as, for example, 5-methylcytosine (5-me-C). Certain nucleobase substitutions, including 5-methylcytosine substitutions, are particularly useful for increasing the binding affinity of an antisense compound for a target nucleic acid. For example, 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278).

Additional unmodified nucleobases include 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (—C≡C—CH_3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

Heterocyclic base moieties may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Nucleobases that are particularly useful for increasing the binding affinity of antisense compounds include 5-substituted pyrimidines, 6-azapyrimidines and N2, N-6 and O-6 substituted purines, including 2 aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

In certain embodiments, antisense compounds targeted to a PTP1B nucleic acid comprise one or more modified nucleobases. In certain embodiments, gap-widened antisense oligonucleotides targeted to a PTP1B nucleic acid comprise one or more modified nucleobases. In certain embodiments, the modified nucleobase is 5-methylcytosine. In certain embodiments, each cytosine is a 5-methylcytosine.

Compositions and Methods for Formulating Pharmaceutical Compositions

Antisense oligonucleotides may be admixed with pharmaceutically acceptable active or inert substance for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

Antisense compound targeted to a PTP1B nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to a PTP1B nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

Pharmaceutically acceptable salts of the compounds described herein may be prepared by methods well-known in the art. For a review of pharmaceutically acceptable salts, see Stahl and Wermuth, Handbook of Pharmaceutical Salts: Properties, Selection and Use (Wiley-VCH, Weinheim, Germany, 2002). Sodium salts of antisense oligonucleotides are useful and are well accepted for therapeutic administration to humans. Accordingly, in one embodiment the compounds described herein are in the form of a sodium salt.

A prodrug can include the incorporation of additional nucleosides at one or both ends of an antisense compound which are cleaved by endogenous nucleases within the body, to form the active antisense compound.

Conjugated Antisense Compounds

Antisense compounds may be covalently linked to one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the resulting antisense oligonucleotides. Typical conjugate groups include cholesterol moieties and lipid moieties. Additional conjugate groups include carbohydrates, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes.

Antisense compounds can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of antisense compounds to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect the antisense compound having terminal nucleic acid 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 are well known in the art and include, for example, inverted deoxy abasic caps. Further 3' and 5'-stabilizing groups that can be used to cap one or both ends of an antisense compound to impart nuclease stability include those disclosed in WO 03/004602 published on Jan. 16, 2003.

Cell Culture and Antisense Compounds Treatment

The effects of antisense compounds on the level, activity or expression of PTP1B nucleic acids can be tested in vitro in a variety of cell types. Cell types used for such analyses are available from commercial vendors (e.g. American Type Culture Collection, Manassus, Va.; Zen-Bio, Inc., Research Triangle Park, N.C.; Clonetics Corporation, Walkersville, Md.) and cells are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, Calif.). Illustrative cell types include, but are not limited to, HepG2 cells, Hep3B cells, primary hepatocytes, A549 cells, GM04281 fibroblasts and LLC-MK2 cells.

In Vitro Testing of Antisense Oligonucleotides

Described herein are methods for treatment of cells with antisense oligonucleotides, which can be modified appropriately for treatment with other antisense compounds.

In general, cells are treated with antisense oligonucleotides when the cells reach approximately 60-80% confluence in culture.

One reagent commonly used to introduce antisense oligonucleotides into cultured cells includes the cationic lipid transfection reagent LIPOFECTIN® (Invitrogen, Carlsbad, Calif.). Antisense oligonucleotides are mixed with LIPOFECTIN® in OPTI-MEM® 1 (Invitrogen, Carlsbad, Calif.) to achieve the desired final concentration of antisense oligonucleotide and a LIPOFECTIN® concentration that typically ranges 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

Another reagent used to introduce antisense oligonucleotides into cultured cells includes LIPOFECTAMINE 2000® (Invitrogen, Carlsbad, Calif.). Antisense oligonucleotide is mixed with LIPOFECTAMINE 2000® in OPTI-MEM® 1 reduced serum medium (Invitrogen, Carlsbad, Calif.) to achieve the desired concentration of antisense oligonucleotide and a LIPOFECTAMINE® concentration that typically ranges 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

Another reagent used to introduce antisense oligonucleotides into cultured cells includes Cytofectin® (Invitrogen, Carlsbad, Calif.). Antisense oligonucleotide is mixed with Cytofectin® in OPTI-MEM® 1 reduced serum medium (Invitrogen, Carlsbad, Calif.) to achieve the desired concentration of antisense oligonucleotide and a Cytofectin® concentration that typically ranges 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation.

Cells are treated with antisense oligonucleotides by routine methods. Cells are typically harvested 16-24 hours after antisense oligonucleotide treatment, at which time RNA or protein levels of target nucleic acids are measured by methods known in the art and described herein. In general, when treatments are performed in multiple replicates, the data are presented as the average of the replicate treatments.

The concentration of antisense oligonucleotide used varies from cell line to cell line. Methods to determine the optimal antisense oligonucleotide concentration for a particular cell line are well known in the art. Antisense oligonucleotides are typically used at concentrations ranging from 1 nM to 300 nM when transfected with LIPOFECTAMINE2000®, Lipofectin or Cytofectin. Antisense oligonucleotides are used at higher concentrations ranging from 625 to 20,000 nM when transfected using electroporation.

RNA Isolation

RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. RNA is prepared using methods well known in the art, for example, using the TRIZOL® Reagent (Invitrogen, Carlsbad, Calif.) according to the manufacturer's recommended protocols.

Analysis of Inhibition of Target Levels or Expression

Inhibition of levels or expression of a PTP1B nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or quantitative real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM® 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, Calif. and used according to manufacturer's instructions.

Quantitative Real-Time PCR Analysis of Target RNA Levels

Quantitation of target RNA levels may be accomplished by quantitative real-time PCR using the ABI PRISM® 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, Calif.) according to manufacturer's instructions. Methods of quantitative real-time PCR are well known in the art.

Prior to real-time PCR, the isolated RNA is subjected to a reverse transcriptase (RT) reaction, which produces complementary DNA (cDNA) that is then used as the substrate for the real-time PCR amplification. The RT and real-time PCR reactions are performed sequentially in the same sample well. RT and real-time PCR reagents are obtained from Invitrogen (Carlsbad, Calif.). RT, real-time-PCR reactions are carried out by methods well known to those skilled in the art.

Gene (or RNA) target quantities obtained by real time PCR are normalized using either the expression level of a gene whose expression is constant, such as cyclophilin A, or by quantifying total RNA using RIBOGREEN® (Invitrogen, Inc. Carlsbad, Calif.). Cyclophilin A expression is quantified by real time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RIBOGREEN® RNA quantification reagent (Invitrogen, Inc. Eugene, Oreg.). Methods of RNA quantification by RIBOGREEN® are taught in Jones, L. J., et al, (Analytical Biochemistry, 1998, 265, 368-374). A CYTOFLUOR® 4000 instrument (PE Applied Biosystems) is used to measure RIBOGREEN® fluorescence.

Probes and primers are designed to hybridize to a PTP1B nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as PRIMER EXPRESS® Software (Applied Biosystems, Foster City, Calif.).

Analysis of Protein Levels

Antisense inhibition of PTP1B nucleic acids can be assessed by measuring PTP1B protein levels. Protein levels of PTP1B can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, Mich.), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art. Antibodies useful for the detection of human and rat PTP1B are commercially available.

In Vivo Testing of Antisense Compounds

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of PTP1B and produce phenotypic changes. Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration. Following a period of treatment with antisense oligonucleotides, RNA is isolated from tissue and changes in PTP1B nucleic acid expression are measured. Changes in PTP1B protein levels are also measured.

Certain Indications

In certain embodiments, provided herein are methods of treating an individual comprising administering one or more pharmaceutical compositions as described herein. In certain embodiments, the individual has metabolic related disease.

As shown in the examples below, compounds targeted to PTP1B, as described herein, have been shown to reduce the severity of physiological symptoms of metabolic related diseases, including metabolic syndrome, diabetes mellitus, insulin resistance, diabetic dyslipidemia, hypertriglyceridemia, obesity and weight gain. In certain of the experiments, the compounds reduced blood glucose levels, e.g., the animals continued to experience symptoms, but the symptoms were less severe compared to untreated animals. In other of the experiments, however, the compounds appear to reduce the symptoms of diabetes; e.g., animals treated for a longer period of time experienced less severe symptoms than those administered the compounds for a shorter period of time. In other of the experiments, however, the compounds appear to inhibit weight gain; e.g., animals treated for a longer period of time experienced less severe symptoms than those administered the compounds for a shorter period of time. In other of the experiments, however, the compounds appear to inhibit hypertriglyceridemia; e.g., animals treated for a longer period of time experienced less severe symptoms than those administered the compounds for a shorter period of time. The ability of the compounds exemplified below to restore function therefore demonstrates that symptoms of the disease may be reversed by treatment with a compound as described herein.

Diabetes mellitus is characterized by numerous physical and physiological symptoms. Any symptom known to one of skill in the art to be associated with Type 2 diabetes can be ameliorated or otherwise modulated as set forth above in the methods described above. In certain embodiments, the symptom is a physical symptom selected from the group consisting of increased glucose levels, increased weight gain, frequent urination, unusual thirst, extreme hunger, extreme fatigue, blurred vision, frequent infections, tingling or numbness at the extremities, dry and itchy skin, weight loss, slow-healing sores, and swollen gums.

In certain embodiments, the symptom is a physiological symptom selected from the group consisting of increased insulin resistance, increased glucose levels, increased fat mass, decreased metabolic rate, decreased glucose clearance, decreased glucose tolerance, decreased insulin sensitivity, decreased hepatic insulin sensitivity, increased adipose tissue size and weight, increased body fat, and increased body weight.

In certain embodiments, the physical symptom is increased weight gain. In certain embodiments, the symptom is frequent urination. In certain embodiments, the symptom is unusual thirst. In certain embodiments, the symptom is extreme hunger. In certain embodiments, the symptom is extreme fatigue. In certain embodiments, the symptom is blurred vision. In certain embodiments, the symptom is frequent infections. In certain embodiments, the symptom is tingling or numbness at the extremities. In certain embodiments, the symptom is dry and itchy skin. In certain embodiments, the symptom is weight loss. In certain embodiments, the symptom is slow-healing sores. In certain embodiments, the symptom is swollen gums. In certain embodiments, the symptom is increased insulin resistance. In certain embodiments, the symptom is increased fat mass. In certain embodiments, the symptom is decreased metabolic rate. In certain embodiments, the symptom is decreased glucose clearance. In certain embodiments, the symptom is decreased glucose tolerance. In certain embodiments, the symptom is decreased insulin sensitivity. In certain embodiments, the symptom is decreased hepatic insulin sensitivity. In certain embodiments, the symptom is increased adipose tissue size and weight. In certain embodiments, the symptom is increased body fat. In certain embodiments, the symptom is increased body weight.

Liu and Chemoff have shown that PTP1B binds to and serves as a substrate for the epidermal growth factor receptor (EGFR) (Liu and Chemoff, Biochem. J., 1997, 327, 139-145). Furthermore, in A431 human epidermoid carcinoma cells, PT1B was found to be inactivated by the presence of H_2O_2 generated by the addition of EGF. These studies indicate that PTP1B can be negatively regulated by the oxidation state of the cell, which is often deregulated during tumorigenesis (Lee et al., J. Biol. Chem., 1998, 273, 15366-15372).

Overexpression of PTP1B has been demonstrated in malignant ovarian cancers and this correlation was accompanied by a concomitant increase in the expression of the associated growth factor receptor (Wiener et al., Am. J. Obstet. Gynecol., 1994, 170, 1177-1183).

PTP1B has been shown to suppress transformation in NIH3T3 cells induced by the neu oncogene (Brown-Shimer et al., Cancer Res., 1992, 52, 478-482), as well as in rat 3Y1 fibroblasts induced by v-srk, v-src, and v-ras (Liu et al., Mol. Cell. Biol., 1998, 18, 250-259) and rat-1 fibroblasts induced by bcr-abl (LaMontagne et al., Proc. Natl. Acad. Sci. U.S.A., 1998, 95, 14094-14099). It has also been shown that PTP1B promotes differentiation of K562 cells, a chronic myelogenous leukemia cell line, in a similar manner as does an inhibitor of the bcr-abl oncoprotein. These studies describe the possible role of PTP1B in controlling the pathogenesis of chronic myeloid leukemia (LaMontagne et al., Proc. Natl. Acad. Sci. U.S.A., 1998, 95, 14094-14099).

Accordingly, provided herein are methods for ameliorating a symptom associated with hyperproliferative disorders in a subject in need thereof. In certain embodiments, the hyperproliferative disorder is cancer. In certain embodiments, provided herein are methods for ameliorating a symptom associated with cancer. In certain embodiments, provided is a method for reducing the rate of onset of a symptom associated with hyperproliferative disorders. In certain embodiments, provided is a method for reducing the rate of onset of a symptom associated with cancer. In certain embodiments, provided is a method for reducing the severity of a symptom associated with hyperproliferative disorders. In certain embodiments, provided is a method for reducing the severity of a symptom associated with cancer. In such embodiments, the methods comprise administering to an individual in need thereof a therapeutically effective amount of a compound targeted to a PTP1B nucleic acid.

In certain embodiments, provided are methods of treating an individual comprising administering one or more pharmaceutical compositions as described herein. In certain embodiments, the individual has metabolic related disease.

In certain embodiments, administration of an antisense compound targeted to a PTP1B nucleic acid results in reduction of PTP1B expression by at least about 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values.

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to transthyretin are used for the preparation of a medicament for treating a patient suffering or susceptible to metabolic related disease.

In certain embodiments, the methods described herein include administering a compound comprising a modified oligonucleotide having a contiguous nucleobases portion as described herein of a sequence recited in SEQ ID NO: 26 (ISIS 404173).

Administration

In certain embodiments, the compounds and compositions as described herein may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. The compounds and compositions as described herein can be administered directly to a tissue or organ.

In certain embodiments, the compounds and compositions as described herein are administered parenterally. "Parenteral administration" means administration through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g. intracerebral administration, intrathecal administration, intraventricular administration, ventricular administration, intracerebroventricular administration, cerebral intraventricular administration or cerebral ventricular administration. Administration can be continuous, or chronic, or short or intermittent.

In certain embodiments, parenteral administration is by injection. The injection can be delivered with a syringe or a pump. In certain embodiments, the injection is a bolus injection. In certain embodiments, the injection is administered directly to a tissue or organ.

In certain embodiments, the compounds and compositions as described herein are administered parenterally.

In certain embodiments, parenteral administration is subcutaneous.

In further embodiments, the formulation for administration is the compounds described herein and saline.

In certain embodiments, an antisense oligonucleotide is delivered by injection or infusion once every month, every two months, every 90 days, every 3 months, every 6 months, twice a year or once a year.

Certain Combination Therapies

In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with one or more other pharmaceutical agents. In certain embodiments, such one or more other pharmaceutical agents are designed to treat the same disease, disorder, or condition as the one or more pharmaceutical compositions described herein. In certain embodiments, such one or more other pharmaceutical agents are designed to treat a different disease, disorder, or condition as the one or more pharmaceutical compositions described herein. In certain embodiments, such one or more other pharmaceutical agents are designed to treat an undesired side effect of one or more pharmaceutical compositions as described herein. In certain embodiments, one or more pharmaceutical compositions are co-administered with another pharmaceutical agent to treat an undesired effect of that other pharmaceutical agent. In certain embodiments, one or more pharmaceutical compositions are co-administered with another pharmaceutical agent to produce a combinational effect. In certain embodiments, one or more pharmaceutical compositions are co-administered with another pharmaceutical agent to produce a synergistic effect.

In certain embodiments, a first agent and one or more second agents are administered at the same time. In certain embodiments, the first agent and one or more second agents are administered at different times. In certain embodiments, the first agent and one or more second agents are prepared together in a single pharmaceutical formulation. In certain embodiments, the first agent and one or more second agents are prepared separately.

In certain embodiments, the second compound is administered prior to administration of a pharmaceutical composition of the present invention. In certain embodiments, the second compound is administered following administration of a pharmaceutical composition of the present invention. In certain embodiments, the second compound is administered at the same time as a pharmaceutical composition of the present invention. In certain embodiments, the dose of a co-administered second compound is the same as the dose that would be administered if the second compound was administered alone. In certain embodiments, the dose of a co-administered second compound is lower than the dose that would be administered if the second compound was administered alone. In certain embodiments, the dose of a co-administered second compound is greater than the dose that would be administered if the second compound was administered alone.

In certain embodiments, the co-administration of a second compound enhances the effect of a first compound, such that co-administration of the compounds results in an effect that is greater than the effect of administering the first compound alone. In certain embodiments, the co-administration results in effects that are additive of the effects of the compounds when administered alone. In certain embodiments, the co-administration results in effects that are supra-additive of the effects of the compounds when administered alone. In certain embodiments, the first compound is an antisense compound. In certain embodiments, the second compound is an antisense compound.

In certain embodiments, second agents include, but are not limited to, a glucose-lowering agent. The glucose lowering agent can include, but is not limited to, a therapeutic lifestyle change, PPAR agonist, a dipeptidyl peptidase (IV) inhibitor, a GLP-1 analog, insulin or an insulin analog, an insulin secretagogue, a SGLT2 inhibitor, a human amylin analog, a biguanide, an alpha-glucosidase inhibitor, or a combination thereof. The glucose-lowering agent can include, but is not limited to metformin, sulfonylurea, rosiglitazone, meglitinide, thiazolidinedione, alpha-glucosidase inhibitor or a combination thereof. The sulfonylurea can be acetohexamide, chlorpropamide, tolbutamide, tolazamide, glimepiride, a glipizide, a glyburide, or a gliclazide. The meglitinide can be nateglinide or repaglinide. The thiazolidinedione can be pioglitazone or rosiglitazone. The alpha-glucosidase can be acarbose or miglitol.

In some embodiments, the glucose-lowering therapeutic is a GLP-1 analog. In some embodiments, the GLP-1 analog is exendin-4 or liraglutide.

In other embodiments, the glucose-lowering therapeutic is a sulfonylurea. In some embodiments, the sulfonylurea is acetohexamide, chlorpropamide, tolbutamide, tolazamide, glimepiride, a glipizide, a glyburide, or a gliclazide.

In some embodiments, the glucose-lowering drug is a biguanide. In some embodiments, the biguanide is metformin, and in some embodiments, blood glucose levels are decreased without increased lactic acidosis as compared to the lactic acidosis observed after treatment with metformin alone.

In some embodiments, the glucose-lowering drug is a meglitinide. In some embodiments, the meglitinide is nateglinide or repaglinide.

In some embodiments, the glucose-lowering drug is a thiazolidinedione. In some embodiments, the thiazolidinedione is pioglitazone, rosiglitazone, or troglitazone. In some embodiments, blood glucose levels are decreased without greater weight gain than observed with rosiglitazone treatment alone.

In some embodiments, the glucose-lowering drug is an alpha-glucosidase inhibitor. In some embodiments, the alpha-glucosidase inhibitor is acarbose or miglitol.

In a certain embodiment, a co-administered glucose-lowering agent is ISIS 113715.

In a certain embodiment, glucose-lowering therapy is therapeutic lifestyle change.

In certain embodiments, second agents include, but are not limited to, lipid-lowering agents. The lipid-lowering agent can include, but is not limited to atorvastatin, simvastatin, rosuvastatin, and ezetimibe. In certain such embodiments, the lipid-lowering agent is administered prior to administration of a pharmaceutical composition of the present invention. In certain such embodiments, the lipid-lowering agent is administered following administration of a pharmaceutical composition of the present invention. In certain such embodiments the lipid-lowering agent is administered at the same time as a pharmaceutical composition of the present invention. In certain such embodiments the dose of a co-administered lipid-lowering agent is the same as the dose that would be administered if the lipid-lowering agent was administered alone. In certain such embodiments the dose of a co-administered lipid-lowering agent is lower than the dose that would be administered if the lipid-lowering agent was administered alone. In certain such embodiments the dose of a co-administered lipid-lowering agent is greater than the dose that would be administered if the lipid-lowering agent was administered alone.

In certain embodiments, a co-administered lipid-lowering agent is a HMG-CoA reductase inhibitor. In certain such embodiments the HMG-CoA reductase inhibitor is a statin. In certain such embodiments the statin is selected from atorvastatin, simvastatin, pravastatin, fluvastatin, and rosuvastatin.

In certain embodiments, a co-administered lipid-lowering agent is a cholesterol absorption inhibitor. In certain such embodiments, cholesterol absorption inhibitor is ezetimibe.

In certain embodiments, a co-administered lipid-lowering agent is a co-formulated HMG-CoA reductase inhibitor and cholesterol absorption inhibitor. In certain such embodiments the co-formulated lipid-lowering agent is ezetimibe/simvastatin.

In certain embodiments, a co-administered lipid-lowering agent is a microsomal triglyceride transfer protein inhibitor (MTP inhibitor).

In certain embodiments, a co-administered lipid-lowering agent is an oligonucleotide targeted to ApoB.

In certain embodiments, second agents include, but are not limited to an anti-obesity drug or agent. Such anti-obesity agents include but are not limited to Orlistat, Sibutramine, or Rimonabant, and may be administered as described above as adipose or body weight lowering agents. In certain embodiments, the antisense compound may be co-administered with appetite suppressants. Such appetite suppressants include but are not limited to diethylpropion tenuate, mazindol, orlistat, phendimetrazine, phentermine, and sibutramine and may be administered as described herein. In certain embodiment, the anti-obesity agents are CNS based such as, but not limited to, sibutramine or GLP-1 based such as, but not limited to, liraglutide.

Formulations

The compounds provided herein may also be admixed, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor-targeted molecules, or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption-assisting formulations include, but are not limited to, U.S. Pat. Nos. 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

The antisense compounds provided herein encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof.

The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds provided herein: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. The term "pharmaceutically acceptable salt" includes a salt prepared from pharmaceutically acceptable non-toxic acids or bases, including inorganic or organic acids and bases. For oligonucleotides, preferred examples of pharmaceutically acceptable salts and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety. Sodium salts have been shown to be suitable forms of oligonucleotide drugs.

The term "pharmaceutically acceptable derivative" encompasses, but is not limited to, pharmaceutically acceptable salts, solvates, hydrates, esters, prodrugs, polymorphs, isomers, isotopically labeled variants of the compounds described herein.

The present invention also includes pharmaceutical compositions and formulations which include the antisense compounds provided herein. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intracerebral administration, intrathecal administration, intraventricular administration, ventricular administration, intracerebroventricular administration, cerebral intraventricular administration or cerebral ventricular administration.

Parenteral administration, is preferred to target PTP1B expression in the liver and plasma. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient (s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

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

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations. The pharmaceutical compositions and formulations of the present invention may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as an embodiment of the present invention. Emulsions and their uses are well known in the art and are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

Formulations of the present invention include liposomal formulations. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes which are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Liposomes and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

In another embodiment, formulations of the present invention include saline formulations. In certain embodiments, a formulation consists of the compounds described herein and saline. In certain embodiments, a formulation consists essentially of the compounds described herein and saline. In certain embodiments, the saline is pharmaceutically acceptable grade saline. In certain embodiments, the saline is buffered saline. In certain embodiments, the saline is phosphate buffered saline (PBS).

In certain embodiments, a formulation excludes liposomes. In certain embodiments, the formulation excludes sterically stabilized liposomes. In certain embodiments, a formulation excludes phospholipids. In certain embodiments, the formulation consists essentially of the compounds described herein and saline and excludes liposomes.

The pharmaceutical formulations and compositions of the present invention may also include surfactants. Surfactants and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

In one embodiment, the present invention employs various penetration enhancers to affect the efficient delivery of nucleic acids, particularly oligonucleotides. Penetration enhancers and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

One of skill in the art will recognize that formulations are routinely designed according to their intended use, i.e. route of administration.

Formulations for topical administration include those in which the oligonucleotides provided herein are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (e.g. dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (e.g. dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g. dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA).

Compositions and formulations for parenteral administration, including intravenous, intraarterial, subcutaneous, intraperitoneal, intramuscular injection or infusion, or intracranial may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Certain embodiments provided herein provide pharmaceutical compositions containing one or more oligomeric compounds and one or more other chemotherapeutic agents which function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to cancer chemotherapeutic drugs such as daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). When used with the compounds provided herein, such chemotherapeutic agents may be used individually (e.g., 5-FU and oligonucleotide), sequentially (e.g., 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (e.g., 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions provided herein. Combinations of antisense compounds and other non-antisense drugs are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

In another related embodiment, compositions provided herein may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. Alternatively, compositions provided herein may contain two or more antisense compounds targeted to different regions of the same nucleic acid target. Numerous examples of antisense compounds are known in the art. Two or more combined compounds may be used together or sequentially.

Dosing

The formulation of therapeutic compositions and their subsequent administration (dosing) is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}s found to be effective in in vitro and in vivo animal models. In general, dosage is from 0.01 μg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or at desired intervals. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μg to 100 g per kg of body weight, once or more daily.

While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same. Each of the references, GenBank accession numbers, and the like recited in the present application is incorporated herein by reference in its entirety.

Certain Compounds

About two hundred and seventy six newly designed antisense compounds of various lengths, motifs and backbone composition were tested for their effect on human PTP1B mRNA in vitro in several cell types. The new compounds were compared with about five hundred previously designed compounds including ISIS 107772, ISIS 107831, ISIS 142025, ISIS 142026, ISIS 142027, ISIS 142028, ISIS 142082, ISIS 146908, and ISIS 146909 which have previously been determined to be some of the most potent antisense compounds in vitro (see e.g., U.S. Patent Publication No. US 2003/0220282 published Nov. 27, 2003 and PCT Patent Publication No. WO2007/131237 published Nov. 15, 2007). Of the about two hundred and eight five newly designed and previously designed antisense compounds, about eleven compounds were selected for further study based on in vitro potency. The selected compounds were tested for dose dependent inhibition in HuVEC, HepG2, HuVEC, LLC-MK2, and cynomolgus primary hepatocytes. Additional oligonucleotides were designed based on microwalk of ISIS 409826, one of the selected compounds which demonstrated significant reduction of

PTP1B mRNA in all the cell lines tested. The oligonucleotides were tested in HuVEC cells (Example 5) along with gapmers from the earlier screen (Example 1). Several antisense oligonucleotides from the screen in Example 5 were selected and tested for dose-dependent inhibition in HuVEC cells (Example 6) and HepG2 cells (Example 7). Additionally, two oligonucleotides were designed as shortmers to ISIS 1428082, one of the selected compounds. These two shortmers (ISIS 446431 and ISIS 446432), as well as five ISIS oligonucleotides selected from the study described in Examples 6 and 7 were tested in HepG2 cells, LLC-MK2 cells, HuVEC cells, and cynomolgus primary hepatocytes (Examples 8-11). ISIS oligonucleotides that demonstrated dose-dependent reduction of PTP1B mRNA in all cell lines were tested for in vivo tolerability. Two more oligonucleotides ISIS 446433 and ISIS 446434, designed as shortmers to ISIS 409826, were included in the in vivo tolerability studies as well.

The twelve gapmers chosen were tested in a mouse model (see Example 12) and a rat model (Example 13). By virtue of their complementary sequence, the compounds are complementary to the regions 3291-3310, 989-1008, 3290-3309, 3287-3306, 3291-3310, 3288-3307, 3292-3309, 3293-3308, 3288-3305, and 3289-3304 of SEQ ID NO: 1. In the in vivo models, body weights and organ weights, the liver metabolic markers, such as alanine transaminase, aspartate transaminase and bilirubin, kidney metabolic markers, such as BUN and creatinine, plasma glucose levels, cholesterol and triglyceride levels, and inflammatory cytokine levels were measured. Of the twelve compounds tested, five compounds, ISIS 142082, ISIS 404173, ISIS 410003, ISIS 446431, and ISIS 446432 were selected and their viscosity was measured (Example 14). All the five oligonucleotides were considered optimal in their viscosity according to the criteria stated for the study.

Final evaluation of these studies (Examples 12-14), led to the selection of four compounds having a nucleobase sequence of a sequence recited in SEQ ID NO: 27 (ISIS 142082), 46 (ISIS 446431), 26 (ISIS 404173), and 23 (ISIS 409826). By virtue of their complementary sequence, the compounds are complementary to the regions 3291-3310, 3292-3309, 3290-3309, 3287-3306 of SEQ ID NO: 1. In certain embodiments, the compounds targeting the listed regions, as further described herein, comprise a modified oligonucleotide having some nucleobase portion of the sequence recited in the SEQ ID NOs, as further described herein, In certain embodiments, the compounds targeting the listed regions or having a nucleobase portion of a sequence recited in the listed SEQ ID NOs can be of various length, as further described herein, and can have one of various motifs, as further described herein. In certain embodiments, a compound targeting a region or having a nucleobase portion of a sequence recited in the listed SEQ ID NOs has the specific length and motif, as indicated by the ISIS NOs: ISIS 142082, ISIS 446431, ISIS 404173, and ISIS 409826.

Three compounds having a nucleobase sequence of a sequence recited in SEQ ID NO: 27 (ISIS 142082), 23 (ISIS 404173), and 46 (ISIS 446431), were further tested in a long-term, six month tolerability study in a mouse model (See Example 15). The half life in the liver of CD1 mice of all four of the compounds having a nucleobase sequence of a sequence recited in SEQ ID NOs: 53 (ISIS 409826), 27 (ISIS 142082), 26 (ISIS 404173), and 46 (ISIS 446431) was also evaluated (Example 16).

These four compounds were tested for efficacy, pharmacokinetic profile and tolerability in cynomolgus monkeys (Example 17). The inhibition studies in these monkeys indicated that treatment with some of these compounds caused reduction of PTP1B mRNA in the liver and fat tissues. Specifically, treatment with ISIS 409826, ISIS 142082, ISIS 446431 and ISIS 404173 caused 45%, 48%, 18 and 22% reduction of PTP1B mRNA in liver tissue, respectively compared to the PBS control. Treatment with ISIS 409826, ISIS 142082, ISIS 446431 and ISIS 404173 caused 21%, 28%, 12% and 31% reduction of PTP1B mRNA in fat tissue, respectively compared to the PBS control. It was noted that ISIS 404173 caused similar reduction of PTP1B mRNA compared to ISIS 142082, even though the two oligonucleotides differ from each other by a single base-pair shift of their target region on SEQ ID NO: 1. Protein analysis of liver tissue was also conducted by western blot analysis. PTP1B mRNA reduction using ISIS 409826, ISIS 142082, ISIS 446431 and ISIS 404173 was measured at a maximum dose of 40 mgk/week for efficacy and at a lower dose of 8 mgk/week for potency (See table 45). Protein analysis at a lower dose of 8 mgk/week demonstrated that ISIS 404173 caused greater reduction (33%) of PTP1B protein than ISIS 142082 (20%) demonstrating that ISIS 404173 was more potent than ISIS 142082 (See Table 47 and FIG. **1**). Protein analysis at the higher dose of 40 mg/week demonstrated that ISIS 404173 (60% protein reduction) was as efficacious as ISIS 142082 (65% protein reduction). Finally, treatment with ISIS 409826 and ISIS 142082 resulted in 22% decrease in triglyceride levels while treatment with ISIS 404173 resulted in 37% decrease in triglyceride levels compared to the PBS control.

Tolerability studies in cynomolgus monkeys (Example 17) indicated that treatment with ISIS 142082 was not as tolerable to the primates as treatment with ISIS 404173. The levels of C-reactive protein, which is synthesized in the liver and which serves as a marker of inflammation, were measured on day 93. At the higher dose of 40 mg/week, treatment with ISIS 142082 caused a significant increase of CRP levels of 12 mg/L compared to the control level of 1.2 mg/L. Treatment with ISIS 404173 at 40 mg/L caused an increase of CRP levels to 1.6 mg/L. The other compounds tested, ISIS 409826 and ISIS 446431 caused increase of CRP levels to 4.8 mg/L and 6.7 mg/L. Therefore, ISIS 404173 caused the least increase in CRP levels indicating that ISIS 404173 is extremely tolerable and non-proinflammatory. Organ weights were also measured to evaluate the tolerability of ISIS oligonucleotides by the monkeys. Treatment with ISIS 142082 at a dose of 40 mg/L caused increases in kidney and liver weights of 21 g and 18 g respectively, which is two-fold increase over the control (kidney 10 g and liver 10.5 g). Treatment with ISIS 409826 caused a two-fold increase in liver weight (18.5 g vs. 10.5 g of control) and a three-fold increase in spleen weight (6.0 g vs. 2.3 g of control). Treatment with ISIS 446431 caused a four-fold increase in spleen weight (9.6 g vs. 2.3 g control). Treatment with ISIS 404173 caused less than one-fold increase in all organs (kidney 14.8 g; liver 15.5 g; spleen 3.7 g) See (FIG. **2**). Hence, treatment with ISIS 142082, ISIS 409826, and ISIS 446431 were not considered tolerable in the monkeys, whereas treatment with ISIS 404173 was tolerable.

Treatment with ISIS 142082 caused increase in organ weights and elevated levels of CRP, indicating an inflammatory state. Treatment with ISIS 409826 also caused elevated levels of CRP and low complement C3 levels, indicating a diseased state. Treatment with ISIS 404173 and ISIS 446431 were considered optimal in terms of their tolerability profiles in cynomolgus monkeys. However, ISIS 446431 demonstrated less potency compared to ISIS 404173.

In case of the pharmacokinetic profile studies of the oligonucleotides in liver and kidney, none of the ISIS oligonucleotides demonstrated any abnormal ratios in concentration in the liver versus the kidney. ISIS 404173 was a better renal accumulator compared to ISIS 142082, as indicated in the results.

Hence, the in vivo studies, particularly in the cynomolgus monkeys, indicate that ISIS 404173 was just as potent and considerably more tolerable compared to the other compounds. The studies demonstrate that ISIS 142082, although shifted from ISIS 407173 by only one nucleobase, was as efficacious but less potent and tolerable than ISIS 404173, as demonstrated by assays for metabolic and inflammatory markers. Overall, ISIS 404173 was more potent and tolerable compared to any other compound.

Accordingly, provided herein are antisense compounds with any one or more of the improved characteristics. In a certain embodiments, provided herein are compounds comprising a modified oligonucleotide as further described herein targeted to or specifically hybridizable with the region of nucleotides of SEQ ID NO: 1.

Accordingly, provided herein are antisense compounds with any one or more of the improved characteristics. In a certain embodiments, provided herein are compounds comprising a modified oligonucleotide as further described herein targeted to or specifically hybridizable with the region of nucleotides of SEQ ID NO: 2.

In certain embodiments, the compounds as described herein are efficacious by virtue of having at least one of an in vitro IC_{50} of less than 0.4 μM, less than 0.35 μM, less than 0.3 μM, less than 2.5 μM, less than 2.0 μM, less than 1.5 μM, less than 1.0 μM, when delivered to a cynomolgus monkey hepatocyte cell line using electroporation as described in Example 11. In certain embodiments, the compounds as described herein are highly tolerable, as demonstrated by having at least one of an increase an ALT or AST value of no more than 4 fold, 3 fold, or 2 fold over saline treated animals; or an increase in liver, spleen or kidney weight of no more than 30%, 20%, 15%, 12%, 10%, 5% or 2%.

EXAMPLES

Non-Limiting Disclosure and Incorporation by Reference

While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same. Each of the references, GenBank accession numbers, and the like recited in the present application is incorporated herein by reference in its entirety.

Example 1

Antisense Inhibition of Human PTP1B mRNA in HuVEC Cells

Antisense oligonucleotides targeted to a human PTP1B nucleic acid were designed and tested for their effect on PTP1B RNA transcript in vitro. ISIS 107772, ISIS 107831, ISIS 142025, ISIS 142026, ISIS 142027, ISIS 142028, ISIS 142082, ISIS 146908, and ISIS 146909, claimed in a previous patent (BIOL001USP2) were included in this assay for comparison. Cultured HuVEC cells at a density of 5,000 cells per well were transfected using LipofectAMINE 2000® with 2 nM antisense oligonucleotide. After approximately 24 hours, RNA was isolated from the cells and PTP1B mRNA levels were measured by quantitative real-time PCR. PTP1B mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of PTP1B mRNA levels, relative to untreated control cells.

The antisense oligonucleotides in Table 1 are 5-10-5 MOE gapmers or 2-13-5 MOE gapmers. The 5-10-5 MOE gapmers have a gap segment comprising ten 2'-deoxynucleosides and two wing segment comprising five 2'-MOE nucleosides. The 2-13-5 MOE gapmers have a gap segment comprising thirteen 2'-deoxynucleosides, a 5' wing segment comprising two 2'-MOE nucleosides, and a 3' wing segment comprising three 2'-MOE nucleosides. The internucleoside linkages throughout each gapmer are phosphorothioate (P═S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. ‘Target start site’ indicates the 5'-most nucleotide to which the antisense oligonucleotide is targeted in the human gene sequence. ‘Target stop site’ indicates the 3'-most nucleotide to which the antisense oligonucleotide is targeted in the human gene sequence. All the antisense oligonucleotides listed in Table 1 target either the mRNA sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_002827.2) or the genomic sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession NT_011362.9 truncated from nucleotides 14178000 to 14256000), or both.

Some of the human oligonucleotides of Table 1 are also fully cross-reactive with rhesus monkey gene sequences. ‘n/a’ indicates that there were more than 3 base mismatches between the human oligonucleotide and the rhesus monkey gene sequence. The greater the complementarity between the human oligonucleotide and the rhesus monkey sequence, the more likely the human oligonucleotide can cross-react with the rhesus monkey sequence. The human oligonucleotides in Table 1 were compared to SEQ ID NO: 3 (exons 1-9, intron 9 and exon 10 from the rhesus monkey PTP1B scaffold). “Rhesus monkey Target start site” indicates the 5'-most nucleotide to which the gapmer is targeted in the rhesus monkey gene sequence. “Rhesus monkey Target stop site” indicates the 3'-most nucleotide to which the gapmer is targeted rhesus monkey gene sequence.

TABLE 1

Inhibition of human PTP1B RNA transcript in HuVEC cells by antisense oligonucleotides targeting SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3

ISIS No	Sequence	Motif	% inhibition	Start Site at SEQ ID NO: 1	Start Site at SEQ ID NO: 2	Start Site at SEQ ID NO: 3	SEQ ID NO
142025	TTGTCGATCTGCTCGAACTC	5-10-5	43	190	1989	197	4
142026	GACTTGTCGATCTGCTCGAA	5-10-5	59	193	1992	989	5

TABLE 1-continued

Inhibition of human PTP1B RNA transcript in HuVEC cells by antisense oligonucleotides targeting SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3

ISIS No	Sequence	Motif	% inhibi-tion	Start Site at SEQ ID NO: 1	Start Site at SEQ ID NO: 2	Start Site at SEQ ID NO: 3	SEQ ID NO
107772	CCCGGACTTGTCGATCTGCT	5-10-5	66	197	1996	3754	6
142027	GCTCCCGGACTTGTCGATCT	5-10-5	55	200	1999	3759	7
142028	CCAGCTCCCGGACTTGTCGA	5-10-5	60	203	2002	4498	8
373125	GGCACCTTCGATCACAGCCA	5-10-5	55	989	70726	4487	9
113715	GCTCCTTCCACTGATCCTGC	5-10-5	50	1035	n/a	4500	10
107831	GGTCATGCACAGGCAGGTTG	5-10-5	70	2360	75039	3753	11
409988	AGGTCATGCACAGGCAGGTT	2-13-5	83	2361	75040	3759	12
409821	GATCAGGTCATGCACAGGCA	5-10-5	86	2365	75044	3575	13
404176	TGATCAGGTCATGCACAGGC	5-10-5	87	2366	75045	4493	14
146908	ACCCTTGGAATGTCTGAGTT	5-10-5	56	2544	75223	3746	15
404169	CCCATACCCTTGGAATGTCT	5-10-5	77	2549	75228	3756	16
409815	TCCCATACCCTTGGAATGTC	5-10-5	72	2550	75229	3566	17
146909	TTCCCATACCCTTGGAATGT	5-10-5	43	2551	75230	3569	18
409845	TATTCCATGGCCATTGTAAA	5-10-5	23	3283	75962	4485	19
410030	TTATTCCATGGCCATTGTAA	2-13-5	24	3284	75963	4486	20
409825	TTTATTCCATGGCCATTGTA	5-10-5	34	3285	75964	192	21
409883	GTTTATTCCATGGCCATTGT	3-14-3	36	3286	75965	198	22
409999	GGTTTATTCCATGGCCATTG	2-13-5	54	3287	75966	190	23
409826	GGTTTATTCCATGGCCATTG	5-10-5	73	3287	75966	201	23
410000	TGGTTTATTCCATGGCCATT	2-13-5	55	3288	75967	194	24
404172	TGGTTTATTCCATGGCCATT	5-10-5	61	3288	75967	192	24
410001	ATGGTTTATTCCATGGCCAT	2-13-5	51	3289	75968	198	25
409827	ATGGTTTATTCCATGGCCAT	5-10-5	44	3289	75968	195	25
410002	AATGGTTTATTCCATGGCCA	2-13-5	0	3290	75969	204	26
404173	AATGGTTTATTCCATGGCCA	5-10-5	48	3290	75969	201	26
410003	AAATGGTTTATTCCATGGCC	2-13-5	64	3291	75970	193	27
142082	AAATGGTTTATTCCATGGCC	5-10-5	52	3291	75970	190	27
410004	AAAATGGTTTATTCCATGGC	2-13-5	46	3292	75971	196	28
409828	AAAATGGTTTATTCCATGGC	5-10-5	44	3292	75971	194	28
409829	AAAAATGGTTTATTCCATGG	5-10-5	36	3293	75972	198	29
404161	GGTCATTTCCATGGCCAGAG	2-13-5	78	n/a	73855	3746	31
409975	GGAGGTCATTTCCATGGCCA	2-13-5	85	n/a	73858	n/a	32
409976	AGGAGGTCATTTCCATGGCC	2-13-5	85	n/a	73859	2379	30

Example 2

Dose-Dependent Antisense Inhibition of Human PTP1B mRNA in HuVEC Cells

Several antisense oligonucleotides, which displayed significant antisense inhibition of PTP1B mRNA in the study described in Example 1 were further tested in HuVEC cells at various doses. Cells were plated at a density of 5,000 cells per well and transfected using LipofectAMINE 2000® with 0.9375 nM, 1.875 nM, 3.75 nM, 7.5 nM, 15 nM, and 30 nM concentrations of each antisense oligonucleotide. After approximately 16 hours, RNA was isolated from the cells and PTP1B mRNA transcript levels were measured by quantitative real-time PCR using primer probe set RTS3000 (forward sequence CTGGTTTAACCTCCTATCCTTGGA, designated herein as SEQ ID NO: 33; reverse sequence CAGAGCAGCTCGCTACCTCTCT, designated herein as SEQ ID NO: 34, probe sequence CAGCTGGCTCTCCACCTTGTTACACATTATGT, designated herein as SEQ ID NO: 35). PTP1B mRNA levels were normalized to total RNA content, as measured by RIBOGREEN®. Results are presented in Table 2 as percent inhibition of PTP1B mRNA, relative to untreated control cells.

TABLE 2

Dose-dependent antisense inhibition of human PTP1B in HuVEC cells

ISIS No	0.9375 nM	1.875 nM	3.75 nM	7.5 nM	15.0 nM	30.0 nM	IC_{50} (nM)
113715	0	0	2	11	23	33	>30
404161	1	0	7	29	42	57	17
404169	0	6	17	37	57	72	7
404176	0	0	20	38	68	79	6
409815	0	0	7	30	48	65	12
409821	0	1	17	41	68	82	5
409826	0	0	10	30	47	64	12
409975	0	0	23	50	74	86	4
409976	0	0	21	46	65	82	5
409988	0	0	23	49	70	83	5
410003	0	0	4	16	28	46	>30

Example 3

Dose-Dependent Antisense Inhibition of PTP1B mRNA in LLC-MK2 Cells

The antisense oligonucleotides from the study described in Example 2 are also cross-reactive with rhesus monkey the gene sequence (SEQ ID NO: 3) and were further tested in rhesus monkey LLC-MK2 cells at various doses. Cells were plated at a density of 3,000 cells per well and transfected using Lipofectin with 3.125 nM, 6.25 nM, 12.5 nM, 25 nM, 50 nM, and 100 nM concentrations of each antisense oligonucleotide. After approximately 16 hours, RNA was isolated from the cells and PTP1B mRNA levels were measured by quantitative real-time PCR using primer probe set RTS198 (forward sequence GGAGTTCGAGCAGATCGACAA, designated herein as SEQ ID NO: 36; reverse sequence GGCCACTCTACATGGGAAGTC, designated herein as SEQ ID NO: 37, probe sequence AGCTGGGCGGCCATTTACCAGGAT, designated herein as SEQ ID NO: 38). PTP1B mRNA levels were normalized to total RNA content, as measured by RIBOGREEN®. Results are presented in Table 3 as percent inhibition of PTP1B mRNA, relative to untreated control cells. The start and stop sites of each oligonucleotide on rhesus monkey SEQ ID NO: 3 (the concatenation of exons 1-9, intron 9 and exon 10 from the rhesus PTP1B scaffold (gene scaffold 240) are presented in Table 4.

TABLE 3

Dose-dependent antisense inhibition of PTP1B mRNA in LLC-MK2 cells

ISIS No	3.125 nM	6.25 nM	12.5 nM	25.0 nM	50.0 nM	100.0 nM	IC_{50} (nM)
113715	9	18	18	42	71	88	12
404161	18	26	37	49	67	79	9
404169	9	33	36	52	70	85	8
404176	4	21	28	52	73	85	10
409815	19	27	32	51	67	83	9
409821	4	20	37	53	74	85	9
409826	7	31	63	46	62	78	8
409975	13	20	28	43	62	74	15
409976	12	20	37	42	65	77	12
409988	3	20	39	56	73	86	8
410003	16	24	36	43	65	80	11

TABLE 4

Target sites of antisense oligonucleotides targeting PTP1B on rhesus monkey gene sequence (SEQ ID NO: 3)

ISIS No	Start Site	Stop Site
113715	1035	1054
404161	2385	2404
409975	2388	2407
409976	2389	2408
409988	3566	3585
409821	3570	3589
404176	3571	3590
404169	3754	3773
409815	3755	3774
409826	4491	4510
410003	4495	4514

Example 4

Dose-Dependent Antisense Inhibition of PTP1B mRNA in Cynomolgus Primary Hepatocytes

Some of the antisense oligonucleotides from the study described in Examples 1, 2 and 3 were further tested in cynomolgus primary hepatocytes at various doses. Cells were plated at a density of 35,000 cells per well and transfected using Lipofectin with 6.25 nM, 12.5 nM, 25 nM, 50 nM, 100 nM, and 200 nM concentrations of each antisense oligonucleotide. After approximately 16 hours, RNA was isolated from the cells and PTP1B mRNA levels were measured by quantitative real-time PCR using primer probe set RTS198. PTP1B mRNA levels were normalized to total RNA content, as measured by RIBOGREEN®. Results are presented in Table 5 as percent inhibition of PTP1B mRNA, relative to untreated control cells.

TABLE 5

Dose-dependent antisense inhibition of PTP1B mRNA in cynomolgus primary hepatocytes							
ISIS No	6.25 nM	12.5 nM	25.0 nM	50.0 nM	100.0 nM	200.0 nM	IC_{50} (nM)
373125	16	4	13	32	48	67	104
404161	7	3	24	40	56	77	72
404169	0	13	27	44	57	77	67
404176	16	17	27	42	64	76	59
409815	0	24	26	40	57	75	69
409821	0	9	25	37	60	73	73
409826	8	28	10	37	56	71	82
409975	13	19	29	38	57	75	67
409976	2	18	13	35	60	80	70
409988	16	22	28	41	59	77	61
410003	17	10	37	46	60	78	56

Example 5

Antisense Inhibition of Human PTP1B mRNA in HuVEC Cells by Oligonucleotides Designed by Microwalk

Additional gapmers were designed based on ISIS 409826 that demonstrated significant inhibition of PTP1B in all cell lines tested. These gapmers were designed by creating gapmers shifted slightly upstream and downstream (i.e. "microwalk") of ISIS 409826. Oligonucleotides were also created with various motifs, e.g. 5-10-5 MOE, 5-8-5 MOE, 2-13-5 MOE, 6-8-6 MOE motifs, or were uniform oligonucleotides with deoxy and MOE units. These gapmers were tested in vitro. ISIS oligonucleotides ISIS 142082, ISIS 113715, ISIS 373125, ISIS 404161, ISIS 404172, ISIS 404173, ISIS 404176, ISIS 409825, ISIS 409827, ISIS 409828, ISIS 409829, ISIS 409845, ISIS 409998, ISIS 409999, ISIS 410000, ISIS 410001, ISIS 410002, ISIS 410003, ISIS 410004, and ISIS 410030 (from Example 1), as well as ISIS 399038, ISIS 404159, and ISIS 404174, from a previous application (CORE0061WO15), were also included in the assay for comparison. Cultured HuVEC cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and PTP1B mRNA levels were measured by quantitative real-time PCR. The human primer probe set RTS3000 was used to measure PTP1B mRNA levels. PTP1B mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of PTP1B mRNA, relative to untreated control cells. The results are presented in Tables 6 and 7.

The 5-10-5 MOE gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleotides each. The 5-8-5 MOE gapmers are 18 nucleotides in length, wherein the central gap segment is comprised of eight 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleotides each. The 2-13-5 MOE gapmers are 20 nucleotides in length, wherein the central gap segment is comprised of thirteen 2'-deoxynucleotides and is flanked on the 5' and the 3' directions with wings comprising two and five nucleotides respectively. The 6-8-6 MOE gapmers are 18 nucleotides in length, wherein the central gap segment is comprised of eight 2'-deoxynucleotides and is flanked on both sides (in the 5' and 3' directions) by wings comprising six nucleotides each. For each of the motifs (5-10-5, 5-8-5, 2-13-5, and 6-8-6), each nucleotide in the 5' wing segment and each nucleotide in the 3' wing segment has a 2'-MOE modification. The uniform oligonucleotides have deoxy and MOE units distributed throughout the length of the oligonucleotide. The symbols for the various unit chemistries in the uniform oligonucleotide sequences are as follows: 'd'=2'-deoxyribose; 'e'=2'-O-methoxyethyl ribose. The internucleoside linkages throughout each gapmer are phosphorothioate (P═S) linkages. All cytidine residues throughout each gapmer are 5-methylcytidines. "Target start site" indicates the 5'-most nucleotide to which the gapmer is targeted. "Target stop site" indicates the 3'-most nucleotide to which the gapmer is targeted. Each gapmer listed in Table 6 is targeted SEQ ID NO: 1 (GENBANK Accession No. NM_002827.2). All the antisense oligonucleotides listed in Table 7 target SEQ ID NO: 2 (GENBANK Accession NT_011362.9 truncated from nucleotides 14178000 to 14256000).

As shown in Tables 6 and 7, several of the gapmers exhibited at least 50% inhibition, including ISIS numbers: 113715, 142082, 373125, 399038, 404159, 404161, 404172, 404173, 404176, 409826, 409827, 409999, 410000, 410001, 410002, 410003, 410004, 438371, 438372, 438373, 438374, 438375, 438377, 438379, 438380, 438381, 438382, 438383, 438384, 438439, 438442, 438443, 438444, 438445, 438450, 438451, 438452, 438453, 438454, 438455, 438456, 438458, 438459, 438460, 438461, 438462, 438464, 438465, 438468, 438469, 438472, 438473, and 438474.

Several of the gapmers exhibited at least 60% inhibition, including ISIS numbers: 113715, 142082, 373125, 399038, 404161, 404172, 404173, 404176, 409826, 409827, 409999, 410000, 410001, 410002, 410003, 438373, 438374, 438380, 438381, 438382, 438442, 438444, 438445, 438450, 438451, 438452, 438453, 438459, 438460, 438461, 438462, 438468, 438469, 438472, and 438474.

Several of the gapmers exhibited at least 70% inhibition, including ISIS numbers: 142082, 373125, 399038, 404161, 404172, 404173, 404176, 409826, 409827, 409999, 410000, 410001, 410002, 410003, 438373, 438374, 438444, 438451, 438452, 438453, 438460, 438461, 438462, 438468, 438469, 438472, and 438474.

Several of the gapmers exhibited at least 80% inhibition, including ISIS numbers: 142082, 404161, 404173, 404176, 409826, 410000, 410001, 410002, 410003, 438451, 438452, 438460, 438461, and 438474.

Several of the gapmers exhibited at least 85% inhibition, including ISIS numbers: 142082, 404161, 404173, 404176, 409826, 410001, 410002, and 410003.

Several of the gapmers exhibited at least 90% inhibition, including ISIS numbers: 142082, 404161, and 409826.

TABLE 6

Inhibition of human PTP1B mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1

Start Site	Stop Site	ISIS No	Sequence	Motif	% inhibition	SEQ ID NO
989	1008	373125	GGCACCTTCGATCACAGCCA	5-10-5 MOE	72	9
1035	1054	113715	GCTCCTTCCACTGATCCTGC	5-10-5 MOE	68	10
2366	2385	404176	TGATCAGGTCATGCACAGGC	5-10-5 MOE	89	14
3283	3302	409845	TATTCCATGGCCATTGTAAA	5-10-5 MOE	32	19
3284	3303	404174	TTATTCCATGGCCATTGTAA	5-10-5 MOE	47	20
3284	3303	410030	TTATTCCATGGCCATTGTAA	2-13-5 MOE	47	20
3284	3303	438377	$T_eT_eA_dT_dT_dC_eC_eA_dT_dG_dG_dC_dC_dA_dT_dT_dG_dT_dA_eA_e$	Deoxy and MOE units	52	20
3284	3303	438439	$T_eT_eA_dT_dT_dC_dC_dA_dT_dG_eG_eC_dC_dA_dT_dT_dG_dT_dA_eA_e$	Deoxy and MOE units	53	20
3284	3303	438448	$T_eT_eA_eT_dT_dC_eC_eA_dT_dG_dG_dC_dC_dA_dT_dT_dG_dT_eA_eA_e$	Deoxy and MOE units	34	20
3284	3303	438457	$T_eT_eA_eT_dT_dC_dC_dA_eT_eG_dG_dC_dC_dA_dT_dT_dG_dT_eA_eA_e$	Deoxy and MOE units	35	20
3284	3303	438466	TTATTCCATGGCCATTGTAA	6-8-6 MOE	25	20
3285	3304	409825	TTTATTCCATGGCCATTGTA	5-10-5 MOE	47	21
3285	3304	409998	TTTATTCCATGGCCATTGTA	2-13-5 MOE	49	21
3285	3302	438368	TATTCCATGGCCATTGTA	5-8-5 MOE	32	39
3285	3304	438378	$T_eT_eT_dA_dT_dT_eC_eC_dA_dT_dG_dG_dC_dC_dA_dT_dT_dG_dT_eA_e$	Deoxy and MOE units	46	21
3285	3304	438440	$T_eT_eT_dA_dT_dT_dC_dC_dA_dT_eG_eG_dC_dC_dA_dT_dT_dG_dT_eA_e$	Deoxy and MOE units	30	21
3285	3304	438449	$T_eT_eT_eA_dT_dT_eC_eC_dA_dT_dG_dG_dC_dC_dA_dT_dT_dG_eT_eA_e$	Deoxy and MOE units	43	21
3285	3304	438458	$T_eT_eT_eA_dT_dT_dC_dC_eA_eT_dG_dG_dC_dC_dA_dT_dT_dG_eT_eA_e$	Deoxy and MOE units	53	21
3285	3304	438467	TTTATTCCATGGCCATTGTA	6-8-6 MOE	33	21
3286	3305	399038	GTTTATTCCATGGCCATTGT	5-10-5 MOE	74	22
3286	3305	404159	GTTTATTCCATGGCCATTGT	2-13-5 MOE	54	22
3286	3303	438369	TTATTCCATGGCCATTGT	5-8-5 MOE	33	40
3286	3305	438379	$G_eT_eT_dT_dA_dT_eT_eC_dC_dA_dT_dG_dG_dC_dC_dA_dT_dT_dG_eT_e$	Deoxy and MOE units	51	22
3286	3305	438441	$G_eT_eT_dT_dA_dT_dT_dC_dC_dA_eT_eG_dG_dC_dC_dA_dT_dT_dG_eT_e$	Deoxy and MOE units	40	22
3286	3305	438450	$G_eT_eT_eT_dA_dT_eT_eC_dC_dA_dT_dG_dG_dC_dC_dA_dT_dT_eG_eT_e$	Deoxy and MOE units	64	22
3286	3305	438459	$G_eT_eT_eT_dA_dT_dT_dC_eC_eA_dT_dG_dG_dC_dC_dA_dT_dT_eG_eT_e$	Deoxy and MOE units	68	22
3286	3305	438468	GTTTATTCCATGGCCATTGT	6-8-6 MOE	76	22
3287	3306	409826	GGTTTATTCCATGGCCATTG	5-10-5 MOE	93	23
3287	3306	409999	GGTTTATTCCATGGCCATTG	2-13-5 MOE	75	23
3287	3304	438370	TTTATTCCATGGCCATTG	5-8-5 MOE	33	41

TABLE 6-continued

Inhibition of human PTP1B mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1

Start Site	Stop Site	ISIS No	Sequence	Motif	% inhibition	SEQ ID NO
3287	3306	438380	$G_eG_eT_dT_dT_dA_eT_eT_dC_dC_dA_dT_dG_dG_dC_dC_dA_dT_dT_eG_e$	Deoxy and MOE units	63	23
3287	3306	438442	$G_eG_eT_dT_dT_dA_dT_dT_dC_dC_eA_eT_dG_dG_dC_dC_dA_dT_dT_eG_e$	Deoxy and MOE units	67	23
3287	3306	438451	$G_eG_eT_eT_dT_dA_eT_eT_dC_dC_dA_dT_dG_dG_dC_dC_dA_dT_eT_eG_e$	Deoxy and MOE units	83	23
3287	3306	438460	$G_eG_eT_eT_dT_dA_dT_dT_eC_eC_dA_dT_dG_dG_dC_dC_dA_dT_eT_eG_e$	Deoxy and MOE units	82	23
3287	3306	438469	GGTTTATTCCATGGCCATTG	6-8-6 MOE	71	23
3288	3307	404172	TGGTTTATTCCATGGCCATT	5-10-5 MOE	76	24
3288	3307	410000	TGGTTTATTCCATGGCCATT	2-13-5 MOE	83	24
3288	3305	438371	GTTTATTCCATGGCCATT	5-8-5 MOE	54	42
3288	3307	438381	$T_eG_eG_dT_dT_dT_eA_eT_dT_dC_dC_dA_dT_dG_dG_dC_dC_dAT_eT_e$	Deoxy and MOE units	69	24
3288	3307	438443	$T_eG_eG_dT_dT_dT_dA_dT_dT_dC_eC_eA_dT_dG_dG_dC_dC_dA_dT_eT_e$	Deoxy and MOE units	50	24
3288	3307	438452	$T_eG_eG_eT_dT_dT_eA_eT_dT_dC_dC_dA_dT_dG_dG_dC_dC_dA_eT_eT_e$	Deoxy and MOE units	82	24
3288	3307	438461	$T_eG_eG_eT_dT_dT_dA_dT_eT_eC_dC_dA_dT_dG_dG_dC_dC_dA_eT_eT_e$	Deoxy and MOE units	81	24
3288	3307	438470	TGGTTTATTCCATGGCCATT	6-8-6 MOE	46	24
3289	3308	409827	ATGGTTTATTCCATGGCCAT	5-10-5 MOE	74	25
3289	3308	410001	ATGGTTTATTCCATGGCCAT	2-13-5 MOE	85	25
3289	3306	438372	GGTTTATTCCATGGCCAT	5-8-5 MOE	52	43
3289	3308	438382	$A_eT_eG_dG_dT_dT_eT_eA_dT_dT_dC_dC_dA_dT_dG_dG_dC_dC_dA_eT_e$	Deoxy and MOE units	65	25
3289	3308	438444	$A_eT_eG_dG_dT_dT_dT_dA_dT_dT_eC_eC_dA_dT_dG_dG_dC_dC_dA_eT_e$	Deoxy and MOE units	72	25
3289	3308	438453	$A_eT_eG_eG_dT_dT_eT_eA_dT_dT_dC_dC_dA_dT_dG_dG_dC_dC_eA_eT_e$	Deoxy and MOE units	72	25
3289	3308	438462	$A_eT_eG_eG_dT_dT_dT_dA_eT_eT_dC_dC_dA_dT_dG_dG_dC_dC_eA_eT_e$	Deoxy and MOE units	70	25
3289	3308	438471	ATGGTTTATTCCATGGCCAT	6-8-6 MOE	45	25
3290	3309	404173	AATGGTTTATTCCATGGCCA	5-10-5 MOE	85	26
3290	3309	410002	AATGGTTTATTCCATGGCCA	2-13-5 MOE	85	26
3290	3307	438373	TGGTTTATTCCATGGCCA	5-8-5 MOE	70	44
3290	3309	438383	$A_eA_eT_dG_dG_dT_eT_eT_dA_dT_dT_dC_dC_dA_dT_dG_dG_dC_dC_eA_e$	Deoxy and MOE units	54	26
3290	3309	438445	$A_eA_eT_dG_dG_dT_dT_dT_dA_dT_eT_eC_dC_dA_dT_dG_dG_dC_dC_eA_e$	Deoxy and MOE units	66	26
3290	3309	438454	$A_eA_eT_eG_dG_dT_eT_eT_dA_dT_dT_dC_dC_dA_dT_dG_dG_dC_eC_eA_e$	Deoxy and MOE units	52	26
3290	3309	438463	$A_eA_eT_eG_dG_dT_dT_dT_eA_eT_dT_dC_dC_dA_dT_dG_dG_dC_eC_eA_e$	Deoxy and MOE units	39	26

TABLE 6-continued

Inhibition of human PTP1B mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 1

Start Site	Stop Site	ISIS No	Sequence	Motif	% inhibition	SEQ ID NO
3290	3309	438472	AATGGTTTATTCCATGGCCA	6-8-6 MOE	73	26
3291	3310	142082	AAATGGTTTATTCCATGGCC	5-10-5 MOE	90	27
3291	3310	410003	AAATGGTTTATTCCATGGCC	2-13-5 MOE	86	27
3291	3308	438374	ATGGTTTATTCCATGGCC	5-8-5 MOE	79	45
3291	3310	438384	$A_eA_eA_dT_dG_dG_eT_eT_dT_dA_dT_dT_dC_dC_dA_dT_dG_dG_dC_eC_e$	Deoxy and MOE units	53	27
3291	3310	438446	$A_eA_eA_dT_dG_dG_dT_dT_dT_dA_eT_eT_dC_dC_dA_dT_dG_dG_dC_eC_e$	Deoxy and MOE units	38	27
3291	3310	438455	$A_eA_eA_eT_dG_dG_eT_eT_dT_dA_dT_dT_dC_dC_dA_dT_dG_dG_eC_eC_e$	Deoxy and MOE units	58	27
3291	3310	438464	$A_eA_eA_eT_dG_dG_dT_dT_eT_eA_dT_dT_dC_dC_dA_dT_dG_dG_eC_eC_e$	Deoxy and MOE units	58	27
3291	3310	438473	AAATGGTTTATTCCATGGCC	6-8-6 MOE	57	27
3292	3311	409828	AAAATGGTTTATTCCATGGC	5-10-5 MOE	43	28
3292	3311	410004	AAAATGGTTTATTCCATGGC	2-13-5 MOE	58	28
3292	3309	438375	AATGGTTTATTCCATGGC	5-8-5 MOE	55	46
3292	3311	438385	$A_eA_eA_dA_dT_dG_eG_eT_dT_dT_dA_dT_dT_dC_dC_dA_dT_dG_dG_eC_e$	Deoxy and MOE units	36	28
3292	3311	438447	$A_eA_eA_dA_dT_dG_dG_dT_dT_dT_eA_eT_dT_dC_dC_dA_dT_dG_dG_eC_e$	Deoxy and MOE units	35	28
3292	3311	438456	$A_eA_eA_eA_dT_dG_eG_eT_dT_dT_dA_dT_dT_dC_dC_dA_dT_dG_eG_eC_e$	Deoxy and MOE units	58	28
3292	3311	438465	$A_eA_eA_eA_dT_dG_dGT_eT_eT_dA_dT_dT_dC_dC_dA_dT_dG_eG_eC_e$	Deoxy and MOE units	51	28
3292	3311	438474	AAAATGGTTTATTCCATGGC	6-8-6 MOE	82	28
3293	3312	409829	AAAAATGGTTTATTCCATGG	5-10-5 MOE	42	29
3293	3310	438376	AAATGGTTTATTCCATGG	5-8-5 MOE	36	47

TABLE 7

Inhibition of human PTP1B mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 2

Start Site	Stop Site	ISIS No	Sequence	Motif	% inhibition	SEQ ID NO
70726	70745	373125	GGCACCTTCGATCACAGCCA	5-10-5 MOE	72	9
73855	73874	404161	GGTCATTTCCATGGCCAGAG	2-13-5 MOE	93	31
75045	75064	404176	TGATCAGGTCATGCACAGGC	5-10-5 MOE	89	14
75962	75981	409845	TATTCCATGGCCATTGTAAA	5-10-5 MOE	32	19
75963	75982	404174	TTATTCCATGGCCATTGTAA	5-10-5 MOE	47	20
75963	75982	410030	TTATTCCATGGCCATTGTAA	2-13-5 MOE	47	20
75963	75982	438377	$T_eT_eA_dT_dT_dC_eC_eA_dT_dG_dG_dC_dC_dA_dT_dT_dG_dT_dA_eA_e$	Deoxy and MOE units	52	20

TABLE 7-continued

Inhibition of human PTP1B mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 2

Start Site	Stop Site	ISIS No	Sequence	Motif	% inhibition	SEQ ID NO
75963	75982	438439	$T_eT_eA_dT_dT_dC_dC_dA_dT_dG_eG_eC_dC_dA_dT_dT_dG_dT_dA_eA_e$	Deoxy and MOE units	53	20
75963	75982	438448	$T_eT_eA_eT_dT_dC_eC_eA_dT_dG_dG_dC_dC_dA_dT_dT_dG_dT_eA_eA_e$	Deoxy and MOE units	34	20
75963	75982	438457	$T_eT_eA_eT_dT_dC_dC_dA_eT_eG_dG_dC_dC_dA_dT_dT_dG_dT_eA_eA_e$	Deoxy and MOE units	35	20
75963	75982	438466	TTATTCCATGGCCATTGTAA	6-8-6 MOE	25	20
75964	75983	409825	TTTATTCCATGGCCATTGTA	5-10-5 MOE	47	21
75964	75983	409998	TTTATTCCATGGCCATTGTA	2-13-5 MOE	49	21
75964	75981	438368	TATTCCATGGCCATTGTA	5-8-5 MOE	32	39
75964	75983	438378	$T_eT_eT_dA_dT_dT_eC_eC_dA_dT_dG_dG_dC_dC_dA_dT_dT_dG_dT_eA_e$	Deoxy and MOE units	46	21
75964	75983	438440	$T_eT_eT_dA_dT_dT_dC_dC_dA_dT_eG_eG_dC_dC_dA_dT_dT_dG_dT_eA_e$	Deoxy and MOE units	30	21
75964	75983	438449	$T_eT_eT_eA_dT_dT_eC_eC_dA_dT_dG_dG_dC_dC_dA_dT_dT_dG_eT_eA_e$	Deoxy and MOE units	43	21
75964	75983	438458	$T_eT_eT_eA_dT_dT_dC_dC_eA_eT_dG_dG_dC_dC_dA_dT_dT_dG_eT_eA_e$	Deoxy and MOE units	53	21
75964	75983	438467	TTTATTCCATGGCCATTGTA	6-8-6 MOE	33	21
75965	75984	399038	GTTTATTCCATGGCCATTGT	5-10-5 MOE	74	22
75965	75984	404159	GTTTATTCCATGGCCATTGT	2-13-5 MOE	54	22
75965	75982	438369	TTATTCCATGGCCATTGT	5-8-5 MOE	33	40
75965	75984	438379	$G_eT_eT_dT_dA_dT_eT_eC_dC_dA_dT_dG_dG_dC_dC_dA_dT_dT_dG_eT_e$	Deoxy and MOE units	51	22
75965	75984	438441	$G_eT_eT_dT_dA_dT_dT_dC_dC_dA_eT_eG_dG_dC_dC_dA_dT_dT_dG_eT_e$	Deoxy and MOE units	40	22
75965	75984	438450	$G_eT_eT_eT_dA_dT_eT_eC_dC_dA_dT_dG_dG_dC_dC_dA_dT_dT_eG_eT_e$	Deoxy and MOE units	64	22
75965	75984	438459	$G_eT_eT_eT_dA_dT_dT_dC_eC_eA_dT_dG_dG_dC_dC_dA_dT_dT_eG_eT_e$	Deoxy and MOE units	68	22
75965	75984	438468	GTTTATTCCATGGCCATTGT	6-8-6 MOE	76	22
75966	75985	409826	GGTTTATTCCATGGCCATTG	5-10-5 MOE	93	23
75966	75985	409999	GGTTTATTCCATGGCCATTG	2-13-5 MOE	75	23
75966	75983	438370	TTTATTCCATGGCCATTG	5-8-5 MOE	33	41
75966	75985	438380	$G_eG_eT_dT_dT_dA_eT_eT_dC_dC_dA_dT_dG_dG_dC_dC_dA_dT_dT_eG_e$	Deoxy and MOE units	63	23
75966	75985	438442	$G_eG_eT_dT_dT_dA_dT_dT_dC_dC_eA_eT_dG_dG_dC_dC_dA_dT_dT_eG_e$	Deoxy and MOE units	67	23
75966	75985	438451	$G_eG_eT_eT_dT_dA_eT_eT_dC_dC_dA_dT_dG_dG_dC_dC_dA_dT_eT_eG_e$	Deoxy and MOE units	83	23
75966	75985	438460	$G_eG_eT_eT_dT_dA_dT_dT_eC_eC_dA_dT_dG_dG_dC_dC_dA_dT_eT_eG_e$	Deoxy and MOE units	82	23
75966	75985	438469	GGTTTATTCCATGGCCATTG	6-8-6 MOE	71	23
75967	75986	404172	TGGTTTATTCCATGGCCATT	5-10-5 MOE	76	24

TABLE 7-continued

Inhibition of human PTP1B mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 2

Start Site	Stop Site	ISIS No	Sequence	Motif	% inhibition	SEQ ID NO
75967	75986	410000	TGGTTTATTCCATGGCCATT	2-13-5 MOE	83	24
75967	75984	438371	GTTTATTCCATGGCCATT	5-8-5 MOE	54	42
75967	75986	438381	$T_eG_eG_dT_dT_dT_eA_eT_dT_dC_dC_dA_dT_dG_dG_dC_dC_dAT_eT_e$	Deoxy and MOE units	69	24
75967	75986	438443	$T_eG_eG_dT_dT_dT_dA_dT_dT_dC_eC_eA_dT_dG_dG_dC_dC_dA_dT_eT_e$	Deoxy and MOE units	50	24
75967	75986	438452	$T_eG_eG_eT_dT_dT_eA_eT_dT_dC_dC_dA_dT_dG_dG_dC_dC_dA_eT_eT_e$	Deoxy and MOE units	82	24
75967	75986	438461	$T_eG_eG_eT_dT_dT_dA_dT_eT_eC_dC_dA_dT_dG_dG_dC_dC_dA_eT_eT_e$	Deoxy and MOE units	81	24
75967	75986	438470	TGGTTTATTCCATGGCCATT	6-8-6 MOE	46	24
75968	75987	409827	ATGGTTTATTCCATGGCCAT	5-10-5 MOE	74	25
75968	75987	410001	ATGGTTTATTCCATGGCCAT	2-13-5 MOE	85	25
75968	75985	438372	GGTTTATTCCATGGCCAT	5-8-5 MOE	52	43
75968	75987	438382	$A_eT_eG_dG_dT_dT_eT_eA_dT_dT_dC_dC_dA_dT_dG_dG_dC_dC_dA_eT_e$	Deoxy and MOE units	65	25
75968	75987	438444	$A_eT_eG_dG_dT_dT_dT_dA_dT_dT_eC_eC_dA_dT_dG_dG_dC_dC_dA_eT_e$	Deoxy and MOE units	72	25
75968	75987	438453	$A_eT_eG_eG_dT_dT_eT_eA_dT_dT_dC_dC_dA_dT_dG_dG_dC_dC_eA_eT_e$	Deoxy and MOE units	72	25
75968	75987	438462	$A_eT_eG_eG_dT_dT_dT_dA_eT_eT_dC_dC_dA_dT_dG_dG_dC_dC_eA_eT_e$	Deoxy and MOE units	70	25
75968	75987	438471	ATGGTTTATTCCATGGCCAT	6-8-6 MOE	45	25
75969	75988	404173	AATGGTTTATTCCATGGCCA	5-10-5 MOE	85	26
75969	75988	410002	AATGGTTTATTCCATGGCCA	2-13-5 MOE	85	26
75969	75986	438373	TGGTTTATTCCATGGCCA	5-8-5 MOE	70	44
75969	75988	438383	$A_eA_eT_dG_dG_dT_eT_eT_dA_dT_dT_dC_dC_dA_dT_dG_dG_dC_dC_eA_e$	Deoxy and MOE units	54	26
75969	75988	438445	$A_eA_eT_dG_dG_dT_dT_dT_dA_dT_eT_eC_dC_dA_dT_dG_dG_dC_dC_eA_e$	Deoxy and MOE units	66	26
75969	75988	438454	$A_eA_eT_eG_dG_dT_eT_eT_dA_dT_dT_dC_dC_dA_dT_dG_dG_dC_eC_eA_e$	Deoxy and MOE units	52	26
75969	75988	438463	$A_eA_eT_eG_dG_dT_dT_dT_eA_eT_dT_dC_dC_dA_dT_dG_dG_dC_eC_eA_e$	Deoxy and MOE units	39	26
75969	75988	438472	AATGGTTTATTCCATGGCCA	6-8-6 MOE	73	26
75970	75989	142082	AAATGGTTTATTCCATGGCC	5-10-5 MOE	90	27
75970	75989	410003	AAATGGTTTATTCCATGGCC	2-13-5 MOE	86	27
75970	75987	438374	ATGGTTTATTCCATGGCC	5-8-5 MOE	79	45
75970	75989	438384	$A_eA_eA_dT_dG_dG_eT_eT_dT_dA_dT_dT_dC_dC_dA_dT_dG_dG_dC_eC_e$	Deoxy and MOE units	53	27
75970	75989	438446	$A_eA_eA_dT_dG_dG_dT_dT_dT_dA_eT_eT_dC_dC_dA_dT_dG_dG_dC_eC_e$	Deoxy and MOE units	38	27
75970	75989	438455	$A_eA_eA_eT_dG_dG_eT_eT_dT_dA_dT_dT_dC_dC_dA_dT_dG_dG_eC_eC_e$	Deoxy and MOE units	58	27

TABLE 7-continued

Inhibition of human PTP1B mRNA levels by chimeric antisense oligonucleotides targeted to SEQ ID NO: 2						
Start Site	Stop Site	ISIS No	Sequence	Motif	% inhibition	SEQ ID NO
75970	75989	438464	$A_eA_eA_eT_dG_dG_dT_dT_eT_eA_dT_dT_dC_dC_dA_dT_dG_dG_eC_eC_e$	Deoxy and MOE units	58	27
75970	75989	438473	AAATGGTTTATTCCATGGCC	6-8-6 MOE	57	27
75971	75990	409828	AAAATGGTTTATTCCATGGC	5-10-5 MOE	43	28
75971	75990	410004	AAAATGGTTTATTCCATGGC	2-13-5 MOE	58	28
75971	75988	438375	AATGGTTTATTCCATGGC	5-8-5 MOE	55	46
75971	75990	438385	$A_eA_eA_dA_dT_dG_eG_eT_dT_dT_dA_dT_dT_dC_dC_dA_dT_dG_dG_eC_e$	Deoxy and MOE units	36	28
75971	75990	438447	$A_eA_eA_dA_dT_dG_dG_dT_dT_dT_eA_eT_dT_dC_dC_dA_dT_dG_dG_eC_e$	Deoxy and MOE units	35	28
75971	75990	438456	$A_eA_eA_eA_dT_dG_eG_eT_dT_dT_dA_dT_dT_dC_dC_dA_dT_dG_eG_eC_e$	Deoxy and MOE units	58	28
75971	75990	438465	$A_eA_eA_eA_dT_dG_dGT_eT_eT_dA_dT_dT_dC_dC_dA_dT_dG_eG_eC_e$	Deoxy and MOE units	51	28
75971	75990	438474	AAAATGGTTTATTCCATGGC	6-8-6 MOE	82	28
75972	75991	409829	AAAAATGGTTTATTCCATGG	5-10-5 MOE	42	29
75972	75989	438376	AAATGGTTTATTCCATGG	5-8-5 MOE	36	47

Example 6

Dose-Dependent Antisense Inhibition of Human PTP1B mRNA in HuVEC Cells

Several antisense oligonucleotides, which displayed significant antisense inhibition of PTP1B mRNA in the study described in Example 5 were further tested in HuVEC cells at various doses. Cells were plated at a density of 2,000 cells per well and transfected by electroporation with 31.25 nM, 62.5 nM, 125 nM, 250 nM, 500 nM, 1000 nM, 2000 nM and 4000 nM concentrations of each antisense oligonucleotide. After approximately 16 hours, RNA was isolated from the cells and PTP1B mRNA levels were measured by quantitative real-time PCR using primer probe set RTS3000. PTP1B mRNA levels were normalized to total RNA content, as measured by RIBOGREEN®. Results are presented in Table 8 as percent inhibition of PTP1B mRNA, relative to untreated control cells.

TABLE 8

Dose-dependent antisense inhibition of human PTP1B in HuVEC cells									
ISIS No	31.25 nM	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	2000.0 nM	4000.0 nM	IC_{50} (μM)
142082	15	30	40	42	71	84	90	94	0.2
404173	15	19	33	54	69	81	86	93	0.2
404176	9	26	34	34	67	80	88	94	0.3
409826	17	16	28	44	60	73	85	95	0.3
410002	0	0	24	52	54	77	90	96	0.4
410003	9	7	19	46	60	80	91	95	0.4
438374	20	22	40	44	59	70	79	85	0.3
438460	14	21	23	42	62	78	85	95	0.3
438474	27	0	13	34	42	68	74	86	0.6

Example 7

Dose-Dependent Antisense Inhibition of Human mRNA PTP1B in HepG2 Cells

The antisense oligonucleotides, tested in the study described in Example 6, were further tested in HepG2 cells at various doses. Cells were plated at a density of 20,000 cells per well and transfected by electroporation with 31.25 nM, 62.5 nM, 125 nM, 250 nM, 500 nM, 1000 nM, 2000 nM and 4000 nM concentrations of each antisense oligonucleotide. After approximately 16 hours, RNA was isolated from the cells and PTP1B mRNA levels were measured by quantitative real-time PCR using primer probe set RTS3000. PTP1B mRNA levels were normalized to total RNA content, as measured by RIBOGREEN®. Results are presented in Table 9 as percent inhibition of PTP1B mRNA, relative to untreated control cells. The mRNA levels were also analyzed using rhesus monkey primer probe set RTS198, and the results are presented in Table 10. The start and stop sites of each oligonucleotide on rhesus monkey SEQ ID NO: 3 are presented in Table 11.

TABLE 9

Analysis of dose-dependent antisense inhibition of human PTP1B in HepG2 cells using RTS3000									
ISIS No	31.25 nM	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	2000.0 nM	4000.0 nM	IC_{50} (μM)
142082	0	0	7	0	26	38	60	82	1.4
404173	2	0	1	19	0	29	47	80	1.9
404176	0	0	5	13	2	33	62	79	1.7
409826	0	0	0	2	15	29	46	76	1.9
410002	13	6	0	11	8	28	44	75	2.0
410003	0	0	9	11	22	33	30	83	1.9
438374	0	0	17	11	23	38	33	61	2.9
438460	4	0	10	11	9	26	52	79	1.8
438474	0	0	2	11	6	20	52	54	2.8

TABLE 10

Analysis of dose-dependent antisense inhibition of human PTP1B in HepG2 cells using RTS198									
ISIS No	31.25 nM	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	2000.0 nM	4000.0 nM	IC_{50} (μM)
142082	14	19	2	0	80	41	63	80	1.5
404173	0	0	2	0	16	26	60	83	1.6
404176	0	0	0	5	0	31	59	80	1.9
409826	0	0	0	16	23	10	49	72	2.3
410002	0	0	0	0	0	0	40	100	>4.0
410003	0	1	12	0	10	41	51	82	1.6
438374	0	0	0	9	43	22	49	55	2.8
438460	0	0	0	9	30	42	47	81	1.4
438474	2	0	9	38	19	31	49	60	2.5

TABLE 11

Target sites of antisense oligonucleotides targeting PTP1B on rhesus monkey gene sequence (SEQ ID NO: 3)			
OligoID	Start Site	Stop Site	SEQ ID NO
142082	4495	4514	27
404173	4494	4513	26
404176	3571	3590	14
409826	4491	4510	23
410002	4494	4513	26
410003	4495	4514	27
438374	4495	4512	45
438460	4491	4510	23
438474	4496	4515	28

Example 8

Dose-Dependent Antisense Inhibition of Human PTP1B mRNA in HepG2 Cells

Short antisense oligonucleotides to the target site of ISIS 142082 were designed. The target sites, motifs and sequence details of these shortmers are presented in Table 12. These antisense oligonucleotides were tested in HepG2 cells at various doses. Some of the antisense oligonucleotides from the study described in Example 7 were included in the assay for comparison. Cells were plated at a density of 20,000 cells per well and transfected by electroporation with 78.125 nM, 156.25 nM, 312.5 nM, 625 nM, 1,250 nM, 2,500 nM, 5,000 nM and 10,000 nM concentrations of each antisense oligonucleotide. After approximately 16 hours, RNA was isolated from the cells and PTP1B mRNA levels were measured by quantitative real-time PCR using primer probe set RTS3000. PTP1B mRNA levels were normalized to total RNA content, as measured by RIBOGREEN®. Results are presented in Table 13 as percent inhibition of PTP1B mRNA, relative to untreated control cells.

TABLE 12

Target sites of antisense oligonucleotides targeting SEQ ID NO: 1					
ISIS No	Start Site	Stop Site	Sequence	Motif	SEQ ID NO
142082	3291	3310	AAATGGTTTATTCCATGGCC	5-10-5	27
446431	3292	3309	AATGGTTTATTCCATGGC	4-10-4	46
446432	3293	3308	ATGGTTTATTCCATGG	3-10-3	48

TABLE 13

Dose-dependent antisense inhibition of human PTP1B in HepG2 cells									
ISIS No	78.125 nM	156.25 nM	312.5 nM	625.0 nM	1250.0 nM	2500.0 nM	5000.0 nM	10000.0 nM	IC_{50} (μM)
113715	6	12	17	17	16	45	61	86	3.3
142082	14	34	23	47	60	81	86	90	0.8
404173	8	22	29	45	60	73	83	88	0.8
409826	19	18	41	56	75	84	89	91	0.5
410003	0	0	19	39	55	81	91	92	1.0
446431	10	24	26	38	57	74	85	92	1.0
446432	0	8	10	10	10	26	40	67	6.0

Example 9

Dose-Dependent Antisense Inhibition of PTP1B mRNA in LLC-MK2 Cells

The antisense oligonucleotides from the study described in Example 8 are also cross-reactive with rhesus monkey PTP1B gene sequence (SEQ ID NO: 3) and were further tested in rhesus monkey LLC-MK2 cells at various doses. Cells were plated at a density of 25,000 cells per well and transfected using electroporation with 78.125 nM, 156.25 nM, 312.5 nM, 625 nM, 1250 nM, 2500 nM, 5,000 nM, and 10,000 nM concentrations of each antisense oligonucleotide. After approximately 16 hours, RNA was isolated from the cells and PTP1B mRNA levels were measured by quantitative real-time PCR using primer probe set RTS198. PTP1B mRNA levels were normalized to total RNA content, as measured by RIBOGREEN®. Results are presented in Table 14 as percent inhibition of PTP1B mRNA, relative to untreated control cells. The start and stop sites of each oligonucleotide on rhesus monkey SEQ ID NO: 3 are presented in Table 15.

TABLE 14

Dose-dependent antisense inhibition of PTP1B mRNA in LLC-MK2 cells									
ISIS No	78.125 nM	156.25 nM	312.5 nM	625.0 nM	1250.0 nM	2500.0 nM	5000.0 nM	10000.0 nM	IC_{50} (μM)
113715	0	0	4	13	27	53	57	70	3.3
142082	2	12	31	41	69	74	80	92	0.9
404173	2	0	22	29	36	61	78	84	1.6
409826	12	0	19	38	66	66	82	92	1.2
410003	0	0	0	26	32	65	81	91	1.8
446431	0	0	9	32	45	70	79	58	1.4
446432	0	0	7	16	10	20	26	43	37.0

TABLE 15

Target sites of antisense oligonucleotides targeting SEQ ID NO: 3				
ISIS No	Start Site	Stop Site	Sequence	SEQ ID NO
113715	1035	1054	GCTCCTTCCACTGATCCTGC	10
142082	4495	4514	AAATGGTTTATTCCATGGCC	27
404173	4494	4513	AATGGTTTATTCCATGGCCA	26
409826	4491	4510	GGTTTATTCCATGGCCATTG	23
410003	4495	4514	AAATGGTTTATTCCATGGCC	27
446431	4496	4513	AATGGTTTATTCCATGGC	46
446432	4497	4512	ATGGTTTATTCCATGG	48

Example 10

Dose-Dependent Antisense Inhibition of Human PTP1B mRNA in HuVEC Cells

The antisense oligonucleotides, tested in the study described in Examples 8 and 9, were further tested in HuVEC cells at various doses. Cells were plated at a density of 20,000 cells per well and transfected by electroporation with 31.25 nM, 62.5 nM, 125 nM, 250 nM, 500 nM, 1000 nM, 2000 nM and 4000 nM concentrations of each antisense oligonucleotide. After approximately 16 hours, RNA was isolated from the cells and PTP1B mRNA levels were measured by quantitative real-time PCR using primer probe set RTS3000. PTP1B mRNA levels were normalized to total RNA content, as measured by RIBOGREEN®. Results are presented in Table 16 as percent inhibition of PTP1B mRNA, relative to untreated control cells.

TABLE 16

Dose-dependent antisense inhibition of human PTP1B in HuVEC cells									
ISIS No	31.25 nM	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	2000.0 nM	4000.0 nM	IC_{50} (μM)
113715	10	0	22	24	65	86	92	98	0.2
142082	52	78	89	93	95	96	98	98	<0.3
404173	35	66	80	89	95	98	97	97	0.05
409826	57	72	82	64	97	98	98	98	<0.3
410003	43	47	75	84	48	95	96	91	0.05
446431	33	63	75	87	96	97	98	98	0.05
446432	0	11	30	45	66	79	85	76	0.3

Example 11

Dose-Dependent Antisense Inhibition of PTP1B mRNA in Cynomolgus Primary Hepatocytes

The antisense oligonucleotides from the study described in Examples 8-10 were further tested in cynomolgus primary hepatocytes at various doses. Cells were plated at a density of 35,000 cells per well and transfected using electroporation with 31.25 nM, 62.5 nM, 125 nM, 250 nM, 500 nM, 1,000 nM, 2,000 nM, and 4,000 nM concentrations of each antisense oligonucleotide. After approximately 16 hours, RNA was isolated from the cells and PTP1B mRNA levels were measured by quantitative real-time PCR using primer probe set RTS198. PTP1B mRNA levels were normalized to total RNA content, as measured by RIBOGREEN®. Results are presented in Table 17 as percent inhibition of PTP1B mRNA, relative to untreated control cells.

TABLE 17

Dose-dependent antisense inhibition of PTP1B mRNA in cynomolgus primary hepatocytes									
ISIS No	31.25 nM	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	2000.0 nM	4000.0 nM	IC_{50} (μM)
113715	4	26	25	43	46	73	82	95	0.4
142082	25	37	50	67	74	87	86	88	0.1
404173	18	20	43	54	67	82	85	89	0.2
409826	34	47	51	65	76	87	88	90	0.1
410003	8	20	44	53	68	79	80	83	0.2
446431	9	14	35	54	57	79	79	88	0.3
446432	4	0	1	3	0	11	6	37	>4.0

Example 12

Tolerability of Antisense Oligonucleotides Targeting Human PTP1B in a Mouse Model

ISIS oligonucleotides that demonstrated dose-dependent inhibition in the studies described in Examples 8-11 were evaluated for tolerability in a mouse model by monitoring changes in the levels of various metabolic markers in CD1 mice. Two more ISIS oligonucleotides, ISIS 446433 (4-10-4 MOE; 5'-GTTTATTCCATGGCCATT-3' (SEQ ID NO: 42); target start site at SEQ ID NO: 1 is 3288) and ISIS 446434 (3-10-3; 5'-TTTATTCCATGGCCAT-3' (SEQ ID NO: 49); target start site at SEQ ID NO: 1 is 3289) were designed as shortmers to ISIS 409826 (target start site at SEQ ID NO: 1 is 3287) and were also evaluated in this study.

Treatment

CD1 mice (available from Jackson Labs, Bar Harbor, Me.) were maintained on a 12-hour light/dark cycle and fed ad libitum normal lab chow (Harlan Laboratories, Indianapolis, Ind.). Animals were acclimated for at least 7 days in the research facility before initiation of the experiment. Antisense oligonucleotides were prepared in PBS and sterilized by filtering through a 0.2 micron filter. Oligonucleotides were dissolved in 0.9% PBS for injection.

Groups of five CD1 mice each were injected subcutaneously twice a week with 100 mg/kg of ISIS 142082, ISIS 373125, ISIS 404173, ISIS 409826, ISIS 410002, ISIS 410003, ISIS 438452, ISIS 438460, ISIS 446431, ISIS 446432, ISIS 446433, or ISIS 446434 for 4 weeks. One group of five CD1 mice was injected subcutaneously twice a week with PBS for 4 weeks. This PBS group served as the control group. Blood samples were collected via tail snipping. Two days after the last dose, body weights were taken, mice were euthanized and organs and plasma were harvested for further analysis.

Body and Organ Weights

The body weights of the mice were measured weekly. The body weights are presented in Table 18. Liver, spleen and kidney weights were measured at the end of the study, and are presented in Table 19. The results demonstrate that none of the ISIS oligonucleotides had any adverse effect on the overall health of the mice.

TABLE 18

Weekly body weights of CD1 mice during antisense oligonucleotide treatment (g)				
	Week 1	Week 2	Week 3	Week 4
PBS	29	31	32	34
ISIS 142082	31	34	34	36
ISIS 373125	29	31	32	35
ISIS 404173	31	33	34	36
ISIS 409826	31	34	34	37
ISIS 410002	32	35	35	36
ISIS 410003	31	34	34	37
ISIS 438452	32	35	36	39

TABLE 18-continued

Weekly body weights of CD1 mice during antisense oligonucleotide treatment (g)				
	Week 1	Week 2	Week 3	Week 4
ISIS 438460	31	34	34	37
ISIS 446431	30	33	33	36
ISIS 446432	27	30	30	33
ISIS 446433	30	33	33	37
ISIS 446434	30	33	34	37

TABLE 19

Organ weights of CD1 mice after antisense oligonucleotide treatment (g)				
	Liver	Fat	Spleen	Kidney
PBS	1.7	0.45	0.11	0.53
ISIS 142082	2.3	0.33	0.18	0.52
ISIS 373125	1.9	0.38	0.16	0.53
ISIS 404173	2.3	0.41	0.23	0.59
ISIS 409826	2.2	0.37	0.17	0.54
ISIS 410002	1.9	0.22	0.30	0.76
ISIS 410003	2.1	0.44	0.22	0.60
ISIS 438452	2.2	0.42	0.18	0.55
ISIS 438460	2.2	0.34	0.17	0.52
ISIS 446431	2.1	0.34	0.19	0.53
ISIS 446432	1.7	0.31	0.13	0.46
ISIS 446433	2.2	0.35	0.17	0.53
ISIS 446434	2.2	0.36	0.18	0.56

Liver Function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured bi-weekly. The results are presented in Tables 20 and 21, and indicate that most of the ISIS oligonucleotides were considered tolerable in the mice, as demonstrated by their liver transaminase profile.

TABLE 20

Effect of antisense oligonucleotide treatment on ALT (IU/L) of CD1 mice			
	Week 0	Week 2	Week 4
PBS	27	26	20
ISIS 142082	38	35	105
ISIS 373125	30	27	51
ISIS 404173	30	31	124
ISIS 409826	26	34	236
ISIS 410002	27	203	219
ISIS 410003	31	29	99
ISIS 438452	32	40	217
ISIS 438460	30	40	216
ISIS 446431	29	38	114
ISIS 446432	26	27	35
ISIS 446433	25	76	115
ISIS 446434	23	44	146

TABLE 21

Effect of antisense oligonucleotide treatment on AST (IU/L) of CD1 mice			
	Week 0	Week 2	Week 4
PBS	54	62	50
ISIS 142082	75	59	103
ISIS 373125	55	57	97
ISIS 404173	52	61	117

TABLE 21-continued

Effect of antisense oligonucleotide treatment on AST (IU/L) of CD1 mice			
	Week 0	Week 2	Week 4
ISIS 409826	49	59	192
ISIS 410002	51	151	417
ISIS 410003	64	47	122
ISIS 438452	59	56	157
ISIS 438460	65	56	217
ISIS 446431	56	66	140
ISIS 446432	50	51	74
ISIS 446433	54	87	121
ISIS 446434	42	64	132

Plasma Glucose Levels

To evaluate the effect of ISIS oligonucleotides on glucose metabolism, plasma levels of glucose were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.). Results are presented in Table 22, expressed in mg/dL. None of the ISIS oligonucleotides had any adverse effects on the glucose metabolism of the mice.

TABLE 22

Effect of antisense oligonucleotide treatment on plasma glucose levels in CD1 mice			
	Week 0	Week 2	Week 4
PBS	175	188	182
ISIS 142082	195	178	161
ISIS 373125	187	201	177
ISIS 404173	185	213	168
ISIS 409826	183	187	187
ISIS 410002	183	164	137
ISIS 410003	198	218	168
ISIS 438452	168	197	175
ISIS 438460	212	203	169
ISIS 446431	192	188	148
ISIS 446432	194	193	175
ISIS 446433	216	198	151
ISIS 446434	199	189	159

Plasma Lipid and Triglyceride Levels

To evaluate the effect of ISIS oligonucleotides on cholesterol and triglyceride metabolism, plasma levels of each were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.). Results are presented in Tables 23 and 24, expressed in mg/dL. Most of the ISIS oligonucleotides did not have any adverse effects of the lipid metabolism of the mice.

TABLE 23

Effect of antisense oligonucleotide treatment on plasma cholesterol levels in CD1 mice			
	Week 0	Week 2	Week 4
PBS	162	145	153
ISIS 142082	156	136	126
ISIS 373125	137	106	104
ISIS 404173	162	124	154
ISIS 409826	153	142	146
ISIS 410002	136	63	47
ISIS 410003	160	131	96
ISIS 438452	143	128	121
ISIS 438460	146	140	129
ISIS 446431	139	124	116
ISIS 446432	146	135	137
ISIS 446433	152	144	145
ISIS 446434	147	147	144

TABLE 24

Effect of antisense oligonucleotide treatment on plasma triglyceride levels in CD1 mice			
	Week 0	Week 2	Week 4
PBS	153	153	162
ISIS 142082	170	153	114
ISIS 373125	142	116	112
ISIS 404173	195	140	107
ISIS 409826	182	120	80
ISIS 410002	137	99	51
ISIS 410003	152	138	102
ISIS 438452	123	134	93
ISIS 438460	165	146	85
ISIS 446431	131	160	123
ISIS 446432	168	194	136
ISIS 446433	186	117	133
ISIS 446434	145	101	84

Cytokine Levels

To evaluate the effect of ISIS oligonucleotides on factors involved in inflammation, blood was collected after the end of the treatment period for measurement of cytokine levels. The samples were sent to Aushon Biosystems (Woburn, Mass.) for analysis. Levels of murine IL-6, JE, MIP-1α, and TNF-α were measured using murine antibodies. The results are presented in Table 25. Most of the ISIS oligonucleotides did not have any adverse effects on the cytokine levels of the mice.

TABLE 25

Effect of antisense oligonucleotide treatment on plasma cytokine levels in CD1 mice				
	mIL-6	mJE	mMIP-1α	mTNF-α
PBS	250	20	1	4
ISIS 142082	225	145	4	23
ISIS 373125	77	91	3	17
ISIS 404173	88	155	1	24
ISIS 409826	33	112	3	15
ISIS 410002	113	225	28	84
ISIS 410003	111	138	4	24
ISIS 438452	62	148	1	15
ISIS 438460	64	184	2	9
ISIS 446431	52	170	1	15
ISIS 446432	57	75	1	3
ISIS 446433	64	138	3	61
ISIS 446434	59	127	0	21

Example 13

Tolerability of Antisense Oligonucleotides Targeting Human PTP1B in a Rat Model

The ISIS oligonucleotides from the study described in Example 12 were further evaluated for tolerability in a rat model by monitoring changes in the levels of various metabolic markers in Sprague Dawley rats.

Treatment

Sprague Dawley rats were maintained on a 12-hour light/dark cycle and fed ad libitum normal lab chow (Harlan Laboratories, Indianapolis, Ind.). Animals were acclimated for at least 7 days in the research facility before initiation of the experiment. Antisense oligonucleotides were prepared in PBS and sterilized by filtering through a 0.2 micron filter. Oligonucleotides were dissolved in 0.9% PBS for injection.

Groups of four rats each were injected subcutaneously twice a week with ISIS 142082, ISIS 373125, ISIS 404173, ISIS 409826, ISIS 410002, ISIS 410003, ISIS 438452, ISIS 438460, ISIS 446431, ISIS 446432, ISIS 446433, or ISIS 446434 at a dose of 50 mg/kg twice a week for 4 weeks, followed by a dose of 30 mg/kg twice a week for 8 weeks. One group of four rats was injected subcutaneously twice a week with PBS for 12 weeks. This PBS group served as the control group. Blood samples were collected via tail snipping. Two days after the last dose, body weights were taken, rats were euthanized and organs and plasma were harvested for further analysis.

Body and Organ Weights

The body weights of the rats were measured weekly. The body weights are presented in Table 26. Liver, spleen and kidney weights were measured at the end of the study, and are presented in Table 27. 'n/a' indicates no data being available for that particular group at that particular time point due to all the rats in the group being euthanized before the time point.

TABLE 26

Bi-weekly body weights of Sprague Dawley rats during antisense oligonucleotide treatment (g)							
	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
PBS	288	369	405	442	463	500	495
ISIS 142082	295	348	318	345	337	347	345
ISIS 373125	304	385	399	419	427	421	448
ISIS 404173	294	346	352	377	384	391	401
ISIS 409826	292	346	350	355	356	373	356
ISIS 410002	297	333	323	324	306	335	n/a
ISIS 410003	299	341	328	320	307	305	301
ISIS 438452	301	327	335	333	327	347	332
ISIS 438460	304	345	346	347	356	377	n/a
ISIS 446431	307	376	340	357	353	357	349
ISIS 446432	287	340	344	363	372	399	404
ISIS 446433	298	331	318	354	n/a	n/a	n/a
ISIS 446434	303	366	356	n/a	n/a	n/a	n/a

TABLE 27

Organ weights of Sprague Dawley rats during antisense oligonucleotide treatment (g)				
	Liver	Fat	Spleen	Kidney
PBS	15.9	2.1	0.8	3.6
ISIS 142082	21.8	0.7	4.5	5.2
ISIS 373125	17.9	1.1	1.9	3.4
ISIS 404173	17.7	1.1	2.3	4.3
ISIS 409826	19.8	0.5	3.6	4.4
ISIS 410003	18.7	0.5	3.8	3.5
ISIS 438452	17.3	0.6	3.1	3.8
ISIS 446431	22.1	0.4	6.1	5.3
ISIS 446432	18.1	1.2	3.3	3.8

Liver Function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured bi-weekly. Plasma levels of bilirubin (mg/dL) were also measured using the same clinical chemistry analyzer. The results are presented in Tables 28, 29 and 30. 'n/a' indicates no data being available for that particular group at that particular time point due to all the rats in the group being euthanized before the time point.

TABLE 28

Effect of antisense oligonucleotide treatment on ALT (IU/L) of Sprague Dawley rats

	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
PBS	58	68	54	48	48	46	46
ISIS 142082	54	75	40	44	49	62	57
ISIS 373125	57	61	60	48	48	45	38
ISIS 404173	49	52	53	41	51	53	42
ISIS 409826	51	59	56	50	42	37	40
ISIS 410002	46	65	75	73	103	126	n/a
ISIS 410003	49	78	62	49	61	59	66
ISIS 438452	46	57	58	53	51	52	50
ISIS 438460	49	88	162	96	114	91	n/a
ISIS 446431	51	57	45	45	40	55	49
ISIS 446432	52	59	48	43	44	49	46
ISIS 446433	53	120	65	86	n/a	n/a	n/a
ISIS 446434	53	76	161	n/a	n/a	n/a	n/a

TABLE 29

Effect of antisense oligonucleotide treatment on AST (IU/L) of Sprague Dawley rats

	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
PBS	84	92	86	79	91	74	81
ISIS 142082	87	89	86	126	136	154	149
ISIS 373125	79	72	93	124	111	95	81
ISIS 404173	75	69	88	75	89	96	83
ISIS 409826	75	74	96	112	108	90	106
ISIS 410002	67	87	155	173	229	245	n/a
ISIS 410003	71	95	106	136	161	160	186
ISIS 438452	70	84	104	157	164	174	167
ISIS 438460	79	122	214	287	216	172	n/a
ISIS 446431	73	79	93	137	129	158	153
ISIS 446432	80	76	86	99	96	105	102
ISIS 446433	77	151	128	234	n/a	n/a	n/a
ISIS 446434	81	137	359	n/a	n/a	n/a	n/a

TABLE 30

Effect of antisense oligonucleotide treatment on Bilirubin (mg/dL) of Sprague Dawley rats

	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
PBS	0.11	0.15	0.14	0.16	0.25	0.16	0.13
ISIS 142082	0.12	0.11	0.18	0.12	0.12	0.15	0.15
ISIS 373125	0.13	0.13	0.15	0.36	0.14	0.15	0.13
ISIS 404173	0.11	0.13	0.14	0.13	0.13	0.14	0.10
ISIS 409826	0.12	0.12	0.15	0.12	0.11	0.10	0.10
ISIS 410002	0.11	0.13	0.18	0.13	0.19	0.54	n/a
ISIS 410003	0.12	0.12	0.14	0.16	0.14	0.17	0.14
ISIS 438452	0.12	0.13	0.15	0.13	0.14	0.13	0.13
ISIS 438460	0.11	0.14	0.22	0.28	0.15	0.17	n/a
ISIS 446431	0.14	0.17	0.19	0.13	0.10	0.16	0.16
ISIS 446432	0.12	0.14	0.13	0.12	0.11	0.14	0.13
ISIS 446433	0.12	0.12	0.18	0.20	n/a	n/a	n/a
ISIS 446434	0.12	0.17	0.20	n/a	n/a	n/a	n/a

Kidney Function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma levels of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.). Results are presented in Tables 31 and 32, expressed in mg/dL. The total urine protein to creatinine ratio was also calculated and the results are presented in Table 33.

TABLE 31

Effect of antisense oligonucleotide treatment on BUN (mg/dL) of Sprague Dawley rats

	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
PBS	17	20	20	18	26	18	16
ISIS 142082	21	23	31	23	31	24	29
ISIS 373125	21	21	24	19	31	21	21
ISIS 404173	18	19	21	19	25	21	20
ISIS 409826	20	21	24	23	28	22	26
ISIS 410002	19	22	25	23	29	32	n/a
ISIS 410003	18	20	23	23	30	29	26
ISIS 438452	19	22	27	25	29	22	24
ISIS 438460	20	23	25	26	31	24	n/a
ISIS 446431	19	21	24	23	29	24	23
ISIS 446432	20	21	24	20	29	23	19
ISIS 446433	18	21	25	53	n/a	n/a	n/a
ISIS 446434	18	23	120	n/a	n/a	n/a	n/a

TABLE 32

Effect of antisense oligonucleotide treatment on creatinine (mg/dL) of Sprague Dawley rats

	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
PBS	0.2	0.3	0.4	0.3	0.5	0.4	0.3
ISIS 142082	0.3	0.3	0.5	0.4	0.5	0.5	0.4
ISIS 373125	0.3	0.5	0.6	0.4	0.6	0.6	0.4
ISIS 404173	0.3	0.3	0.5	0.4	0.5	0.6	0.4
ISIS 409826	0.3	0.4	0.6	0.4	0.5	0.5	0.4
ISIS 410002	0.3	0.3	0.6	0.4	0.5	0.5	n/a
ISIS 410003	0.3	0.3	0.6	0.4	0.6	0.6	0.4
ISIS 438452	0.3	0.3	0.6	0.4	0.5	0.5	0.4
ISIS 438460	0.3	0.4	0.5	0.3	0.5	0.5	n/a
ISIS 446431	0.3	0.3	0.5	0.4	0.5	0.5	0.4
ISIS 446432	0.3	0.3	0.6	0.4	0.5	0.5	0.4
ISIS 446433	0.3	0.3	0.5	0.4	n/a	n/a	n/a
ISIS 446434	0.3	0.4	0.8	n/a	n/a	n/a	n/a

TABLE 33

Effect of antisense oligonucleotide treatment on total urine protein to urine creatinine ratio of Sprague Dawley rats

	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
PBS	1.5	1.4	1.2	1.6	1.3	1.2	1.2
ISIS 142082	1.2	4.3	4.0	7.7	6.6	7.5	7.4
ISIS 373125	1.3	3.9	3.8	6.7	6.2	8.8	9.7
ISIS 404173	1.1	4.8	5.5	6.4	7.4	10.2	11.9
ISIS 409826	1.2	3.7	3.8	5.9	9.8	28.8	37.6
ISIS 410002	1.2	3.9	4.1	6.0	9.7	26.3	n/a
ISIS 410003	1.3	4.5	5.3	5.9	7.6	10.8	18.0
ISIS 438452	1.4	3.3	3.1	5.1	8.0	9.2	10.5
ISIS 438460	1.3	4.0	4.5	7.3	16.0	53.9	n/a
ISIS 446431	1.2	4.5	5.0	5.3	7.2	8.0	8.8
ISIS 446432	1.3	4.2	4.4	6.2	9.1	7.6	9.2
ISIS 446433	1.1	3.4	5.7	81.5	n/a	n/a	n/a
ISIS 446434	1.1	3.7	25.8	n/a	n/a	n/a	n/a

Plasma Glucose Levels

To evaluate the effect of ISIS oligonucleotides on glucose metabolism, plasma levels of glucose were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.). Results are presented in Table 34, expressed in mg/dL.

TABLE 34

Effect of antisense oligonucleotide treatment on plasma glucose levels in Sprague Dawley rats							
	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
PBS	150	140	155	152	163	138	144
ISIS 142082	153	137	155	145	142	130	135
ISIS 373125	153	135	136	143	133	106	135
ISIS 404173	162	138	149	152	145	144	154
ISIS 409826	155	141	150	145	143	132	135
ISIS 410002	152	139	148	151	130	124	n/a
ISIS 410003	152	138	146	140	132	126	143
ISIS 438452	166	134	162	153	135	143	147
ISIS 438460	166	140	151	156	150	130	n/a
ISIS 446431	154	143	155	147	153	139	145
ISIS 446432	159	141	155	152	152	138	153
ISIS 446433	158	138	141	118	n/a	n/a	n/a
ISIS 446434	166	149	124	n/a	n/a	n/a	n/a

Plasma Lipid and Triglyceride Levels

To evaluate the effect of ISIS oligonucleotides on total cholesterol and triglyceride levels, plasma levels of each were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.). Results are presented in Tables 35 and 36, expressed in mg/dL.

TABLE 35

Effect of antisense oligonucleotide treatment on plasma cholesterol levels (mg/dL) in Sprague Dawley rats							
	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
PBS	55	57	64	51	73	64	53
ISIS 142082	62	41	54	62	70	64	62
ISIS 373125	73	59	66	51	66	61	39
ISIS 404173	57	41	68	62	87	85	75
ISIS 409826	57	42	79	65	86	122	110
ISIS 410002	69	57	75	65	73	96	n/a
ISIS 410003	72	44	70	67	89	76	73
ISIS 438452	63	33	53	51	71	70	61
ISIS 438460	64	40	98	81	94	146	n/a
ISIS 446431	64	41	56	54	63	68	59
ISIS 446432	62	44	70	50	80	80	65
ISIS 446433	59	63	95	139	n/a	n/a	n/a
ISIS 446434	63	48	91	n/a	n/a	n/a	n/a

TABLE 36

Effect of antisense oligonucleotide treatment on plasma triglyceride levels (mg/dL) in Sprague Dawley rats						
	Week 0	Week 2	Week 4	Week 6	Week 8	Week 12
PBS	66	73	82	80	98	106
ISIS 142082	92	30	71	44	25	28
ISIS 373125	66	28	20	24	24	28
ISIS 404173	48	28	28	35	31	49
ISIS 409826	68	29	28	25	31	68
ISIS 410002	71	23	23	27	71	n/a
ISIS 410003	78	22	22	37	30	64
ISIS 438452	89	33	39	34	50	35
ISIS 438460	98	20	34	35	33	n/a
ISIS 446431	72	29	38	36	35	48
ISIS 446432	n/a	41	37	31	37	53
ISIS 446433	68	21	29	129	n/a	n/a
ISIS 446434	69	27	103	n/a	n/a	n/a

Cytokine Levels

To evaluate the effect of ISIS oligonucleotides on factors involved in inflammation, blood was collected after the end of the treatment period for measurement of cytokine levels.

The samples were sent to Aushon Biosystems (Woburn, Mass.) for analysis. Levels of rat IL-6, MCP-1, MIP-1α, and TNF-α were measured with their respective antibodies. The results are presented in Table 37.

TABLE 37

Effect of antisense oligonucleotide treatment on plasma cytokine levels in Sprague Dawley rats				
	rIL-6	rMCP-1	rMIP-1α	rTNF-α
PBS	315	403	6	77
ISIS 142082	74	3082	38	697
ISIS 373125	<25	2215	7	15
ISIS 404173	125	2244	60	499
ISIS 409826	<25	6041	52	100
ISIS 410003	245	3315	40	444
ISIS 438452	105	4513	26	519
ISIS 446431	924	3104	54	402
ISIS 446432	29	2007	46	610

Example 14

Measurement of Viscosity of ISIS Antisense Oligonucleotides Targeting Human PTP1B

The viscosity of the antisense oligonucleotides selected from studies described in Examples 12 and 13 was measured with the aim of screening out antisense oligonucleotides which have a viscosity more than 40 cP at a concentration of 165-185 mg/mL. Oligonucleotides having a viscosity greater than 40 cP would be too viscous to be administered to any subject.

ISIS oligonucleotides (32-35 mg) were weighed into a glass vial, 120 μL of water was added and the antisense oligonucleotide was dissolved into solution by heating the vial at 50° C. Part of (75 μL) the pre-heated sample was pipetted to a micro-viscometer (Cambridge). The temperature of the micro-viscometter was set to 25° C. and the viscosity of the sample was measured. Another part (20 μL) of the pre-heated sample was pipetted into 10 mL of water for UV reading at 260 nM at 85° C. (Cary UV instrument). The results are presented in Table 38 and indicate that all the antisense oligonucleotides solutions are optimal in their viscosity under the criterion stated above.

TABLE 38

Viscosity and concentration of ISIS antisense oligonucleotides targeting human PTP1B		
ISIS No.	Viscosity (cP)	Concentration (mg/mL)
142082	3.8	188
404173	3.8	163
410003	4.5	176
446431	3.2	180
446432	2.4	175

Example 15

Six Month Tolerability Study of Antisense Oligonucleotides Targeting Human PTP1B in a Mouse Model

ISIS oligonucleotides selected from the studies described in Examples 12-14 were evaluated for long-term tolerability in a mouse model by monitoring changes in the levels of various metabolic markers in CD1 mice.

Treatment

Male CD1 mice were maintained on a 12-hour light/dark cycle and fed ad libitum normal lab chow (Harlan Laboratories, Indianapolis, Ind.). Animals were acclimated for at least 7 days in the research facility before initiation of the experiment. Antisense oligonucleotides were prepared in PBS and sterilized by filtering through a 0.2 micron filter. Oligonucleotides were dissolved in 0.9% PBS for injection.

Groups of ten CD1 mice each were injected subcutaneously twice a week with 25 mg/kg of ISIS 142082, ISIS 404173, or ISIS 446431 for 24 weeks. One group of ten CD1 mice was injected subcutaneously twice a week with PBS for 24 weeks. This PBS group served as the control group. Blood samples were collected on days 140 via mandibular bleeds. On day 168, blood was collected via terminal cardiac puncture under CO_2 anesthesia, the mice were euthanized and organs were harvested for further analysis.

Plasma Glucose Levels

To evaluate the effect of ISIS oligonucleotides on glucose metabolism, plasma levels of glucose were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.). Results are presented in Table 39, expressed in mg/dL.

TABLE 39

Effect of antisense oligonucleotide treatment on plasma glucose levels on day 168

	Glucose
PBS	214
ISIS 142082	177
ISIS 404173	204
ISIS 446431	191

Liver Function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) on day 168 were measured. Plasma levels of bilirubin (mg/dL) were also measured using the same clinical chemistry analyzer. Alkaline phosphatase, which is synthesized in increased amounts by damaged liver cells, is also a marker of liver disease (Narayanan, S. Ann. Clin. Lab. Sci. 21: 12-8, 1991) and was similarly measured. Albumin, which is typically decreased in liver disease (Oettl, K. et al., Biochim Biophys. Acta. 1782: 469-73, 2008), was also similarly measured. The results are presented in Table 40.

TABLE 40

Effect of antisense oligonucleotide treatment on liver metabolic markers on day 168

	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)	Alkaline phos-phatase (IU/L)	Albumin (g/dL)
PBS	50	82	0.2	44	2.5
ISIS 142082	148	197	0.1	56	2.3
ISIS 404173	68	137	0.1	57	2.5
ISIS 446431	115	173	0.1	42	2.4

Cardiac Function

To evaluate the effect of ISIS oligonucleotides on heart function, plasma levels of creatine phosphokinase (CPK) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.) on day 168. An increased level of this marker indicates heart muscle injury (Barohn, R. J. In: Goldman L, Ausiello D, eds. Cecil Medicine. 23rd ed. Philadelphia, Pa.: Saunders Elsevier; 2007: chapter 447). The results are presented in Table 41.

TABLE 41

Effect of antisense oligonucleotide treatment on cardiac marker CPK on day 168

	CPK (IU/L)
PBS	98
ISIS 142082	120
ISIS 404173	107
ISIS 446431	159

Pancreatic Function

To evaluate the effect of ISIS oligonucleotides on pancreatic function, plasma levels of amylase were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.) on day 168. An increased level of this marker indicates acute pancreatitis (Sternby, B. et al., Mayo Clin. Proc. 71: 1138-44, 1996). The results are presented in Table 42.

TABLE 42

Effect of antisense oligonucleotide treatment on pancreatic marker amylase on day 168

	Amylase (IU/L)
PBS	1101
ISIS 142082	1374
ISIS 404173	1280
ISIS 446431	1232

Kidney Function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma levels of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, N.Y.). Results are presented in Table 43, expressed in mg/dL.

TABLE 43

Effect of antisense oligonucleotide treatment on kidney metabolic markers on day 168

	BUN	Creatinine
PBS	20	0.3
ISIS 142082	24	0.2
ISIS 404173	21	0.2
ISIS 446431	19	0.3

Example 16

Measurement of Half-Life of Antisense Oligonucleotide in CD1 Mouse Liver

CD1 mice were treated with the ISIS antisense oligonucleotides selected from studies described in Example 14, and the oligonucleotide half-life as well as the elapsed time for oligonucleotide degradation and elimination from the liver was evaluated.

Treatment

Groups of ten CD1 mice each were injected subcutaneously twice per week for 2 weeks with 50 mg/kg of ISIS 142082, ISIS 446431, ISIS 404173, or ISIS 409826. Five mice from each group were sacrificed 3 days and 56 days following the final dose. Livers were harvested for analysis.

Measurement of Oligonucleotide Concentration

The concentration of the full-length oligonucleotide was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 50) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μg/g. Half-lives were then calculated using WinNonlin software (PHARSIGHT).

The results are presented in Table 44. Antisense oligonucleotides with half-lives within 11-34 days were chosen for further studies.

TABLE 44

Full-length oligonucleotide concentration (μg/g) and half-life (days) of oligonucleotide in CD1 mouse liver

	Days	Full length conc. (mg/g)	Half-life (days)
142082		265	19.8
	56	42	
446431	3	293	19.6
	56	45	
404173	3	281	14.8
	56	24	
409826	3	304	18.4
	56	41	

Example 17

Effect of ISIS Antisense Oligonucleotides Targeting Human PTP1B in Cynomolgus Monkeys

Cynomolgus monkeys were treated with ISIS antisense oligonucleotides from studies described in Examples 15 and 16. Antisense oligonucleotide efficacy and tolerability, as well as their pharmacokinetic profile in the liver and kidney, were evaluated.

Treatment

Prior to the study, the monkeys were kept in quarantine for a 30-day time period, during which standard panels of serum chemistry and hematology, examination of fecal samples for ova and parasites, and a tuberculosis test, were conducted to screen out abnormal or ailing monkeys. Six groups of randomly assigned three male and two female cynomolgus monkeys each were injected subcutaneously thrice per week for the first week, and subsequently, once a week for the next 12 weeks with either 8 mg/kg or 40 mg/kg of ISIS 142082, ISIS 446431, or ISIS 404173. One group of three male and two female cynomolgus monkeys was injected subcutaneously thrice per week for the first week,

and subsequently, once a week for the next 12 weeks with 40 mg/kg of ISIS 409826. A control group of three male and two female cynomolgus monkeys was injected subcutaneously thrice per week for the first week, and subsequently, once a week for the next 12 weeks with PBS. Terminal sacrifices of all groups were conducted 48 hours after the last dose, on day 93.

During the study period, the monkeys were observed daily for signs of illness or distress. Any animal showing adverse effects to the treatment was removed and referred to the veterinarian and Study Director.

Inhibition Studies

RNA Analysis

RNA was extracted from liver and the abdominal adipose tissues for real-time PCR analysis of PTP1B using primer probe set 1 (forward sequence GACCAGCTGCGCTTCTCCTA, designated herein as SEQ ID NO: 51; reverse sequence CAGAGGAGTCCCCCATGATG, designated herein as SEQ ID NO: 52; probe sequence TTGGCTGTGATCGAAGGTGCCAAA, designated herein as SEQ ID NO: 53) or primer probe set 2 (forward sequence GGGCCCTTTGCCTAACACA, designated herein as SEQ ID NO: 54; reverse sequence CGACACCCCTGCTTTTCTG, designated herein as SEQ ID NO: 55; probe sequence CGGTCACTTTTGGGAGATGGTGTGG, designated herein as SEQ ID NO: 56), each targeting different regions of the PTP1B mRNA. Results are presented as percent reduction of PTP1B mRNA, relative to PBS control, normalized with RIBOGREEN®. As shown in Table 45, treatment with ISIS antisense oligonucleotides resulted in significant reduction of PTP1B mRNA in comparison to the PBS control. Treatment with ISIS 404173 caused reduction of PTP1B mRNA levels similar to that with treatment with ISIS 142082.

TABLE 45

Inhibition of PTP1B mRNA in the cynomolgus monkey liver and fat tissue relative to the PBS control

ISIS No.	Dose (mg/kg)	% inhibition in liver (probe set 1)	% inhibition in liver (probe set 2)	% inhibition in fat (probe set 1)	% inhibition in fat (probe set 2)
409826	40	38	45	17	21
142082	8	12	14	13	16
	40	46	48	34	28
446831	8	0	8	6	1
	40	8	18	14	12
404173	8	4	13	8	4
	40	26	22	38	31

Protein Analysis

Tissue was extracted from liver For measuring PTP1B protein levels by western blot analysis. Specifically, PTP1B protein samples from monkeys treated with ISIS 404173 were compared with those treated with ISIS 142082. The results are presented in Tables 46, 47 and 48 expressed as percentage reduction compared to control levels. The levels of PTP1B levels were normalized against total protein levels, as well as against a constitutively expressed protein, IR-β. Treatment with ISIS 404173 caused greater reduction of PTP1B liver protein to that with treatment with ISIS 142082 at the lower dose of 8 mg/kg (Table 47).

TABLE 46

PTP1B protein level reduction after treatment with ISIS 404173 in the cynomolgus monkey liver		
Dose (mg/kg)	% inhibition (normalized to total protein)	% inhibition (normalized to IR-β)
8	49	42
40	67	66

TABLE 47

PTP1B protein level reduction after treatment with ISIS 404173 or ISIS 142082 at 8 mg/kg in the cynomolgus monkey liver		
ISIS No	% inhibition (normalized to total protein)	% inhibition (normalized to IR-β)
142082	20	4
404173	33	27

TABLE 48

PTP1B protein level reduction after treatment with ISIS 404173 or ISIS 142082 at 40 mg/kg in the cynomolgus monkey liver		
ISIS No	% inhibition (normalized to total protein)	% inhibition (normalized to IR-β)
142082	65	63
404173	60	56

Tolerability Studies

Body and Organ Weight Measurements

To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body and organ weights were measured after terminal sacrifice. Body weights were measured and compared to that of the PBS control animals. Organ weights were measured and treatment group weights were compared to the corresponding PBS control weights. The data is presented in Table 49. Treatment with ISIS 142082 did cause increases in liver and kidney weights at the higher dose.

TABLE 49

Final body and organ weight weights in the cynomolgus monkey relative to the control						
	Dose (mg/kg)	Body weight (kg)	Kidney (g)	Liver (g)	Spleen (g)	Gastocnemius muscle (g)
PBS	—	2.2	9.6	10.5	2.3	10.6
409826	40	2.3	14.0	18.5	6.0	8.8
142082	8	2.3	10.6	13.0	3.7	9.5
	40	2.2	20.9	17.7	7.0	9.0
446431	8	2.3	11.6	12.7	4.8	10.2
	40	2.3	15.9	16.1	9.6	7.7
404173	8	2.3	11.6	12.8	4.8	11.1
	40	2.2	14.8	15.5	3.7	8.7

Liver Function

To evaluate the effect of ISIS oligonucleotides on hepatic function, blood samples were collected from all the study groups 7 days before the start of treatment, as well as on days 30, 58, and 93 of the treatment period. The blood samples were collected in tubes without any anticoagulant for serum separation. The tubes were kept at room temperature for 90 min and then centrifuged (3000 rpm for 10 min at room temperature) to obtain serum.

Levels of transaminases were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Tables 50 and 51, expressed in IU/L. Alkaline phosphatase, which is synthesized in increased amounts by damaged liver cells and is also a marker of liver disease and was similarly measured, and the data is presented in Table 52. The levels of AST, ALT and alkaline phosphatase in all the treatment groups were similar to that of the PBS control group.

C-reactive protein (CRP), which is synthesized in the liver and which serves as a marker of inflammation, was also similarly measured, and the data is presented in Table 53. Treatment with ISIS 142082 and ISIS 409826 at the higher dose resulted in high levels of CRP, suggesting liver inflammation.

Bilirubin is also a liver metabolic marker and was similarly measured and is presented in Table 54, expressed in mg/dL. Bilurubin levels of all the treatment groups were found to be similar to that of the PBS control group. Gamma-glutamyltransferase (GGT) is an enzyme produced in the liver and is a useful laboratory marker for early liver damage or cholestatic disease (Betro, M. G. et al., Am. J. Clin. Pathol. 60: 672-8, 1973). GGT levels were measured and the results are presented in Table 55, and demonstrate no difference between the PBS control and the treatment groups.

TABLE 50

Effect of antisense oligonucleotide treatment on ALT (IU/L) in cynomolgus monkey serum					
	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS	—	37	44	42	35
409826	40	39	51	86	74
142082	8	37	39	45	36
	40	49	62	59	69
446431	8	52	54	67	86
	40	38	58	87	99
404173	8	34	50	41	45
	40	44	50	63	73

TABLE 51

Effect of antisense oligonucleotide treatment on AST (IU/L) in cynomolgus monkey serum					
	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS		40	49	55	44
409826	40	48	53	73	59
142082	8	44	45	49	42
	40	54	70	72	69
446431	8	57	43	48	50
	40	41	60	63	81
404173	8	44	53	57	59
	40	46	65	71	74

TABLE 52

Effect of antisense oligonucleotide treatment on alkaline phosphatase (IU/L) in cynomolgus monkey serum

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS	—	784	834	1021	838
409826	40	728	883	1178	981
142082	8	718	739	788	688
	40	666	656	711	774
446431	8	742	745	885	908
	40	778	759	768	735
404173	8	888	957	1135	1155
	40	931	958	1135	1263

TABLE 53

Effect of antisense oligonucleotide treatment on CRP (mg/L) in cynomolgus monkey plasma

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS		1.0	1.8	1.4	1.2
409826	40	1.0	4.3	4.8	4.8
142082	8	0.8	1.2	0.9	1.0
	40	0.8	2.6	3.4	12.1
446431	8	1.4	1.4	0.9	1.1
	40	0.8	2.4	2.2	6.7
404173	8	1.4	1.9	1.5	1.8
	40	3.1	1.6	1.2	1.6

TABLE 54

Effect of antisense oligonucleotide treatment on bilirubin (mg/dL) in cynomolgus monkey plasma

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS	—	0.19	0.20	0.20	0.17
409826	40	0.17	0.13	0.13	0.10
142082	8	0.17	0.18	0.16	0.14
	40	0.16	0.13	0.13	0.08
446431	8	0.21	0.17	0.18	0.15
	40	0.19	0.18	0.15	0.12
404173	8	0.23	0.19	0.20	0.16
	40	0.22	0.15	0.14	0.13

TABLE 55

Effect of antisense oligonucleotide treatment on GGT (IU/L) in cynomolgus monkey plasma

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS	—	65	74	79	71
409826	40	84	86	94	87
142082	8	63	67	68	62
	40	67	72	71	61
446431	8	60	62	62	63
	40	61	58	62	60
404173	8	57	66	66	68
	40	56	63	69	79

Kidney Function

To evaluate the effect of ISIS oligonucleotides on kidney function, blood samples were collected from all the study groups. The blood samples were collected in tubes without any anticoagulant for serum separation. The tubes were kept at room temperature for 90 min and then centrifuged (3000 rpm for 10 min at room temperature) to obtain serum. Levels of BUN and creatinine were measured 7 days before the start of treatment, as well as on days 30, 58, and 93 of the treatment period using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Results are presented in Tables 56 and 57, expressed in mg/dL. Treatment with ISIS oligonucleotides had no adverse effect on either BUN or creatinine levels.

TABLE 56

Effect of antisense oligonucleotide treatment on serum BUN levels (mg/dL) in cynomolgus monkeys

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS	—	28	28	25	28
409826	40	32	30	28	32
142082	8	26	25	24	26
	40	28	28	25	25
446431	8	28	27	25	26
	40	28	27	25	28
404173	8	28	30	24	27
	40	28	24	25	23

TABLE 57

Effect of antisense oligonucleotide treatment on serum creatinine levels (mg/dL) in cynomolgus monkeys

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS		0.83	0.88	0.94	0.78
409826	40	0.77	0.84	0.92	0.82
142082	8	0.74	0.78	0.79	0.71
	40	0.72	0.80	0.86	0.73
446431	8	0.79	0.75	0.83	0.71
	40	0.76	0.83	0.88	0.77
404173	8	0.81	0.91	0.87	0.82
	40	0.76	0.84	0.92	0.76

Cholesterol and Triglyceride Levels

To evaluate the effect of ISIS oligonucleotides on lipid metabolism, blood samples were collected from all the study groups. The blood samples were collected in tubes without any anticoagulant for serum separation. The tubes were kept at room temperature for 90 min and then centrifuged (3000 rpm for 10 min at room temperature) to obtain serum. Concentrations of cholesterol and triglycerides were measured 7 days before the start of treatment, as well as on days 30, 58, and 93 of the treatment period using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Results are presented in Tables 58 and 59, expressed in mg/dL. Treatment with ISIS oligonucleotides had no adverse effect on either cholesterol or triglyceride levels.

TABLE 58

Effect of antisense oligonucleotide treatment on scrum cholesterol levels (mg/dL) in cynomolgus monkeys

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS	—	135	163	162	143
409826	40	153	150	140	116
142082	8	116	151	159	141
	40	110	140	138	128

TABLE 58-continued

Effect of antisense oligonucleotide treatment on serum cholesterol levels (mg/dL) in cynomolgus monkeys

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
446431	8	125	144	141	133
	40	93	99	95	81
404173	8	123	147	149	136
	40	135	135	125	124

TABLE 59

Effect of antisense oligonucleotide treatment on serum triglyceride levels (mg/dL) in cynomolgus monkeys

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS	—	47	55	45	54
409826	40	30	29	33	42
142082	8	23	31	37	32
	40	28	28	35	42
446431	8	24	46	34	93
	40	31	44	47	56
404173	8	28	38	25	28
	40	30	38	45	34

Hematology

To evaluate any inflammatory effect of ISIS oligonucleotides in cynomolgus monkeys, blood samples were approximately 0.5 mL of blood was collected from each of the available study animals in tubes containing the potassium salt of EDTA. Samples were analyzed for red blood cell (RBC) count, white blood cells (WBC) count, platelet count and hemoglobin content, using an ADVIAl20 hematology analyzer (Bayer, USA). The data is presented in Tables 60-63. Treatment with ISIS oligonucleotides did not significantly alter the blood cell count or hemoglobin levels, compared to the control.

TABLE 60

Effect of antisense oligonucleotide treatment on WBC ($\times 10^3/\mu L$) in cynomolgus monkeys

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS	—	11.6	12.6	11.1	8.9
409826	40	12.4	12.7	15.3	10.8
142082	8	11.3	15.2	12.8	9.7
	40	11.9	13.2	12.5	8.5
446431	8	9.7	13.4	12.7	9.3
	40	10.7	11.5	11.9	10.2
404173	8	14.9	18.9	14.9	11.8
	40	11.1	14.2	12.9	10.8

TABLE 61

Effect of antisense oligonucleotide treatment on ABC ($\times 10^6/\mu L$) in cynomolgus monkeys

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS	—	5.5	5.6	5.8	5.1
409826	40	5.6	5.8	6.1	5.6
142082	8	5.4	5.4	5.6	5.2
	40	5.7	5.6	5.8	5.5

TABLE 61-continued

Effect of antisense oligonucleotide treatment on ABC ($\times 10^6/\mu L$) in cynomolgus monkeys

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
446431	8	5.5	5.3	5.5	5.4
	40	5.5	5.4	5.8	5.3
404173	8	5.9	5.9	6.1	5.7
	40	5.1	5.4	5.5	5.5

TABLE 62

Effect of antisense oligonucleotide treatment on platelets ($\times 10^3/\mu L$) in cynomolgus monkeys

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS	—	592	555	571	516
409826	40	536	493	400	338
142082	8	439	477	349	284
	40	461	454	401	263
446431	8	438	397	359	282
	40	516	337	369	323
404173	8	489	491	420	355
	40	520	470	389	316

TABLE 63

Effect of antisense oligonucleotide treatment on hemoglobin levels (g/dL) in cynomolgus monkeys

	Dose (mg/kg)	Day −7	Day 30	Day 58	Day 93
PBS	—	12.5	12.8	13.1	11.9
409826	40	12.6	12.8	13.3	12.5
142082	8	12.3	12.5	13.0	12.2
	40	12.1	12.0	12.1	11.5
446431	8	12.3	12.1	12.4	12.6
	40	12.7	12.6	13.3	12.4
404173	8	12.7	13.0	13.3	12.7
	40	11.6	12.3	12.4	12.6

Analysis of Factors of Inflammation

To evaluate the effect of ISIS oligonucleotides for complement C3 analysis as an inflammation factor, blood was collected from all available animals in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for 90 min and then centrifuged (3000 rpm for 10 min at room temperature) to obtain serum. Complement C3 was measured using an automatic analyzer (Toshiba 200 FR NEO chemistry analyzer, Toshiba co., Japan). The data is presented in Table 64, expressed in mg/dL. Treatment with ISIS 409826 resulted in low complement C3 levels, indicating a diseased state.

TABLE 64

Effect of antisense oligonucleotide treatment on serum C3 levels (mg/dL) in cynomolgus monkeys

	Dose (mg/kg)	Day −7	Day −1	Day 30	Day 58	Day 93
PBS	—	136	138	148	149	129
409826	40	129	131	101	101	90
142082	8	126	135	126	127	111
	40	133	134	106	121	111
446431	8	130	144	128	132	125
	40	129	130	111	117	114

TABLE 64-continued

Effect of antisense oligonucleotide treatment on serum C3 levels (mg/dL) in cynomolgus monkeys						
	Dose (mg/kg)	Day −7	Day −1	Day 30	Day 58	Day 93
404173	8	127	136	137	136	127
	40	125	134	101	103	102

Analysis of Insulin Levels

To evaluate the effect of ISIS oligonucleotides on the thyroid gland, blood was collected on days 42, 84 and 91 from animals fasted overnight in tubes treated with EDTA. The tubes were kept on ice and plasma was obtained after centrifugation (3000 rpm for 10 min at 4° C.) within 30 min of blood collection. Insulin levels were measured using an automatic analyzer (Toshiba 200 FR NEO chemistry analyzer, Toshiba co., Japan). The data is presented in Table 65, expressed in ng/mL.

TABLE 65

Effect of antisense oligonucleotide treatment on plasma insulin levels (ng/mL) in cynomolgus monkeys				
	Dose (mg/kg)	Day 42	Day 84	Day 91
PBS	—	29	26	26
409826	40	14	22	15
142082	8	8	4	8
	40	8	10	10
404173	8	9	10	7
	40	6	3	2

Pharmacokinetic Studies

Measurement of Oligonucleotide Concentration

The concentration of the full-length oligonucleotide as well as the total oligonucleotide concentration (including the degraded form) was measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-β-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 50) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μg/g. The results are presented in Tables 66 and 67, expressed as ug/g tissue. The ratio of the concentrations in the kidney versus the liver was calculated. Treatment with ISIS oligonucleotides did not result in any abnormality in the ratio. The results indicate that ISIS 404173 is a better renal accumulator than ISIS 142082 at the higher dose.

TABLE 66

Total oligonucleotide concentration (μg/g) in the liver of cynomolgus monkey				
ISIS No.	Dose (mg/kg)	Kidney	Liver	Kidney/ Liver ratio
409826	40	4424	954	4.64
142082	8	1688	1044	1.62
	40	6385	1774	3.60
446431	8	1323	641	2.06
	40	6662	1159	5.75
404173	8	971	712	1.36
	40	7180	1464	4.90

TABLE 67

Full-length oligonucleotide concentration (μg/g) in the liver of cynomolgus monkey				
ISIS No.	Dose (mg/kg)	Kidney	Liver	Kidney/ Liver ratio
409826	40	3472	728	4.77
142082	8	1232	653	1.89
	40	4103	1244	3.30
446431	8	1204	416	2.89
	40	5645	846	6.67
404173	8	650	424	1.53
	40	5039	1094	4.61

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<400> SEQUENCE: 6

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<400> SEQUENCE: 13

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aggaggtcat ttccatggcc 20

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<400> SEQUENCE: 31

ggtcatttcc atggccagag 20

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<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 32

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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 33

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<210> SEQ ID NO 34
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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 34

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<210> SEQ ID NO 35
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<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 35

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<400> SEQUENCE: 37

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<400> SEQUENCE: 38

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<400> SEQUENCE: 47

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<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 50

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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 51

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<223> OTHER INFORMATION: Synthetic Primer

<400> SEQUENCE: 52

cagaggagtc ccccatgatg 20

<210> SEQ ID NO 53
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<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 53

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<210> SEQ ID NO 54
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<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 56

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What is claimed is:

1. A method of treating diabetes in a subject comprising administering to the subject a pharmaceutical composition suitable for parenteral administration to an animal comprising an aqueous solution, said aqueous solution comprising:

a pharmaceutically acceptable carrier or diluent; and

a single stranded modified oligonucleotide targeted to PTP1B consisting of 20 linked nucleosides **[**having**]** *consisting of* a nucleobase sequence consisting of SEQ ID NO: 26, or salt thereof,

wherein the carrier or diluent is sterile and the aqueous solution is suitably viscous for parenteral administration and wherein the composition is administered parenterally, thereby treating diabetes in the subject.

2. The method of claim **1**, wherein the aqueous solution suitably viscous for parenteral administration has a viscosity level less than 40 centipoise (cP).

3. The method of claim **2**, wherein the parenteral administration is subcutaneous injection.

4. The method of claim **2**, wherein the parenteral administration is intravenous infusion.

5. The method of claim **1**, wherein the *20 linked nucleosides of the single stranded* modified oligonucleotide **[**comprises**]** *consist of*:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of five linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, wherein each internucleoside linkage is a phosphorothioate linkage, and wherein each cytosine of the *single stranded* modified oligonucleotide is a 5-methylcytosine.

6. The method of claim **5**, wherein the aqueous solution suitably viscous for parenteral administration has a viscosity level less than 40 centipoise (cP).

7. The method of claim **6**, wherein the aqueous solution comprises the *single stranded* modified oligonucleotide targeted to PTP1B at a concentration of about 165-185 mg/mL.

8. The method of claim **7**, wherein the aqueous solution has a temperature of about 25° C.

9. The method of claim **8**, wherein the parenteral administration is subcutaneous injection.

10. The method of claim **8**, wherein the parenteral administration is intravenous infusion.

11. The method of claim **1**, wherein the pharmaceutically acceptable carrier or diluent is water.

12. The method of claim **[5]** *11*, wherein the *20 linked nucleosides of the single stranded* modified oligonucleotide **[**comprises**]** *consist of*:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of five linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, wherein each internucleoside linkage is a phosphorothioate linkage, and wherein each cytosine of the *single stranded* modified oligonucleotide is a 5-methylcytosine.

13. The method of claim **12**, wherein the aqueous solution suitably viscous for parenteral administration has a viscosity level less than 40 centipoise (cP).

14. The method of claim **13**, wherein the aqueous solution comprises the *single stranded* modified oligonucleotide targeted to PTP1B at a concentration of about 165-185 mg/mL.

15. The method of claim **14**, wherein the aqueous solution has a temperature of about 25° C.

16. The method of claim **15**, wherein the parenteral administration is subcutaneous injection.

17. The method of claim **16**, wherein the parenteral administration is intravenous infusion.

18. The method of claim 1, wherein the subject is human.

19. The method of claim 18, wherein the diabetes is Type II diabetes.

20. The method of claim 19, wherein administering the [compound] *composition* decreases blood glucose levels in the human.

21. The method of claim 12, wherein the subject is human.

22. The method of claim 21, wherein the diabetes is Type II diabetes.

23. The method of claim 22, wherein administering the [compound] *composition* decreases blood glucose levels in the human.

24. The method of claim 13, wherein the subject is human.

25. The method of claim 24, wherein the diabetes is Type II diabetes.

26. The method of claim 25, wherein administering the [compound] *composition* decreases blood glucose levels in the human.

27. A method of decreasing blood glucose levels in a subject comprising administering to the subject a compound consisting of a single stranded modified oligonucleotide targeted to PTP1B consisting of 20 linked nucleosides [having] *consisting of* a nucleobase sequence consisting of SEQ ID NO: 26, wherein the *20 linked nucleosides of the single stranded* modified oligonucleotide [comprises] *consist of*:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of five linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine of the *single stranded* modified oligonucleotide is a 5-methylcytosine; and

wherein the compound is administered to the subject parenterally, thereby decreasing blood glucose levels in the subject.

28. The method of claim 27, wherein the subject is human.

29. The method of claim 28, wherein the human has diabetes.

30. The method of claim 29, wherein the diabetes is Type II diabetes.

31. The method of claim 29, wherein administering the compound treats diabetes.

32. The method of claim 30, wherein administering the compound treats diabetes.

33. A single stranded modified oligonucleotide consisting of 20 linked nucleosides [having] *consisting of* a nucleobase sequence consisting of SEQ ID NO: 26 [and comprising], *wherein the 20 linked nucleosides of the single stranded modified oligonucleotide consist of*:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of five linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine of the *single stranded* modified oligonucleotide is a 5-methylcytosine.

34. A compound [consisting of] *comprising* a pharmaceutically acceptable salt of [the single-stranded] *a single stranded* oligonucleotide [of claim 33] *consisting of 20 linked nucleosides consisting of a nucleobase sequence consisting of SEQ ID NO: 26, wherein the 20 linked nucleosides of the single stranded modified oligonucleotide consist of*:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of five linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine of the single stranded modified oligonucleotide is a 5-methylcytosine.

35. The compound of claim 34, wherein the pharmaceutically acceptable salt is a sodium salt.

36. The compound of claim 34, wherein the pharmaceutically acceptable salt is a potassium salt.

37. A compound consisting of a sodium salt of a single stranded modified oligonucleotide consisting of 20 linked nucleosides [having] *consisting of* a nucleobase sequence consisting of SEQ ID NO: 26 [and comprising], *wherein the 20 linked nucleosides of the single stranded modified oligonucleotide consist of*:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of five linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine of the *single stranded* modified oligonucleotide is a 5-methylcytosine.

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