(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 September 2017 (21.09.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/161172 A1

(51) International Patent Classification:
A61P 9/10 (2006.01) *A61P 25/28* (2006.01)
A61P 11/06 (2006.01) *C07H 21/02* (2006.01)
A61P 25/00 (2006.01)

(21) International Application Number:
PCT/US2017/022788

(22) International Filing Date:
16 March 2017 (16.03.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/309,360 16 March 2016 (16.03.2016) US
62/394,663 14 September 2016 (14.09.2016) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2017/161172 A1

(54) **Title**: METHODS OF MODULATING KEAP1

(57) **Abstract**: Provided herein are methods, compounds, and compositions for reducing expression of KEAP1 in animal. Such methods, compounds, and compositions are useful to treat, prevent, delay, or ameliorate oxidative stress or a disease, disorder or condition related to oxidative stress an animal in need. Diseases, disorders or conditions related to oxidative stress include NASH, NAFLD, asthma and insulin resistance.

METHODS OF MODULATING KEAP1

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 BIOL0291WOSEQ_ST25.txt, created on March 16, 2017 which is 72 MB in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present embodiments provide methods, compounds, and compositions for treating, preventing, or ameliorating a disease related to oxidative stress such as insulin resistance, non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD), by administering a Kelch-like ECH-associated protein 1 (KEAP1) specific inhibitor to an individual.

BACKGROUND

Oxidative stress, which results from an imbalance between the generation of electrophiles (e.g., free radicals) and the antioxidant response in a body, has been linked to a number of pathologic processes including diabetes, insulin resistance, obesity, non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD). Oxidative stress linked complications of diabetes include stroke, neuropathy, retinopathy and nephropathy.

Kelch-like ECH-associated protein 1 (KEAP1) functions as an oxidative stress-sensitive substrate adaptor protein for a Cul3-based E3 unbiquitin ligase complex. KEAP1 and the substrates it binds are key regulators of the antioxidant response and KEAP1 is a key defence against oxidative stress.

Transcription factor NF-E2-related factor 2 (NRF2) is a substrate of KEAP1. KEAP1 acts upstream of Nrf2 in the cellular response to oxidative and xenobiotic stress. KEAP1 binds NRF2 and, under normal quiescent conditions, KEAP1 sequesters NRF2 in the cytoplasm and targets NRF2 for ubiquitination and degradation. However, in the presence of xenobiotics or oxidative stress (for example the presence of electrophiles such as reactive oxygen species (ROS)), KEAP1 releases NRF2 which then translocates to the nucleus where it binds to antioxidant response elements (AREs) and initiates transcription of a battery of cytoprotective detoxifying and antioxidant genes including glutathione S-transferase alpha 1 (GSTA1) and NAD(P)H: Quinone Oxidoreductase 1 (NQO1). Thus, KEAP1 represses Nrf2 function by detecting oxidative stress signals, and the stress signals release KEAP1 repression of Nrf2 (Chartoumpekis, *et al.*, 2015, *Biochem. Soc. Trans.* 43:639-644; Padmanabhan, *et al.*, 2006, Molecular Cell 21:689-700; Wakabayashi, *et al.*, 2003, *Nature Genetics* 35:238-245).

Phosphoglycerate mutase family member 5 (PGAM5) is another substrate of KEAP1. KEAP1 interaction with PGAM5 targets it for ubiquitination and degradation and is another defense mechanism against oxidative stress (Lo and Hannink, *J Biol Chem*, 2006, 281(49): 37893-37903).

It has been shown in several mouse models that reducing KEAP1 expression leads to a heightened antioxidant response, making it an attractive pharmacologic target. Studies have been conducted with KEAP1 knockout mice (Ramadori *et al., Free Radic Biol Med*, 2016, 91:114-126; Xu *et al., PLoS One*, 2013 8(11):e79841; Kulkarni *et al., Pharm Res*, 2013, 30(9):2221-31), small molecule inhibitors (Shimozono *et al., Mol Pharmacol*, 2013, 84(1):62-70) and nucleic acid inhibitors such as siRNA (Xu *et al., Cancer Res.* 2013, 73(14): 4406–4417; Soares *et al., Diabetes*, 2016 65(3)633-646; Gonzalez-Rodriguez *et al., Dis Model Mech*, 2014, 7(9):1093-100; Zhong *et al., Invest Ophthalmol Vis Sci*, 2013, 54(6):3941-8; US Patent 8,785,408) to treat a disease related to oxidative stress. However, to date, no therapeutic strategies to target KEAP1 are commercially available. Accordingly, there is an unmet need to develop KEAP1 inhibitors for use in treating KEAP1 related diseases or conditions.

SUMMARY

Certain embodiments disclosed herein provide compounds or compositions comprising a KEAP1 modulator. In certain embodiments, the KEAP1 modulator is a KEAP1 specific inhibitor. In certain embodiments, the KEAP1 specific inhibitor is a nucleic acid, polypeptide, antibody, small molecules, or other agent capable of inhibiting the expression of KEAP1. In certain embodiments, the nucleic acid is a compound or composition targeting KEAP1. In certain embodiments, the compound or composition comprises deoxyribonucleotides. In certain embodiments, the compound or composition comprises ribonucleotides. In certain embodiments, the compound or composition is single stranded. In certain embodiments, the compound or composition is double stranded. In certain embodiments, the compound or composition targeting KEAP1 comprises an oligonucleotide. In certain embodiments, the oligonucleotide is single stranded. In certain embodiments, the oligonucleotide is double stranded. In certain embodiments, the oligonucleotide is a modified oligonucleotide. In certain embodiments, the modified oligonucleotide is single stranded. In certain embodiments, the modified oligonucleotide is double stranded. In any of the foregoing embodiments, the compound can be an antisense compound or oligomeric compound.

Certain embodiments disclosed herein provide a method of reducing KEAP1 expression in an animal comprising administering to the animal a compound or composition comprising a KEAP1 specific inhibitor.

Certain embodiments disclosed herein provide a method of reducing oxidative stress in an animal comprising administering to the animal a therapeutically effective amount of a compound or composition comprising a KEAP1 specific inhibitor.

Certain embodiments disclosed herein provide a method of treating, preventing, delaying or ameliorating a disease, disorder or condition related to oxidative stress in an animal comprising administering

to the animal a therapeutically effective amount of a compound or composition comprising a KEAP1 specific inhibitor.

Certain embodiments disclosed herein provide a method of treating an animal at risk for a disease, disorder or condition related to oxidative stress comprising administering to the animal a therapeutically effective amount of a compound or composition comprising a KEAP1 specific inhibitor.

In certain embodiments, the KEAP1 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of KEAP1. In certain embodiments, the KEAP1 specific inhibitor comprises a compound or composition described herein. In certain embodiments, the compound or composition comprises an antisense compound or an oligomeric compound. In certain embodiments, the compound or composition comprises a modified oligonucleotide. In certain embodiments, the compound or composition comprises a modified oligonucleotide 8 to 80 linked nucleosides in length and having a nucleobase sequence comprising at least an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobase portion complementary to the sequence of any one of SEQ ID NOs: 1-6. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides.

In certain embodiments, the disease, disorder or condition related to oxidative stress is a metabolic disease, disorder or condition. In certain embodiments, the metabolic disease, disorder or condition is insulin resistance. In certain embodiments, the disease, disorder or condition related to oxidative stress is NASH and/or NAFLD.

DETAILED DESCRIPTION OF THE INVENTION

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 embodiments, 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.

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, treatises, and GenBank and NCBI reference sequence records are hereby expressly incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

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, compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Compounds described by ISIS number (ISIS #) indicate

a combination of nucleobase sequence, chemical modification, and motif.

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

"2'-deoxynucleoside" means a nucleoside comprising 2'-H(H) furanosyl sugar moiety, as found in naturally occurring deoxyribonucleic acids (DNA). In certain embodiments, a 2'-deoxynucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (uracil).

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

"2'-MOE nucleoside" (also 2'-O-methoxyethyl nucleoside) means a nucleoside comprising a 2'-MOE modified sugar moiety.

"2'-substituted nucleoside" or "2-modified nucleoside" means a nucleoside comprising a 2'-substituted or 2'-modified sugar moiety. As used herein, "2'-substituted" or "2-modified" in reference to a sugar moiety means a sugar moiety comprising at least one 2'-substituent group other than H or OH.

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

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

"5-methylcytosine" means a cytosine with a methyl group attached to the 5 position.

"About" means within ±10% of a value. For example, if it is stated, "the compounds affected about 70% inhibition of a KEAP1", it is implied that KEAP1 levels are inhibited within a range of 60% and 80%.

"Administration" or "administering" refers to routes of introducing a compound or composition provided herein to an individual to perform its intended function. An example of a route of administration that can be used includes, but is not limited to parenteral administration, such as subcutaneous, intravenous, or intramuscular injection or infusion.

"Administered concomitantly" or "co-administration" means administration of two or more compounds in any manner in which the pharmacological effects of both are manifest in the patient. Concomitant administration does not require that both compounds be administered in a single pharmaceutical composition, in the same dosage form, by the same route of administration, or at the same time. The effects of both compounds need not manifest themselves at the same time. The effects need only be overlapping for a period of time and need not be coextensive. Concomitant administration or co-administration encompasses administration in parallel or sequentially.

"Amelioration" refers to an improvement or lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. In certain embodiments, amelioration includes a delay or slowing in the progression or severity of one or more indicators of a condition or disease. The progression or severity of indicators may 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 and/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 compared to target nucleic acid levels or target protein levels in the absence of the antisense compound to the target.

"Antisense compound" means a compound comprising an oligonucleotide and optionally one or more additional features, such as a conjugate group or terminal group. Examples of antisense compounds include single stranded and double stranded compounds, such as, oligonucleotides, ribozymes, siRNAs, shRNAs, ssRNAs, and occupancy-based compounds.

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

"Antisense mechanisms" are all those mechanisms involving hybridization of a compound with target nucleic acid, wherein the outcome or effect of the hybridization is either target degradation or target occupancy with concomitant stalling of the cellular machinery involving, for example, transcription or splicing.

"Antisense oligonucleotide" means an oligonucleotide having a nucleobase sequence that is complementary to a target nucleic acid or region or segment thereof. In certain embodiments, an antisense oligonucleotide is specifically hybridizable to a target nucleic acid or region or segment thereof.

"Bicyclic nucleoside" or "BNA" means a nucleoside comprising a bicyclic sugar moiety. "Bicyclic sugar" or "bicyclic sugar moiety" means a modified sugar moiety comprising two rings, wherein the second ring is formed via a bridge connecting two of the atoms in the first ring thereby forming a bicyclic structure. In certain embodiments, the first ring of the bicyclic sugar moiety is a furanosyl moiety. In certain embodiments, the bicyclic sugar moiety does not comprise a furanosyl moiety.

"Branching group" means a group of atoms having at least 3 positions that are capable of forming covalent linkages to at least 3 groups. In certain embodiments, a branching group provides a plurality of reactive sites for connecting tethered ligands to an oligonucleotide via a conjugate linker and/or a cleavable moiety.

"Cell-targeting moiety" means a conjugate group or portion of a conjugate group that is capable of binding to a particular cell type or particular cell types.

"cEt" or "constrained ethyl" means a bicyclic furanosyl sugar moiety comprising a bridge connecting the 4'-carbon and the 2'-carbon, wherein the bridge has the formula: 4'-$CH(CH_3)$-O-2'.

"Constrained ethyl nucleoside" (also cEt nucleoside) means a nucleoside comprising a cEt.

"Chemical modification" in a compound describes the substitutions or changes through chemical reaction, of any of the units in the compound. "Modified nucleoside" means a nucleoside having,

independently, a modified sugar moiety and/or modified nucleobase. "Modified oligonucleotide" means an oligonucleotide comprising at least one modified internucleoside linkage, a modified sugar, and/or a modified nucleobase.

"Chemically distinct region" refers to a region of a compound that is in some way chemically different than another region of the same 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 compounds" means antisense compounds that have at least 2 chemically distinct regions, each position having a plurality of subunits.

"Cleavable bond" means any chemical bond capable of being split. In certain embodiments, a cleavable bond is selected from among: an amide, a polyamide, an ester, an ether, one or both esters of a phosphodiester, a phosphate ester, a carbamate, a di-sulfide, or a peptide.

"Cleavable moiety" means a bond or group of atoms that is cleaved under physiological conditions, for example, inside a cell, an animal, or a human.

"Complementary" in reference to an oligonucleotide means the nucleobase sequence of such oligonucleotide or one or more regions thereof matches the nucleobase sequence of another oligonucleotide or nucleic acid or one or more regions thereof when the two nucleobase sequences are aligned in opposing directions. Nucleobase matches or complementary nucleobases, as described herein, are limited to the following pairs: adenine (A) and thymine (T), adenine (A) and uracil (U), cytosine (C) and guanine (G), and 5-methyl cytosine (^{m}C) and guanine (G) unless otherwise specified. Complementary oligonucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside and may include one or more nucleobase mismatches. By contrast, "fully complementary" or "100% complementary" in reference to oligonucleotides means that such oligonucleotides have nucleobase matches at each nucleoside without any nucleobase mismatches.

"Conjugate group" means a group of atoms that is attached to an oligonucleotide. Conjugate groups include a conjugate moiety and a conjugate linker that attaches the conjugate moiety to the oligonucleotide.

"Conjugate linker" means a group of atoms comprising at least one bond that connects a conjugate moiety to an oligonucleotide.

"Conjugate moiety" means a group of atoms that is attached to an oligonucleotide via a conjugate linker.

"Contiguous" in the context of an oligonucleotide refers to nucleosides, nucleobases, sugar moieties, or internucleoside linkages that are immediately adjacent to each other. For example, "contiguous nucleobases" means nucleobases that are immediately adjacent to each other in a sequence.

"Designing" or "Designed to" refer to the process of designing a compound that specifically hybridizes with a selected nucleic acid molecule.

"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.

"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.

"Differently modified" mean chemical modifications or chemical substituents that are different from one another, including absence of modifications. Thus, for example, a MOE nucleoside and an unmodified DNA nucleoside are "differently modified," even though the DNA nucleoside is unmodified. Likewise, DNA and RNA are "differently modified," even though both are naturally-occurring unmodified nucleosides. Nucleosides that are the same but for comprising different nucleobases are not differently modified. For example, a nucleoside comprising a 2'-OMe modified sugar and an unmodified adenine nucleobase and a nucleoside comprising a 2'-OMe modified sugar and an unmodified thymine nucleobase are not differently modified.

"Dose" means a specified quantity of a compound or pharmaceutical agent provided in a single administration, or in a specified time period. In certain embodiments, a dose may be administered in two or more boluses, tablets, or injections. For example, in certain embodiments, where subcutaneous administration is desired, the desired dose may require a volume not easily accommodated by a single injection. In such embodiments, two or more injections may be used to achieve the desired dose. In certain embodiments, a dose may be administered in two or more injections to minimize injection site reaction in an individual. In other embodiments, the compound or pharmaceutical agent is administered by infusion over an extended period of time or continuously. Doses may be stated as the amount of pharmaceutical agent per hour, day, week or month.

"Dosing regimen" is a combination of doses designed to achieve one or more desired effects.

"Double stranded compound" means a compound comprising two oligomeric compounds that are complementary to each other and form a duplex, and wherein one of the two said oligomeric compounds comprises an oligonucleotide.

"Effective amount" means the amount of compound sufficient to effectuate a desired physiological outcome in an individual in need of the compound. The effective amount may 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.

"Ensembl ID" is an identification number consisting of letters and numbers assigned to a gene sequence by Ensembl, which is a joint project between EMBL-EBI and the Wellcome Trust Sanger Institute to develop a software system that produces and maintans automatic annotation of selected eukaryotic genomes. Ensembl annotation helps identify a gene location in a particular genome and can be used to configure the equivalent gene on another species' genome.

"Expression" includes all the functions by which a gene's coded information is converted into structures present and operating in a cell. Such structures include, but are not limited to the products of transcription and translation.

"Gapmer" means an oligonucleotide comprising an internal region having a plurality of nucleosides that support RNase H cleavage 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 may be referred to as the "gap" and the external regions may be referred to as the "wings."

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

"Glucose tolerance test" or "GTT" is a test to measure how well an animal breaks down glucose. Glucose is administered to the animal by injection or oral ingestion ("oral glucose tolerance test" or "OGTT") and blood samples are then drawn periodically and measured for the amount of glucose present.

"Hybridization" means annealing of oligonucleotides and/or nucleic acids. While not limited to a particular mechanism, the most common mechanism of hybridization involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense compound and a nucleic acid target. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an oligonucleotide and a nucleic acid target.

"Immediately adjacent" means there are no intervening elements between the immediately adjacent elements of the same kind (e.g. no intervening nucleobases between the immediately adjacent nucleobases).

"Individual" 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 relative to the expression of activity in an untreated or control sample 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 cells, e.g., fat, muscle and/or 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" means a group or bond that forms a covalent linkage between adjacent nucleosides in an oligonucleotide. "Modified internucleoside linkage" means any internucleoside linkage other than a naturally occurring, phosphate internucleoside linkage. Non-phosphate linkages are referred to herein as modified internucleoside linkages.

"Intravenous administration" means administration into a vein.

"Lengthened oligonucleotides" are those that have one or more additional nucleosides relative to an oligonucleotide disclosed herein, e.g. a parent oligonucleotide.

"Linked nucleosides" means adjacent nucleosides linked together by an internucleoside linkage.

"Linker-nucleoside" means a nucleoside that links an oligonucleotide to a conjugate moiety. Linker-nucleosides are located within the conjugate linker of a compound. Linker-nucleosides are not considered part of the oligonucleotide portion of a compound even if they are contiguous with the oligonucleotide.

"KEAP1" means any nucleic acid or protein of KEAP1. "KEAP1 nucleic acid" means any nucleic acid encoding KEAP1. For example, in certain embodiments, a KEAP1 nucleic acid includes a DNA sequence encoding KEAP1, a RNA sequence transcribed from DNA encoding KEAP1 (including genomic DNA comprising introns and exons), including a non-protein encoding (*i.e.* non-coding) RNA sequence, and an mRNA sequence encoding KEAP1. "KEAP1 mRNA" means a mRNA encoding a KEAP1 protein. The target may be referred to in either upper or lower case.

"KEAP1 specific inhibitor" refers to any agent capable of specifically inhibiting KEAP1 expression or activity at the molecular level. For example, KEAP1-specific inhibitors include nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of KEAP1.

"Metabolic disorder" or "metabolic disease" 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 disorders include, but are not limited to, hyperglycemia, prediabetes, diabetes (type I and type 2), obesity, insulin resistance, NASH, NAFLD, metabolic syndrome and dyslipidemia due to type 2 diabetes.

"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" means a nucleobase of a first oligonucleotide that is not complementary to the corresponding nucleobase of a second oligonucleotide or target nucleic acid when the first and second oligonucleotides are aligned. For example, nucleobases including but not limited to a universal nucleobase, inosine, and hypoxanthine, are capable of hybridizing with at least one nucleobase but are still mismatched or non-complementary with respect to nucleobase to which it hybridized. As another example, a nucleobase of a first oligonucleotide that is not capable of hybridizing to the corresponding nucleobase of a second oligonucleotide or target nucleic acid when the first and second oligonucleotides are aligned is a mismatch or non-complementary nucleobase.

"Modulating" refers to changing or adjusting a feature in a cell, tissue, organ or organism. For example, modulating a KEAP1 can mean to increase or decrease the level of the KEAP1 in a cell, tissue, organ or organism. A "modulator" effects the change in the cell, tissue, organ or organism. For example, a compound can be a modulator of KEAP1 that decreases the amount of KEAP1 in a cell, tissue, organ or organism.

"MOE" means methoxyethyl.

"Monomer" refers to a single unit of an oligomer. Monomers include, but are not limited to, nucleosides and nucleotides.

"Motif" means the pattern of unmodified and/or modified sugar moieties, nucleobases, and/or internucleoside linkages, in an oligonucleotide.

"Non-alcoholic fatty liver disease" or "NAFLD" means a condition characterized by fat accumulation in 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 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. It has been suggested that increased liver triglycerides lead to increased oxidative stress in hepatocytes of animals and humans, indicating a potential cause-and-effect relationship between hepatic triglyceride accumulation, oxidative stress, and the progression of hepatic steatosis to NASH (Browning and Horton, *J Clin Invest,* 2004, *114*, 147-152). Hypertriglyceridemia and hyperfattyacidemia can cause triglyceride accumulation in peripheral tissues (Shimamura et al., *Biochem Biophys Res Commun,* 2004, *322*, 1080-1085).

"Natural" or "naturally occurring" means found in nature.

"Non-bicyclic modified sugar" or "non-bicyclic modified sugar moiety" means a modified sugar moiety that comprises a modification, such as a substituent, that does not form a bridge between two atoms of the sugar to form a second ring.

"Nucleic acid" refers to molecules composed of monomeric nucleotides. A nucleic acid includes, but is not limited to, ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single stranded nucleic acids, and double stranded nucleic acids.

"Nucleobase" means a heterocyclic moiety capable of pairing with a base of another nucleic acid. As used herein a "naturally occurring nucleobase" is adenine (A), thymine (T), cytosine (C), uracil (U), and guanine (G). A "modified nucleobase" is a naturally occurring nucleobase that is chemically modified. A "universal base" or "universal nucleobase" is a nucleobase other than a naturally occurring nucleobase and modified nucleobase, and is capable of pairing with any nucleobase.

"Nucleobase sequence" means the order of contiguous nucleobases in a nucleic acid or oligonucleotide independent of any sugar or internucleoside linkage.

"Nucleoside" means a compound comprising a nucleobase and a sugar moiety. The nucleobase and sugar moiety are each, independently, unmodified or modified. "Modified nucleoside" means a nucleoside comprising a modified nucleobase and/or a modified sugar moiety. Modified nucleosides include abasic nucleosides, which lack a nucleobase.

"Oligomeric compound" means a compound comprising a single oligonucleotide and optionally one or more additional features, such as a conjugate group or terminal group.

"Oligonucleotide" means a polymer of linked nucleosides each of which can be modified or unmodified, independent one from another. Unless otherwise indicated, oligonucleotides consist of 8-80 linked nucleosides. "Modified oligonucleotide" means an oligonucleotide, wherein at least one sugar, nucleobase, or internucleoside linkage is modified. "Unmodified oligonucleotide" means an oligonucleotide that does not comprise any sugar, nucleobase, or internucleoside modification.

"Parent oligonucleotide" means an oligonucleotide whose sequence is used as the basis of design for more oligonucleotides of similar sequence but with different lengths, motifs, and/or chemistries. The newly designed oligonucleotides may have the same or overlapping sequence as the parent oligonucleotide.

"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.

"Pharmaceutically acceptable carrier or diluent" means any substance suitable for use in administering to an animal. For example, a pharmaceutically acceptable carrier can be a sterile aqueous solution, such as PBS or water-for-injection.

"Pharmaceutically acceptable salts" means physiologically and pharmaceutically acceptable salts of compounds, such as oligomeric compounds or oligonucleotides, i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

"Pharmaceutical agent" means a compound that provides a therapeutic benefit when administered to an individual.

"Pharmaceutical composition" means a mixture of substances suitable for administering to an individual. For example, a pharmaceutical composition may comprise one or more compounds or salt thereof and a sterile aqueous solution.

"Phosphorothioate linkage" means a modified phosphate linkage in which one of the non-bridging oxygen atoms is replaced with a sulfur atom. A phosphorothioate internucleoside linkage is a modified internucleoside linkage.

"Phosphorus moiety" means a group of atoms comprising a phosphorus atom. In certain embodiments, a phosphorus moiety comprises a mono-, di-, or tri-phosphate, or phosphorothioate.

"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 oligomeric compound.

"Prevent" refers to delaying or forestalling the onset, development or progression of a disease, disorder, or condition for a period of time from minutes to indefinitely.

"Prodrug" means a compound in a form outside the body which, when administered to an individual, is metabolized to another form within the body or cells thereof. In certain embodiments, the metabolized form is the active, or more active, form of the compound (e.g., drug). Typically conversion of a prodrug within the body is facilitated by the action of an enzyme(s) (e.g., endogenous or viral enzyme) or chemical(s) present in cells or tissues, and/or by physiologic conditions.

"Reduce" means to bring down to a smaller extent, size, amount, or number.

"RefSeq No." is a unique combination of letters and numbers assigned to a sequence to indicate the sequence is for a particular target transcript (e.g., target gene). Such sequence and information about the target gene (collectively, the gene record) can be found in a genetic sequence database. Genetic sequence databases include the NCBI Reference Sequence database, GenBank, the European Nucleotide Archive, and the DNA Data Bank of Japan (the latter three forming the International Nucleotide Sequence Database Collaboration or INSDC).

"Region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic.

"RNAi compound" means an antisense compound that acts, at least in part, through RISC or Ago2, but not through RNase H, to modulate a target nucleic acid and/or protein encoded by a target nucleic acid. RNAi compounds include, but are not limited to double stranded siRNA, single stranded RNA (ssRNA), and microRNA, including microRNA mimics.

"Segments" are defined as smaller or sub-portions of regions within a nucleic acid.

"Side effects" means physiological disease and/or conditions 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 may

indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

"Single stranded" in reference to a compound means the compound has only one oligonucleotide. "Self-complementary" means an oligonucleotide that at least partially hybridizes to itself. A compound consisting of one oligonucleotide, wherein the oligonucleotide of the compound is self-complementary, is a single stranded compound. A single stranded compound may be capable of binding to a complementary compound to form a duplex.

"Sites," are defined as unique nucleobase positions within a target nucleic acid.

"Specifically hybridizable" refers to an oligonucleotide having a sufficient degree of complementarity between the oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids. In certain embodiments, specific hybridization occurs under physiological conditions.

"Specifically inhibit" a target nucleic acid means to reduce or block expression of the target nucleic acid while exhibiting fewer, minimal, or no effects on non-target nucleic acids reduction and does not necessarily indicate a total elimination of the target nucleic acid's expression.

"Standard cell assay" means assay(s) described in the Examples and reasonable variations thereof.

"Standard in vivo experiment" means the procedure(s) described in the Example(s) and reasonable variations thereof.

"Sugar moiety" means an unmodified sugar moiety or a modified sugar moiety. "Unmodified sugar moiety" or "unmodified sugar" means a 2'-OH(H) furanosyl moiety, as found in RNA (an "unmodified RNA sugar moiety"), or a 2'-H(H) moiety, as found in DNA (an "unmodified DNA sugar moiety"). Unmodified sugar moieties have one hydrogen at each of the 1', 3', and 4' positions, an oxygen at the 3' position, and two hydrogens at the 5' position. "Modified sugar moiety" or "modified sugar" means a modified furanosyl sugar moiety or a sugar surrogate. "Modified furanosyl sugar moiety" means a furanosyl sugar comprising a non-hydrogen substituent in place of at least one hydrogen of an unmodified sugar moiety. In certain embodiments, a modified furanosyl sugar moiety is a 2'-substituted sugar moiety. Such modified furanosyl sugar moieties include bicyclic sugars and non-bicyclic sugars.

"Sugar surrogate" means a modified sugar moiety having other than a furanosyl moiety that can link a nucleobase to another group, such as an internucleoside linkage, conjugate group, or terminal group in an oligonucleotide. Modified nucleosides comprising sugar surrogates can be incorporated into one or more positions within an oligonucleotide and such oligonucleotides are capable of hybridizing to complementary oligomeric compounds or nucleic acids.

"Subcutaneous administration" means administration just below the skin.

"Target gene" refers to a gene encoding a target.

"Targeting" means specific hybridization of a compound that to a target nucleic acid in order to induce a desired effect.

"Target nucleic acid," "target RNA," "target RNA transcript" and "nucleic acid target" all mean a nucleic acid capable of being targeted by compounds described herein.

"Target region" means a portion of a target nucleic acid to which one or more compounds is targeted.

"Target segment" means the sequence of nucleotides of a target nucleic acid to which a compound described herein 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.

"Terminal group" means a chemical group or group of atoms that is covalently linked to a terminus of an oligonucleotide.

"Therapeutically effective amount" means an amount of a compound, pharmaceutical agent, or composition that provides a therapeutic benefit to an individual.

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

Certain Embodiments

Certain embodiments provide KEAP1 nucleic acids disclosed herein. In certain embodiments disclosed herein, KEAP1 has the human sequence as set forth in GenBank Accession No. NM_203500.1 (incorporated herein as SEQ ID NO: 1). In certain embodiments disclosed herein, KEAP1 has the human sequence as set forth in GenBank Accession No. NM_012289.3 (incorporated herein as SEQ ID NO: 2). In certain embodiments, KEAP1 has the human sequence as set forth in GenBank Accession No. NC_000019.10 nucleotides 10483001_10506000 (incorporated herein as SEQ ID NO: 3). In certain embodiments disclosed herein, KEAP1 has the mouse sequence as set forth in GenBank Accession No. NM_001110305.1 (incorporated herein as SEQ ID NO: 4). In certain embodiments disclosed herein, KEAP1 has the mouse sequence as set forth in GenBank Accession No. NM_016679.4 (incorporated herein as SEQ ID NO: 5). In certain embodiments, KEAP1 has the mouse sequence as set forth in GenBank Accession No. NC_000075.6 nucleotides 21227001_21242000 (incorporated herein as SEQ ID NO: 6).

Certain embodiments provide methods, compounds, and compositions for modulating a disease related to oxidative stress, or a symptom thereof, in an animal by administering a therapeutically effective amount of a compound or composition to the animal, wherein the compound or composition comprises a KEAP1 modulator. Modulation of a KEAP1 can lead to a decrease in KEAP1 level or expression in order to treat, prevent, ameliorate or delay oxidative stress or a disease, disorder or condition related to oxidative stress, or a symptom thereof. In certain embodiments, the KEAP1 modulator is a KEAP1 specific inhibitor. In certain embodiments, KEAP1 specific inhibitors are nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of the KEAP1.

Certain embodiments disclosed herein provide compounds or compositions comprising a KEAP1 modulator. In certain embodiments, the KEAP1 modulator is a KEAP1 specific inhibitor. In certain embodiments, the KEAP1 specific inhibitor is a nucleic acid, polypeptide, antibody, small molecules, or other

agent capable of inhibiting the expression of KEAP1. In certain embodiments, the nucleic acid is a compound or composition targeting KEAP1. In certain embodiments, the compound or composition comprises deoxyribonucleotides. In certain embodiments, the compound or composition comprises ribonucleotides. In certain embodiments, the compound or composition is single stranded. In certain embodiments, the compound or composition is double stranded. In certain embodiments, the compound or composition targeting KEAP1 comprises an oligonucleotide. In certain embodiments, the oligonucleotide is single stranded. In certain embodiments, the oligonucleotide is double stranded. In certain embodiments, the oligonucleotide is a modified oligonucleotide. In certain embodiments, the modified oligonucleotide is single stranded. In certain embodiments, the modified oligonucleotide is double stranded. In any of the foregoing embodiments, the compound can be an antisense compound or oligomeric compound.

In any of the foregoing embodiments, the compound or composition can consist of 8 to 80, 10 to 30, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 22, 18 to 24, 18 to 30, 18 to 50, 19 to 22, 19 to 30, 19 to 50, or 20 to 30 linked nucleosides. In certain embodiments, these compounds are oligonucleotides.

Certain embodiments disclosed herein provide a compound or composition comprising a modified oligonucleotide that is 10 to 30 linked nucleosides in length targeted to a KEAP1. The KEAP1 target can have a nucleobase sequence selected from any one of SEQ ID NOs: 1-6. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 70%, 75%, 80%, 85%, 90%, 95% or 100% complementary to the nucleobase sequences recited in any one of SEQ ID NOs: 1-6 as measured over the entirety of the modified oligonucleotide. In certain embodiments, the modified oligonucleotide comprises a nucleobase sequence comprising a portion of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, or 16 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 1-6.

Certain embodiments provide a compound comprising a modified oligonucleotide 8 to 80 linked nucleosides in length and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 7-9. In certain embodiments, the compound is an antisense compound or oligomeric compound. In certain embodiments, the compound is single stranded. In certain embodiments, the compound is double stranded. In certain embodiments, the modified oligonucleotide consists of 12 to 30 linked nucleosides.

Certain embodiments provide a compound comprising a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 7-9. In certain embodiments, the compound is an antisense compound or oligomeric compound. In certain embodiments, the compound is single stranded. In certain embodiments, the compound is double stranded.

In certain embodiments, the modified oligonucleotide consists of 10 to 50, 10 to 30, 12 to 30, 13 to 24, 14 to 24, 15 to 30, 15 to 24, 15 to 20, 15 to 18, 16 to 30, 16 to 24, 16 to 20, 16 to 18, 18 to 24 or 19 to 22 linked nucleosides. 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 or 30 linked nucleosides or a range defined by any two of these values.

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

In certain embodiments, any of the foregoing oligonucleotides comprises at least one modified sugar. In certain embodiments, at least one modified sugar comprises a 2'-O-methoxyethyl group. In certain embodiments, at least one modified sugar is a bicyclic sugar, such as a 4'-$CH(CH_3)$-O-2' group, a 4'-CH_2-O-2' group, or a 4'-$(CH_2)_2$-O-2'group.

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

Certain embodiments disclosed herein provide a compound or composition comprising a modified oligonucleotide with: 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 sugar comprises a bicyclic sugar or 2'-O-methoxyethyl group. In certain embodiments, the bicyclic sugar comprises a 4'-$CH(CH_3)$-O-2' group, a 4'-CH_2-O-2' group or a 4'-$(CH_2)_2$-O-2'group. In certain embodiments, at least one internucleoside linkage is a phosphorothioate linkage. In certain embodiments, each internucleoside linkage is a phosphorothioate linkage. In certain embodiments, at least one cytosine is a 5-methylcytosine. In certain embodiments, each cytosine is a 5-methylcytosine.

Certain embodiments disclosed herein provide a compound or composition comprising a modified oligonucleotide 16 nucleobases in length with: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of three linked nucleosides; and c) a 3' wing segment consisting of three 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 sugar comprises a bicyclic sugar or 2'-O-methoxyethyl group. In certain embodiments, the bicyclic sugar comprises a 4'-$CH(CH_3)$-O-2' group, a 4'-CH_2-O-2' group or a 4'-$(CH_2)_2$-O-2'group. In certain embodiments, at least one internucleoside linkage is a phosphorothioate linkage. In certain embodiments, each internucleoside linkage is a phosphorothioate linkage. In certain embodiments, at least one cytosine is a 5-methylcytosine. In certain embodiments, each cytosine is a 5-methylcytosine.

Certain embodiments disclosed herein provide a compound or composition comprising a modified oligonucleotide 20 nucleobases in length with: 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 and each nucleoside of each wing segment comprises a modified sugar. In certain embodiments, the modified sugar comprises a bicyclic sugar or 2'-O-methoxyethyl group. In certain embodiments, the bicyclic sugar comprises a 4'-$CH(CH_3)$-O-2' group, a 4'-CH_2-O-2' group or a 4'-$(CH_2)_2$-O-2'group. In certain embodiments, at least one internucleoside linkage is a phosphorothioate linkage. In certain embodiments, each internucleoside linkage is a phosphorothioate linkage. In certain embodiments, at least one cytosine is a 5-methylcytosine. In certain embodiments, each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions disclosed herein further comprise a conjugate group. In certain embodiments, the conjugate group is a carbohydrate group. In certain embodiments, the conjugate group is a GalNAc group.

In certain embodiments, the compounds or compositions disclosed herein comprise a salt of the compound. In certain embodiments, the compounds or compositions disclosed herein comprise a salt of the modified oligonucleotide. In certain embodiments, the salt is a sodium salt. In certain embodiments, the salt is a potassium salt.

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

Certain embodiments disclosed herein provide a method of reducing KEAP1 expression in an animal comprising administering to the animal a compound or composition comprising a KEAP1 specific inhibitor. Certain embodiments disclosed herein provide use of a KEAP1 specific inhibitor for reducing KEAP1 expression in an animal. In certain embodiments, the KEAP1 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of KEAP1. In certain embodiments, the KEAP1 specific inhibitor comprises a compound or composition described herein. In certain embodiments, the compound or composition comprises an antisense compound or an oligomeric compound. In certain embodiments, the compound or composition comprises a modified oligonucleotide. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides.

Certain embodiments disclosed herein provide a method of reducing oxidative stress in an animal comprising administering to the animal a therapeutically effective amount of a compound or composition comprising a KEAP1 specific inhibitor. Certain embodiments disclosed herein provide use of a KEAP1 specific inhibitor for reducing oxidative stress in an animal. In certain embodiments, the KEAP1 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of KEAP1. In certain embodiments, the KEAP1 specific inhibitor comprises a compound or composition described herein. In certain embodiments, the compound or composition comprises an antisense compound or an oligomeric compound. In certain embodiments, the compound or composition comprises a modified oligonucleotide. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked

nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides.

Certain embodiments disclosed herein provide a method of treating, preventing, delaying or ameliorating a disease, disorder or condition related to oxidative stress in an animal comprising administering to the animal a therapeutically effective amount of a compound or composition comprising a KEAP1 specific inhibitor. Certain embodiments disclosed herein provide use of a KEAP1 specific inhibitor for treating, preventing, delaying or ameliorating a disease, disorder or condition related to oxidative stress. In certain embodiments, the KEAP1 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of KEAP1. In certain embodiments, the KEAP1 specific inhibitor comprises a compound or composition described herein. In certain embodiments, the compound or composition comprises an antisense compound or an oligomeric compound. In certain embodiments, the compound or composition comprises a modified oligonucleotide. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides.

Certain embodiments disclosed herein provide a method of treating an animal at risk for a disease, disorder or condition related to oxidative stress comprising administering to the animal a therapeutically effective amount of a compound or composition comprising a KEAP1 specific inhibitor. Certain embodiments disclosed herein provide use of a KEAP1 specific inhibitor for treating an animal at risk for a disease, disorder or condition related to oxidative stress. In certain embodiments, the KEAP1 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of KEAP1. In certain embodiments, the KEAP1 specific inhibitor comprises a compound or composition described herein. In certain embodiments, the compound or composition comprises an antisense compound or an oligomeric compound. In certain embodiments, the compound or composition comprises a modified oligonucleotide. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides.

In certain embodiments the disease, disorder or condition related to oxidative stress is a metabolic disease, disorder or condition. In certain embodiments the metabolic disease, disorder or condition is metabolic syndrome, type 2 diabetes, diabetic nephropathy, diabetic cardiomyopathy and/or insulin resistance. In certain embodiments the disease, disorder or condition related to oxidative stress is a fatty liver disease, disorder or condition. In certain embodiments the fatty liver disease, disorder or condition is hepatic steatosis, NASH and/or NAFLD. In certain embodiments the disease, disorder or condition related to oxidative stress is an inflammatory disease. In certain embodiments the inflammatory disease, disorder or condition is asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and/or airway hyperresponsiveness. In certain embodiments the disease, disorder or condition related to oxidative stress is

an allergic disease, disorder or condition. In certain embodiments the allergic disease, disorder or condition is asthma. In certain embodiments the disease, disorder or condition related to oxidative stress is a fibrotic disease, disorder or condition. In certain embodiments, the fibrotic disease, disorder or condition is pulmonary fibrosis, renal fibrosis, cystic fibrosis, or hepatic fibrosis. In certain embodiments the disease, disorder or condition related to oxidative stress is cancer, age related macular degeneration, atherosclerosis, hyperlipidemia, and neurologic disorders.

Certain embodiments disclosed herein provide a method of treating, preventing, delaying or ameliorating organ damage in an animal comprising administering to the animal a therapeutically effective amount of a compound or composition comprising a KEAP1 specific inhibitor. Certain embodiments disclosed herein provide use of a KEAP1 specific inhibitor for treating, preventing, delaying or ameliorating a disease, disorder or condition related to organ damage. In certain embodiments, the organ damage is due to fibrosis. In certain embodiments, the fibrotic organ is lung or liver. In certain embodiments, the fibrotic disease, disorder or condition is hepatic fibrosis, NAFLD, NASH, pulmonary fibrosis, cystic fibrosis, or hepatic fibrosis. In certain embodiments, the KEAP1 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of KEAP1. In certain embodiments, the KEAP1 specific inhibitor comprises a compound or composition described herein. In certain embodiments, the compound or composition comprises an antisense compound or an oligomeric compound. In certain embodiments, the compound or composition comprises a modified oligonucleotide. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides

Certain embodiments provide methods of using the compounds and compositions described herein for inhibiting a KEAP1 expression. In certain embodiments, the compounds or compositions inhibit KEAP1 by at least 5%, at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90%.

Certain embodiments provide methods of using the compounds and compositions described herein for use in therapy. In certain embodiments, the therapy is used in treating, preventing, delaying the onset or slowing progression of a disease, disorder or condition related to KEAP1. In certain embodiments, the KEAP1 disease, disorder or condition is related to oxidative stress. In certain embodiments the disease, disorder or condition related to oxidative stress is a metabolic disease, disorder or condition. In certain embodiments the metabolic disease, disorder or condition is metabolic syndrome, type 2 diabetes, diabetic nephropathy, diabetic cardiomyopathy and/or insulin resistance. In certain embodiments the disease, disorder or condition related to oxidative stress is a fatty liver disease, disorder or condition. In certain embodiments the fatty liver disease, disorder or condition is hepatic steatosis, NASH and/or NAFLD. In certain embodiments the disease, disorder or condition related to oxidative stress is an inflammatory disease. In certain embodiments the inflammatory disease, disorder or condition is asthma, chronic obstructive

pulmonary disease (COPD), cystic fibrosis (CF) and/or airway hyperresponsiveness. In certain embodiments the disease, disorder or condition related to oxidative stress is an allergic disease, disorder or condition. In certain embodiments the allergic disease, disorder or condition is asthma. In certain embodiments the disease, disorder or condition related to oxidative stress is a fibrotic disease, disorder or condition. In certain embodiments, the fibrotic disease, disorder or condition is pulmonary fibrosis, renal fibrosis, cystic fibrosis, or hepatic fibrosis. In certain embodiments the disease, disorder or condition related to oxidative stress is cancer, age related macular degeneration, atherosclerosis, hyperlipidemia, and neurologic disorders.

Certain embodiments provide methods of using the compounds and compositions described herein for use in the manufacture of a medicament. In certain embodiments, the medicament is used in treating, preventing, delaying the onset or slowing progression of a disease, disorder or condition related to KEAP1. In certain embodiments, the disease is related to oxidative stress. In certain embodiments the disease, disorder or condition related to oxidative stress is a metabolic disease, disorder or condition. In certain embodiments the metabolic disease, disorder or condition is metabolic syndrome, type 2 diabetes, diabetic nephropathy, diabetic cardiomyopathy and/or insulin resistance. In certain embodiments the disease, disorder or condition related to oxidative stress is a fatty liver disease, disorder or condition. In certain embodiments the fatty liver disease, disorder or condition is hepatic steatosis, NASH and/or NAFLD. In certain embodiments the disease, disorder or condition related to oxidative stress is an inflammatory disease. In certain embodiments the inflammatory disease, disorder or condition is asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and/or airway hyperresponsiveness. In certain embodiments the disease, disorder or condition related to oxidative stress is an allergic disease, disorder or condition. In certain embodiments the allergic disease, disorder or condition is asthma. In certain embodiments the disease, disorder or condition related to oxidative stress is a fibrotic disease, disorder or condition. In certain embodiments, the fibrotic disease, disorder or condition is pulmonary fibrosis, renal fibrosis, cystic fibrosis, or hepatic fibrosis. In certain embodiments the disease, disorder or condition related to oxidative stress is cancer, age related macular degeneration, atherosclerosis, hyperlipidemia, and neurologic disorders.

In certain embodiments, the animal is a human.

In any of the foregoing methods or uses, the compound or composition can be administered parenterally. For example, in certain embodiments the compound or composition can be administered through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration.

In certain embodiments, the compounds or compositions disclosed herein are designated as a first agent and the methods or uses disclosed herein further comprise administering a second agent. In certain embodiments, the first agent and the second agent are co-administered. In certain embodiments the first agent and the second agent are co-administered sequentially or concomitantly.

Certain embodiments provide a method of using a compound or composition disclosed herein to reduce a biomarker of oxidative stress by at least 5%, at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 45% or at least 50%. In certain embodiments, the biomarker reduced is liver triglyceride, liver cholesterol, liver ballooning degeneration, fibrosis (e.g., fibrosis in an organ such as the liver or lung), body weight, organ weight, hyperinsulinemia, fasting hyperleptinemia, liver hydroxyproline, collagen (e.g., Col1a2), liver glutathione (GSH), asthma, airway hyperresponsiveness (AHR), eosinophil infiltration into the lungs, mucus production (e.g., as measured by expression of GOB5, SPDEF and/or MUC5B) and inflammation (e.g., as measured by macrophage-restricted F4/80). In certain embodiments, the biomarker of fibrosis is hydroxyproline or collagen (e.g., Col1a2). Certain embodiments provide a method of using a compound or composition disclosed herein to increase an antioxidant biomarker by at least 5%, at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 45% or at least 50%. In certain embodiments, the biomarker increased is insulin sensitivity, glucose tolerance, NRF2, PGAM5, Glutathione S-transferases (GSTs e.g., GSTA1), GCLM, NQO1, NQO2, HO-1.

Certain embodiments provided herein relate to methods of using the KEAP1 targeting compounds described herein in combination with one or more agent or therapy to treat oxidative stress or a disease, disorder or condition related to oxidative stress. In certain embodiments, the agents are antioxidants. In certain embodiments, the agents are anti-inflammatory drugs. In certain embodiments, the agents are anti-allergy drugs. In certain embodiments, the agents are insulin sensitizing drugs. In certain embodiments, the agents are glucose absorption inhibitors. In certain embodiments, the agents are glucose-lowering drugs. In some embodiments, the drugs include, but are not limited to: a PPAR agonist (gamma, dual, or pan), 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, or an alpha-glucosidase inhibitor. In certain embodiments, the drug is metformin, sulfonylurea, or rosiglitazone. Agents or therapies can be administered concomitantly or sequentially to an animal.

In certain embodiments, provided is a kit for treating, preventing, or ameliorating an oxidative stress related disease and/or condition, disease, disorder or condition, wherein the kit comprises: (i) a KEAP1 specific inhibitor as described herein; and optionally (ii) an additional agent or therapy as described herein.

A kit of the present invention may further include instructions for using the kit to treat, prevent, or ameliorate an oxidative stress related disease, disorder or condition as described herein.

Certain Indications

Certain embodiments provided herein relate to methods of inhibiting KEAP1 expression, which can be useful for treating, preventing, or ameliorating a disease, disorder or condition associated with a KEAP1 in an individual, by administration of a compound that specifically targets KEAP1. An example of a disease, disorder or condition associated with KEAP1 is oxidative stress.

Certain embodiments provided herein relate to methods of reducing, treating or ameliorating oxidative stress in an animal by administration of a compound that specifically targets KEAP1 to the animal suffering from oxidative stress.

Certain embodiments provided herein relate to methods of preventing oxidative stress in an animal by administration of a compound that specifically targets KEAP1 to an animal suffering prone to oxidative stress.

Certain embodiments provided herein relate to methods of reducing oxidative stress in an animal, which can be useful for treating, preventing, or ameliorating a disease, disorder or condition associated with oxidative stress, by administration of a compound that specifically targets KEAP1 to the animal suffering from the disease, disorder or condition associated with oxidative stress.

In certain embodiments the disease, disorder or condition related to oxidative stress is a metabolic disease, disorder or condition. In certain embodiments the metabolic disease, disorder or condition is metabolic syndrome, type 2 diabetes, diabetic nephropathy, diabetic cardiomyopathy and/or insulin resistance.

In certain embodiments the disease, disorder or condition related to oxidative stress is a fatty liver disease, disorder or condition. In certain embodiments the fatty liver disease, disorder or condition is hepatic steatosis, NASH and/or NAFLD.

In certain embodiments the disease, disorder or condition related to oxidative stress is an inflammatory disease. In certain embodiments the inflammatory disease, disorder or condition is asthma, chronic obstructive pulmonary disease (COPD), fibrosis (e.g., hepatic fibrosis, cystic fibrosis (CF), renal fibrosis) and/or airway hyperresponsiveness.

In certain embodiments the disease, disorder or condition related to oxidative stress is an allergic disease, disorder or condition. In certain embodiments the allergic disease, disorder or condition is asthma.

In certain embodiments the disease, disorder or condition related to oxidative stress is a fibrotic disease, disorder or condition. In certain embodiments, the fibrotic disease, disorder or condition is pulmonary fibrosis, renal fibrosis, cystic fibrosis, or hepatic fibrosis.

In certain embodiments the disease, disorder or condition related to oxidative stress is cancer, age related macular degeneration, atherosclerosis, hyperlipidemia, and neurologic disorders.

In certain embodiments, the compound targeting KEAP1 is a KEAP1 specific inhibitor. In certain embodiments, the compound is an antisense compound or an oligomeric compound targeted to a KEAP1. In certain embodiments, the compound comprises an oligonucleotide targeted to a KEAP1 nucleic acid. In certain embodiments, the compound comprises a modified oligonucleotide targeted to a KEAP1 nucleic acid. In certain embodiments disclosed herein, the KEAP1 target has the human or murine sequences as recited in any one of SEQ ID NOs: 1-6.

Certain Compounds

In certain embodiments, compounds described herein are antisense compounds. In certain embodiments, the antisense compound comprises or consists of an oligomeric compound. In certain embodiments, the oligomeric compound comprises a modified oligonucleotide. In certain embodiments, the modified oligonucleotide has a nucleobase sequence complementary to that of a target nucleic acid.

In certain embodiments, a compound described herein comprises or consists of a modified oligonucleotide. In certain embodiments, the modified oligonucleotide has a nucleobase sequence complementary to that of a target nucleic acid.

In certain embodiments, a compound or antisense compound is single stranded. Such a single stranded compound or antisense compound comprises or consists of an oligomeric compound. In certain embodiments, such an oligomeric compound comprises or consists of an oligonucleotide and optionally a conjugate group. In certain embodiments, the oligonucleotide is an antisense oligonucleotide. In certain embodiments, the oligonucleotide is modified. In certain embodiments, the oligonucleotide of a single stranded antisense compound or oligomeric compound comprises a self-complementary nucleobase sequence.

In certain embodiments, compounds are double stranded. Such double stranded compounds comprise a first modified oligonucleotide having a region complementary to a target nucleic acid and a second modified oligonucleotide having a region complementary to the first modified oligonucleotide. In certain embodiments, the modified oligonucleotide is an RNA oligonucleotide. In such embodiments, the thymine nucleobase in the modified oligonucleotide is replaced by a uracil nucleobase. In certain embodiments, compound comprises a conjugate group. In certain embodiments, one of the modified oligonucleotides is conjugated. In certain embodiments, both the modified oligonucleotides are conjugated. In certain embodiments, the first modified oligonucleotide is conjugated. In certain embodiments, the second modified oligonucleotide is conjugated. In certain embodiments, each modified oligonucleotide is 12-30 linked nucleosides in length.

In certain embodiments, compounds are double stranded. Such double stranded compounds comprise a first oligomeric compound having a region complementary to a target nucleic acid and a second oligomeric compound having a region complementary to the first oligomeric compound. The first oligomeric compound of such double stranded compounds typically comprises or consists of a modified oligonucleotide and optionally a conjugate group. The oligonucleotide of the second oligomeric compound of such double stranded compound may be modified or unmodified. Either or both oligomeric compounds of a double stranded compound may comprise a conjugate group. The oligomeric compounds of double stranded compounds may include non-complementary overhanging nucleosides.

Examples of single stranded and double stranded compounds include but are not limited to oligonucleotides, siRNAs, microRNA targeting oligonucleotides, and single stranded RNAi compounds, such as small hairpin RNAs (shRNAs), single stranded siRNAs (ssRNAs), and microRNA mimics.

In certain embodiments, a compound described herein 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, a compound described herein comprises an oligonucleotide 10 to 30 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 12 to 30 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 12 to 22 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 14 to 30 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 14 to 20 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 15 to 30 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 15 to 20 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 16 to 30 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 16 to 20 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 17 to 30 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 17 to 20 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 18 to 30 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 18 to 21 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 18 to 20 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide is 20 to 30 linked subunits in length. In other words, such oligonucleotides are from 12 to 30 linked subunits, 14 to 30 linked subunits, 14 to 20 subunits, 15 to 30 subunits, 15 to 20 subunits, 16 to 30 subunits, 16 to 20 subunits, 17 to 30 subunits, 17 to 20 subunits, 18 to 30 subunits, 18 to 20 subunits, 18 to 21 subunits, 20 to 30 subunits, or 12 to 22 linked subunits, respectively. In certain embodiments, a compound described herein comprises an oligonucleotide 14 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 16 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 17 linked subunits in length. In certain embodiments, compound described herein comprises an oligonucleotide 18 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 19 linked subunits in length. In certain embodiments, a compound described herein comprises an oligonucleotide 20 linked subunits in length. In other embodiments, a compound described herein comprises an oligonucleotide 8 to 80, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 22, 18 to 24, 18 to 30, 18 to 50, 19 to 22, 19 to 30, 19 to 50, or 20 to 30 linked subunits. In certain such embodiments, the compound described herein comprises an oligonucleotide 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 subunits in length,

or a range defined by any two of the above values. In some embodiments the linked subunits are nucleotides, nucleosides, or nucleobases.

In certain embodiments, the compound may further comprise additional features or elements, such as a conjugate group, that are attached to the oligonucleotide. In certain embodiments, such compounds are antisense compounds. In certain embodiments, such compounds are oligomeric compounds. In embodiments where a conjugate group comprises a nucleoside (*i.e.* a nucleoside that links the conjugate group to the oligonucleotide), the nucleoside of the conjugate group is not counted in the length of the oligonucleotide.

In certain embodiments, compounds may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated compound targeted to a KEAP1 nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end, of the compound. Alternatively, the deleted nucleosides may be dispersed throughout the compound.

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

It is possible to increase or decrease the length of a compound, such as an oligonucleotide, and/or introduce mismatch bases without eliminating activity (Woolf et al. (Proc. Natl. Acad. Sci. USA 89:7305-7309, 1992; Gautschi *et al. J. Natl. Cancer Inst.* 93:463-471, March 2001; Maher and Dolnick *Nuc. Acid. Res.* 16:3341-3358,1988). However, seemingly small changes in oligonucleotide sequence, chemistry and motif can make large differences in one or more of the many properties required for clinical development (Seth et al. *J. Med. Chem.* 2009, *52*, 10; Egli et al. *J. Am. Chem. Soc.* 2011, *133*, 16642).

In certain embodiments, compounds described herein are interfering RNA compounds (RNAi), which include double stranded RNA compounds (also referred to as short-interfering RNA or siRNA) and single stranded RNAi compounds (or ssRNA). Such compounds work at least in part through the RISC pathway to degrade and/or sequester a target nucleic acid (thus, include microRNA/microRNA-mimic compounds). As used herein, the term siRNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics.

In certain embodiments, a double stranded compound described herein can comprise any of the oligonucleotide sequences targeted to a KEAP1 described herein. In certain embodiments, a double stranded

compound comprises a first strand comprising sequence complementary to any one of SEQ ID NOs: 1-6 and a second strand.

In certain embodiments, a double stranded compound comprises a first strand comprising the nucleobase sequence complementary to any one of SEQ ID NOs: 1-6 and a second strand. In certain embodiments, the double stranded compound comprises ribonucleotides in which the first strand has uracil (U) in place of thymine (T) and is complementary to any one of SEQ ID NOs: 1-6. In certain embodiments, a double stranded compound comprises (i) a first strand comprising a nucleobase sequence complementary to the site on a KEAP1 of any of SEQ ID NOs: 1-6, and (ii) a second strand. In certain embodiments, the double stranded compound comprises one or more modified nucleotides in which the 2' position in the sugar contains a halogen (such as fluorine group; 2'-F) or contains an alkoxy group (such as a methoxy group; 2'-OMe). In certain embodiments, the double stranded compound comprises at least one 2'-F sugar modification and at least one 2'-OMe sugar modification. In certain embodiments, the at least one 2'-F sugar modification and at least one 2'-OMe sugar modification are arranged in an alternating pattern for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases along a strand of the dsRNA compound. In certain embodiments, the double stranded compound comprises one or more linkages between adjacent nucleotides other than a naturally-occurring phosphodiester linkage. Examples of such linkages include phosphoramide, phosphorothioate, and phosphorodithioate linkages. The double stranded compounds may also be chemically modified nucleic acid molecules as taught in U.S. Pat. No. 6,673,661. In other embodiments, the dsRNA contains one or two capped strands, as disclosed, for example, by WO 00/63364, filed Apr. 19, 2000. In certain embodiments, the first strand of the double stranded compound is an siRNA guide strand and the second strand of the double stranded compound is an siRNA passenger strand. In certain embodiments, the second strand of the double stranded compound is complementary to the first strand. In certain embodiments, each strand of the double stranded compound consists of 16, 17, 18, 19, 20, 21, 22, or 23 linked nucleosides. In certain embodiments, the first or second strand of the double stranded compound can comprise a conjugate group.

In certain embodiments, a single stranded compound described herein can comprise any of the oligonucleotide sequences targeted to a KEAP1 described herein. In certain embodiments, such a single stranded compound is a single stranded RNAi (ssRNAi) compound. In certain embodiments, a ssRNAi compound comprises at least an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobase portion complementary to any one of SEQ ID NOs: 7-9. In certain embodiments, a ssRNAi compound comprises the nucleobase sequence complementary to any one of SEQ ID NOs: 1-6. In certain embodiments, the ssRNAi compound comprises ribonucleotides in which uracil (U) is in place of thymine (T). In certain embodiments, ssRNAi compound comprises a nucleobase sequence complementary to the site on a KEAP1 with any of the the sequences of SEQ ID NOs: 1-6. In certain embodiments, a ssRNAi compound comprises one or more modified nucleotides in which the 2' position in the sugar contains a halogen (such as fluorine group; 2'-F) or contains an alkoxy group (such as a methoxy group; 2'-OMe). In certain embodiments, a ssRNAi compound comprises at least one 2'-F sugar modification and at least one 2'-OMe sugar

modification. In certain embodiments, the at least one 2'-F sugar modification and at least one 2'-OMe sugar modification are arranged in an alternating pattern for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases along a strand of the ssRNAi compound. In certain embodiments, the ssRNAi compound comprises one or more linkages between adjacent nucleotides other than a naturally-occurring phosphodiester linkage. Examples of such linkages include phosphoramide, phosphorothioate, and phosphorodithioate linkages. The ssRNAi compounds may also be chemically modified nucleic acid molecules as taught in U.S. Pat. No. 6,673,661. In other embodiments, the ssRNAi contains a capped strand, as disclosed, for example, by WO 00/63364, filed Apr. 19, 2000. In certain embodiments, the ssRNAi compound consists of 16, 17, 18, 19, 20, 21, 22, or 23 linked nucleosides. In certain embodiments, the ssRNAi compound can comprise a conjugate group.

In certain embodiments, compounds described herein comprise modified oligonucleotides. Certain modified oligonucleotides have one or more asymmetric center and thus give rise to enantiomers, diastereomers, and other stereoisomeric configurations that may be defined, in terms of absolute stereochemistry, as (R) or (S), as α or β such as for sugar anomers, or as (D) or (L) such as for amino acids etc. Included in the modified oligonucleotides provided herein are all such possible isomers, including their racemic and optically pure forms, unless specified otherwise. Likewise, all cis- and trans-isomers and tautomeric forms are also included.

Certain Mechanisms

In certain embodiments, compounds described herein comprise or consist of modified oligonucleotides. In certain embodiments, compounds described herein are antisense compounds. In certain embodiments, such antisense compounds comprise oligomeric compounds. In certain embodiments, compounds described herein are capable of hybridizing to a target nucleic acid, resulting in at least one antisense activity. In certain embodiments, compounds described herein selectively affect one or more target nucleic acid. Such selective compounds comprise a nucleobase sequence that hybridizes to one or more target nucleic acid, resulting in one or more desired antisense activity and does not hybridize to one or more non-target nucleic acid or does not hybridize to one or more non-target nucleic acid in such a way that results in a significant undesired antisense activity.

In certain antisense activities, hybridization of a compound described herein to a target nucleic acid results in recruitment of a protein that cleaves the target nucleic acid. For example, certain compounds described herein result in RNase H mediated cleavage of the target nucleic acid. RNase H is a cellular endonuclease that cleaves the RNA strand of an RNA:DNA duplex. The DNA in such an RNA:DNA duplex need not be unmodified DNA. In certain embodiments, compounds described herein are sufficiently "DNA-like" to elicit RNase H activity. Further, in certain embodiments, one or more non-DNA-like nucleoside in the gap of a gapmer is tolerated.

In certain antisense activities, compounds described herein or a portion of the compound is loaded into an RNA-induced silencing complex (RISC), ultimately resulting in cleavage of the target nucleic acid. For example, certain compounds described herein result in cleavage of the target nucleic acid by Argonaute. Compounds that are loaded into RISC are RNAi compounds. RNAi compounds may be double stranded (siRNA) or single stranded (ssRNA).

In certain embodiments, hybridization of compounds described herein to a target nucleic acid does not result in recruitment of a protein that cleaves that target nucleic acid. In certain such embodiments, hybridization of the compound to the target nucleic acid results in alteration of splicing of the target nucleic acid. In certain embodiments, hybridization of the compound to a target nucleic acid results in inhibition of a binding interaction between the target nucleic acid and a protein or other nucleic acid. In certain such embodiments, hybridization of the compound to a target nucleic acid results in alteration of translation of the target nucleic acid.

Antisense activities may be observed directly or indirectly. In certain embodiments, observation or detection of an antisense activity involves observation or detection of a change in an amount of a target nucleic acid or protein encoded by such target nucleic acid, a change in the ratio of splice variants of a nucleic acid or protein, and/or a phenotypic change in a cell or animal.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

In certain embodiments, compounds described herein comprise or consist of an oligonucleotide comprising a region that is complementary to a target nucleic acid. In certain embodiments, the target nucleic acid is an endogenous RNA molecule. In certain such embodiments, the target nucleic acid is selected from: an mRNA and a pre-mRNA, including intronic, exonic and untranslated regions. In certain embodiments, the target nucleic acid is a pre-mRNA. In certain such embodiments, the target region is entirely within an intron. In certain embodiments, the target region spans an intron/exon junction. In certain embodiments, the target region is at least 50% within an intron.

Human sequences that encode KEAP1 include, without limitation, the following sequences: GenBank Accession No. NM_203500.1 (incorporated herein as SEQ ID NO: 1), GenBank Accession No. NM_012289.3 (incorporated herein as SEQ ID NO: 2) and GenBank Accession No. NC_000019.10 nucleotides 10483001_10506000 (incorporated herein as SEQ ID NO: 3).

Mouse sequences that encode KEAP1 include, without limitation, the following sequences: GenBank Accession No. NM_001110305.1 (incorporated herein as SEQ ID NO: 4), GenBank Accession No. NM_016679.4 (incorporated herein as SEQ ID NO: 5), GenBank Accession No. NC_000075.6 nucleotides 21227001_21242000 (incorporated herein as SEQ ID NO: 6).

Hybridization

In some embodiments, hybridization occurs between a compound disclosed herein and a KEAP1 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. Hybridization 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 compounds provided herein are specifically hybridizable with a KEAP1 nucleic acid.

Complementarity

An oligonucleotide is said to be complementary to another nucleic acid when the nucleobase sequence of such oligonucleotide or one or more regions thereof matches the nucleobase sequence of another oligonucleotide or nucleic acid or one or more regions thereof when the two nucleobase sequences are aligned in opposing directions. Nucleobase matches or complementary nucleobases, as described herein, are limited to adenine (A) and thymine (T), adenine (A) and uracil (U), cytosine (C) and guanine (G), and 5-methyl cytosine (mC) and guanine (G) unless otherwise specified. Complementary oligonucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside and may include one or more nucleobase mismatches. An oligonucleotide is fully complementary or 100% complementary when such oligonucleotides have nucleobase matches at each nucleoside without any nucleobase mismatches.

In certain embodiments, compounds described herein comprise or consist of modified oligonucleotides. In certain embodiments, compounds described herein are antisense compounds. In certain embodiments, compounds comprise oligomeric compounds. Non-complementary nucleobases between a compound and a KEAP1 nucleic acid may be tolerated provided that the compound remains able to specifically hybridize to a target nucleic acid. Moreover, a compound may hybridize over one or more segments of a KEAP1 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 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 KEAP1 nucleic acid, a target region, target segment, or specified portion thereof. Percent complementarity of a compound with a target nucleic acid can be determined using routine methods.

For example, a compound in which 18 of 20 nucleobases of the compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining non-complementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As

such, a compound which is 18 nucleobases in length having four non-complementary 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 a 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, compounds described herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, a compound may be fully complementary to a KEAP1 nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of a compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase 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 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 compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase compound 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 compound. At the same time, the entire 30 nucleobase compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the compound are also complementary to the target sequence.

In certain embodiments, compounds described herein comprise one or more mismatched nucleobases relative to the target nucleic acid. In certain such embodiments, antisense activity against the target is reduced by such mismatch, but activity against a non-target is reduced by a greater amount. Thus, in certain such embodiments selectivity of the compound is improved. In certain embodiments, the mismatch is specifically positioned within an oligonucleotide having a gapmer motif. In certain such embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, or 8 from the 5'-end of the gap region. In certain such embodiments, the mismatch is at position 9, 8, 7, 6, 5, 4, 3, 2, 1 from the 3'-end of the gap region. In certain such embodiments, the mismatch is at position 1, 2, 3, or 4 from the 5'-end of the wing region. In certain such embodiments, the mismatch is at position 4, 3, 2, or 1 from the 3'-end of the wing region. In certain embodiments, the mismatch is specifically positioned within an oligonucleotide not having a gapmer motif. In certain such embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 from the 5'-end of the oligonucleotide. In

certain such embodiments, the mismatch is at position, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 from the 3'-end of the oligonucleotide.

The location of a non-complementary nucleobase may be at the 5' end or 3' end of the compound. Alternatively, the non-complementary nucleobase or nucleobases may be at an internal position of the 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 oligonucleotide.

In certain embodiments, compounds described herein that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 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 KEAP1 nucleic acid, or specified portion thereof.

In certain embodiments, compounds described herein that are, or are up to 11, 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 KEAP1 nucleic acid, or specified portion thereof.

In certain embodiments, compounds described 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 a compound. In certain embodiments, the compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 9 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 10 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least an 11 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 13 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 14 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 15 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 16 nucleobase portion of a target segment. Also contemplated are 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 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. In certain embodiments, compounds described herein are antisense compounds or oligomeric compounds. In certain

embodiments, compounds described herein are modified oligonucleotides. As used herein, a 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 compounds described herein as well as compounds having non-identical bases relative to the compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the compound. Percent identity of an 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, compounds described herein, or portions thereof, are, or 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 compounds or SEQ ID NOs, or a portion thereof, disclosed herein. In certain embodiments, compounds described herein are about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical, or any percentage between such values, to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific Isis number, or portion thereof, in which the compounds comprise an oligonucleotide having one or more mismatched nucleobases. In certain such embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 from the 5'-end of the oligonucleotide. In certain such embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 from the 3'-end of the oligonucleotide.

In certain embodiments, compounds described herein are antisense compounds. In certain embodiments, a portion of the compound is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

In certain embodiments, compounds described herein are oligonucleotides. In certain embodiments, a portion of the oligonucleotide is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

Certain Modified Compounds

In certain embodiments, compounds described herein comprise or consist of oligonucleotides consisting of linked nucleosides. Oligonucleotides may be unmodified oligonucleotides (RNA or DNA) or may be modified oligonucleotides. Modified oligonucleotides comprise at least one modification relative to unmodified RNA or DNA (i.e., comprise at least one modified nucleoside (comprising a modified sugar moiety and/or a modified nucleobase) and/or at least one modified internucleoside linkage).

A. Modified Nucleosides

Modified nucleosides comprise a modified sugar moiety or a modified nucleobase or both a modifed sugar moiety and a modified nucleobase.

1. Modified Sugar Moieties

In certain embodiments, sugar moieties are non-bicyclic modified sugar moieties. In certain embodiments, modified sugar moieties are bicyclic or tricyclic sugar moieties. In certain embodiments, modified sugar moieties are sugar surrogates. Such sugar surrogates may comprise one or more substitutions corresponding to those of other types of modified sugar moieties.

In certain embodiments, modified sugar moieties are non-bicyclic modified sugar moieties comprising a furanosyl ring with one or more acyclic substituent, including but not limited to substituents at the 2', 4', and/or 5' positions. In certain embodiments one or more acyclic substituent of non-bicyclic modified sugar moieties is branched. Examples of 2'-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to: 2'-F, 2'-OCH_3 ("OMe" or "O-methyl"), and 2'-$O(CH_2)_2OCH_3$ ("MOE"). In certain embodiments, 2'-substituent groups are selected from among: halo, allyl, amino, azido, SH, CN, OCN, CF_3, OCF_3, O-C_1-C_{10} alkoxy, O-C_1-C_{10} substituted alkoxy, O-C_1-C_{10} alkyl, O-C_1-C_{10} substituted alkyl, S-alkyl, N(R_m)-alkyl, O-alkenyl, S-alkenyl, N(R_m)-alkenyl, O-alkynyl, S-alkynyl, N(R_m)-alkynyl, O-alkylenyl-O-alkyl, alkynyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(R_m)(R_n)$ or $OCH_2C(=O)$-$N(R_m)(R_n)$, where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_1-C_{10} alkyl, and the 2'-substituent groups described in Cook et al., U.S. 6,531,584; Cook et al., U.S. 5,859,221; and Cook et al., U.S. 6,005,087. Certain embodiments of these 2'-substituent groups can be further substituted with one or more substituent groups independently selected from among: hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro (NO_2), thiol, thioalkoxy, thioalkyl, halogen, alkyl, aryl, alkenyl and alkynyl. Examples of 4'-substituent groups suitable for linearlynon-bicyclic modified sugar moieties include but are not limited to alkoxy (*e.g.*, methoxy), alkyl, and those described in Manoharan et al., WO 2015/106128. Examples of 5'-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to: 5'-methyl (R or S), 5'-vinyl, and 5'-methoxy. In certain embodiments, non-bicyclic modified sugars comprise more than one non-bridging sugar substituent, for example, 2'-F-5'-methyl sugar moieties and the modified sugar moieties and modified nucleosides described in Migawa et al., WO 2008/101157 and Rajeev et al., US2013/0203836.

In certain embodiments, a 2'-substituted nucleoside or 2'- non-bicyclic modified nucleoside comprises a sugar moiety comprising a linear 2'-substituent group selected from: F, NH_2, N_3, OCF_3, OCH_3, $O(CH_2)_3NH_2$, $CH_2CH{=}CH_2$, $OCH_2CH{=}CH_2$, $OCH_2CH_2OCH_3$, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(R_m)(R_n)$, $O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and N-substituted acetamide ($OCH_2C(=O)$-$N(R_m)(R_n)$), where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_1-C_{10} alkyl.

In certain embodiments, a 2'-substituted nucleoside or 2'- non-bicyclic modified nucleoside comprises a sugar moiety comprising a linear 2'-substituent group selected from: F, OCF_3, OCH_3, $OCH_2CH_2OCH_3$, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(CH_3)_2$, $O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and $OCH_2C(=O)$-$N(H)CH_3$ ("NMA").

In certain embodiments, a 2'-substituted nucleoside or 2'- non-bicyclic modified nucleoside comprises a sugar moiety comprising a linear 2'-substituent group selected from: F, OCH_3, and $OCH_2CH_2OCH_3$.

Nucleosides comprising modified sugar moieties, such as non-bicyclic modified sugar moieties, are referred to by the position(s) of the substitution(s) on the sugar moiety of the nucleoside. For example, nucleosides comprising 2'-substituted or 2-modified sugar moieties are referred to as 2'-substituted nucleosides or 2-modified nucleosides.

Certain modifed sugar moieties comprise a bridging sugar substituent that forms a second ring resulting in a bicyclic sugar moiety. In certain such embodiments, the bicyclic sugar moiety comprises a bridge between the 4' and the 2' furanose ring atoms. Examples of such 4' to 2' bridging sugar substituents include but are not limited to: 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2' ("LNA"), 4'-CH_2-S-2', 4'-$(CH_2)_2$-O-2' ("ENA"), 4'-$CH(CH_3)$-O-2' (referred to as "constrained ethyl" or "cEt" when in the *S* configuration), 4'-CH_2-O-CH_2-2', 4'-CH_2-N(R)-2', 4'-$CH(CH_2OCH_3)$-O-2' ("constrained MOE" or "cMOE") and analogs thereof (*see, e.g.,* Seth et al., U.S. 7,399,845, Bhat et al., U.S. 7,569,686, Swayze et al., U.S. 7,741,457, and Swayze et al., U.S. 8,022,193), 4'-$C(CH_3)(CH_3)$-O-2' and analogs thereof (*see, e.g.,* Seth et al., U.S. 8,278,283), 4'-CH_2-$N(OCH_3)$-2' and analogs thereof (*see, e.g.,* Prakash et al., U.S. 8,278,425), 4'-CH_2-O-$N(CH_3)$-2' (*see, e.g.,* Allerson et al., U.S. 7,696,345 and Allerson et al., U.S. 8,124,745), 4'-CH_2-$C(H)(CH_3)$-2' (*see, e.g.,* Zhou, *et al., J. Org. Chem.,*2009, *74*, 118-134), 4'-CH_2-$C(=CH_2)$-2' and analogs thereof (*see e.g.,*, Seth et al., U.S. 8,278,426), 4'-$C(R_aR_b)$-N(R)-O-2', 4'-$C(R_aR_b)$-O-N(R)-2', 4'-CH_2-O-N(R)-2', and 4'-CH_2-N(R)-O-2', wherein each R, R_a, and R_b is, independently, H, a protecting group, or C_1-C_{12} alkyl (*see, e.g.* Imanishi et al., U.S. 7,427,672).

In certain embodiments, such 4' to 2' bridges independently comprise from 1 to 4 linked groups independently selected from: -$[C(R_a)(R_b)]_n$-, -$[C(R_a)(R_b)]_n$-O-, -$C(R_a)$=$C(R_b)$-, -$C(R_a)$=N-, -$C(=NR_a)$-, -C(=O)-, -C(=S)-, -O-, -$Si(R_a)_2$-, -$S(=O)_x$-, and -$N(R_a)$-;

wherein:

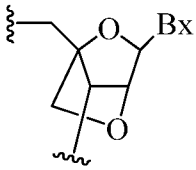
x is 0, 1, or 2;

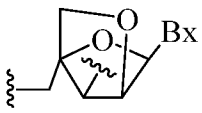
n is 1, 2, 3, or 4;

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.

Additional bicyclic sugar moieties are known in the art, see, for example: Freier *et al., Nucleic Acids Research*, 1997, *25*(*22*), 4429-4443, Albaek *et al., J. Org. Chem.*, 2006, *71*, 7731-7740, 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.*, 20017, *129,* 8362-8379; 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; Wengel et al.,U.S. 7,053,207, Imanishi et al., U.S. 6,268,490, Imanishi et al. U.S.. 6,770,748, Imanishi et al., U.S. RE44,779; Wengel et al., U.S. 6,794,499, Wengel et al., U.S. 6,670,461; Wengel et al., U.S.7,034,133, Wengel et al., U.S. 8,080,644; Wengel et al., U.S. 8,034,909; Wengel et al., U.S. 8,153,365; Wengel et al., U.S. 7,572,582; and Ramasamy et al., U.S. 6,525,191, Torsten et al., WO 2004/106356, Wengel et al., WO 91999/014226; Seth et al.,WO 2007/134181; Seth et al., U.S. 7,547,684; Seth et al., U.S. 7,666,854; Seth et al., U.S. 8,088,746; Seth et al., U.S. 7,750,131; Seth et al., U.S. 8,030,467; Seth et al., U.S. 8,268,980; Seth et al., U.S. 8,546,556; Seth et al., U.S. 8,530,640; Migawa et al., U.S. 9,012,421; Seth et al., U.S. 8,501,805; and U.S. Patent Publication Nos. Allerson et al., US2008/0039618 and Migawa et al., US2015/0191727.

In certain embodiments, bicyclic sugar moieties and nucleosides incorporating such bicyclic sugar moieties are further defined by isomeric configuration. For example, an LNA nucleoside (described herein) may be in the α-L configuration or in the β-D configuration.





LNA (β-D-configuration) bridge = 4'-CH_2-O-2'

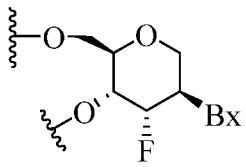
α-*L*-LNA (α-*L*-configuration) bridge = 4'-CH_2-O-2'

α-L-methyleneoxy (4'-CH_2-O-2') or α-L-LNA bicyclic nucleosides have been incorporated into oligonucleotides that showed antisense activity (Frieden et al., *Nucleic Acids Research,* 2003, *21*, 6365-6372). Herein, general descriptions of bicyclic nucleosides include both isomeric configurations. When the positions of specific bicyclic nucleosides (*e.g.*, LNA or cEt) are identified in exemplified embodiments herein, they are in the β-D configuration, unless otherwise specified.

In certain embodiments, modified sugar moieties comprise one or more non-bridging sugar substituent and one or more bridging sugar substituent (e.g., 5'-substituted and 4'-2' bridged sugars).

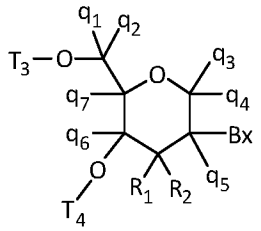
In certain embodiments, modified sugar moieties are sugar surrogates. In certain such embodiments, the oxygen atom of the sugar moiety is replaced, *e.g.,* with a sulfur, carbon or nitrogen atom. In certain such embodiments, such modified sugar moieties also comprise bridging and/or non-bridging substituents as described herein. For example, certain sugar surrogates comprise a 4'-sulfur atom and a substitution at the 2'-position (*see*, *e.g.,* Bhat et al., U.S. 7,875,733 and Bhat et al., U.S. 7,939,677) and/or the 5' position.

In certain embodiments, sugar surrogates comprise rings having other than 5 atoms. For example, in certain embodiments, a sugar surrogate comprises a six-membered tetrahydropyran ("THP"). Such tetrahydropyrans may be further modified or substituted. Nucleosides comprising such modified tetrahydropyrans include but are not limited to hexitol nucleic acid ("HNA"), anitol nucleic acid ("ANA"), manitol nucleic acid ("MNA") (*see* e.g., Leumann, CJ. *Bioorg. & Med. Chem.* 2002, *10*, 841-854), fluoro HNA:



F-HNA

("F-HNA", *see e.g.,* Swayze et al., U.S. 8,088,904; Swayze et al., U.S. 8,440,803; Swayze et al., U.S. ; and Swayze et al., U.S. 9,005,906, F-HNA can also be referred to as a F-THP or 3'-fluoro tetrahydropyran), and nucleosides comprising additional modified THP compounds having the formula:

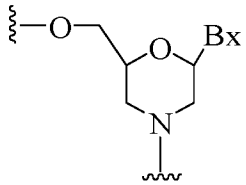


wherein, independently, for each of said modified THP nucleoside: Bx is a nucleobase moiety; T_3 and T_4 are each, independently, an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide or one of T_3 and T_4 is an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide 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 each of R_1 and R_2 is independently selected from among: hydrogen, halogen, substituted or unsubstituted alkoxy, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$, 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, modified THP nucleosides are provided wherein q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is other than H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is methyl. In certain embodiments, modified THP nucleosides are provided wherein one of R_1 and R_2 is F. In certain embodiments, R_1 is F and R_2 is H, in

certain embodiments, R_1 is methoxy and R_2 is H, and in certain embodiments, R_1 is methoxyethoxy and R_2 is H.

In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example, nucleosides comprising morpholino sugar moieties and their use in oligonucleotides have been reported (*see, e.g.,* Braasch et al., Biochemistry, 2002, *41*, 4503-4510 and Summerton et al., U.S. 5,698,685; Summerton et al., U.S. 5,166,315; Summerton et al., U.S.5,185,444; and Summerton et al., U.S. 5,034,506). As used here, the term "morpholino" means a sugar surrogate having the following structure:



In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are refered to herein as "modifed morpholinos."

In certain embodiments, sugar surrogates comprise acyclic moieites. Examples of nucleosides and oligonucleotides comprising such acyclic sugar surrogates include but are not limited to: peptide nucleic acid ("PNA"), acyclic butyl nucleic acid (*see, e.g.,* Kumar et al., *Org. Biomol. Chem.*, 2013, *11*, 5853-5865), and nucleosides and oligonucleotides described in Manoharan et al., WO2011/133876.

Many other bicyclic and tricyclic sugar and sugar surrogate ring systems are known in the art that can be used in modified nucleosides.

2. *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 can impart nuclease stability, binding affinity or some other beneficial biological property to compounds described herein.

In certain embodiments, compounds described herein comprise modified oligonucleotides. In certain embodiments, modified oligonucleotides comprise one or more nucleoside comprising an unmodified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleoside comprising a modified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleoside that does not comprise a nucleobase, referred to as an abasic nucleoside.

In certain embodiments, modified nucleobases are selected from: 5-substituted pyrimidines, 6-azapyrimi¬dines, alkyl or alkynyl substituted pyrimidines, alkyl substituted purines, and N-2, N-6 and O-6 substituted purines. In certain embodiments, modified nucleobases are selected from: 2-aminopropyladenine, 5-hydroxymethyl cytosine, 5-methylcytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-N-methylguanine,

6-N-methyladenine, 2-propyladenine , 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl (C≡C-CH3) uracil, 5-propynylcytosine, 6-azouracil, 6-azocytosine, 6-azothymine, 5-ribosyluracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, 8-aza and other 8-substituted purines, 5-halo, particularly 5-bromo, 5-trifluoromethyl, 5-halouracil, and 5-halocytosine, 7-methylguanine, 7-methyladenine, 2-F-adenine, 2-aminoadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine, 6-N-benzoyladenine, 2-N-isobutyrylguanine, 4-N-benzoylcytosine, 4-N-benzoyluracil, 5-methyl 4-N-benzoylcytosine, 5-methyl 4-N-benzoyluracil, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. Further modified nucleobases include tricyclic pyrimidines, such as 1,3-diazaphenoxazine-2-one, 1,3-diazaphenothiazine-2-one and 9-(2-aminoethoxy)-1,3-diazaphenoxazine-2-one (G-clamp). Modified nucleobases 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. Further nucleobases include those disclosed in Merigan et al., U.S. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990, 858-859; Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613; Sanghvi, Y.S., Chapter 15, Antisense Research and Applications, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993, 273-288; and those disclosed in Chapters 6 and 15, Antisense Drug Technology, Crooke S.T., Ed., CRC Press, 2008, 163-166 and 442-443.

Publications that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include without limitation, Manoharan et al., US2003/0158403, Manoharan et al., US2003/0175906; Dinh et al., U.S. 4,845,205; Spielvogel et al., U.S. 5,130,302; Rogers et al., U.S. 5,134,066; Bischofberger et al., U.S. 5,175,273; Urdea et al., U.S. 5,367,066; Benner et al., U.S. 5,432,272; Matteucci et al., U.S. 5,434,257; Gmeiner et al., U.S. 5,457,187; Cook et al., U.S. 5,459,255; Froehler et al., U.S. 5,484,908; Matteucci et al., U.S. 5,502,177; Hawkins et al., U.S. 5,525,711; Haralambidis et al., U.S. 5,552,540; Cook et al., U.S. 5,587,469; Froehler et al., U.S. 5,594,121; Switzer et al., U.S. 5,596,091; Cook et al., U.S. 5,614,617; Froehler et al., U.S. 5,645,985; Cook et al., U.S. 5,681,941; Cook et al., U.S. 5,811,534; Cook et al., U.S. 5,750,692; Cook et al., U.S. 5,948,903; Cook et al., U.S. 5,587,470; Cook et al., U.S. 5,457,191; Matteucci et al., U.S. 5,763,588; Froehler et al., U.S. 5,830,653; Cook et al., U.S. 5,808,027; Cook et al., 6,166,199; and Matteucci et al., U.S. 6,005,096.

In certain embodiments, compounds targeted to a KEAP1 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.

Modified Internucleoside Linkages

The naturally occuring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. In certain embodiments, compounds described herein having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over 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.

In certain embodiments, compounds targeted to a KEAP1 nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, at least one internucleoside linkage of the compound is a phosphorothioate internucleoside linkage. In certain embodiments, each internucleoside linkage of the compound is a phosphorothioate internucleoside linkage.

In certain embodiments, compounds described herein comprise oligonucleotides. 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, nucleosides of modified oligonucleotides may be linked together using any internucleoside linkage. The two main classes of internucleoside linking groups are defined by the presence or absence of a phosphorus atom. Representative phosphorus-containing internucleoside linkages include but are not limited to phosphates, which contain a phosphodiester bond ("P=O") (also referred to as unmodified or naturally occurring linkages), phosphotriesters, methylphosphonates, phosphoramidates, and phosphorothioates ("P=S"), and phosphorodithioates ("HS-P=S"). Representative non-phosphorus containing internucleoside linking groups include but are not limited to methylenemethylimino (-CH2-N(CH3)-O-CH2-), thiodiester, thionocarbamate (-O-C(=O)(NH)-S-); siloxane (-O-SiH2-O-); and N,N'-dimethylhydrazine (-CH2-N(CH3)-N(CH3)-). Modified internucleoside linkages, compared to naturally occurring phosphate linkages, can be used to alter, typically increase, nuclease resistance of the oligonucleotide. In certain embodiments, internucleoside linkages having a chiral atom can be prepared as a racemic mixture, or as separate enantiomers. Representative chiral internucleoside linkages include but are not limited to alkylphosphonates and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing internucleoside linkages are well known to those skilled in the art.

Neutral internucleoside linkages include, without limitation, phosphotriesters, methylphosphonates, MMI (3'-CH2-N(CH3)-O-5'), amide-3 (3'-CH2-C(=O)-N(H)-5'), amide-4 (3'-CH2-N(H)-C(=O)-5'), formacetal (3'-O-CH2-O-5'), methoxypropyl, and thioformacetal (3'-S-CH2-O-5'). Further neutral internucleoside linkages include nonionic linkages comprising siloxane (dialkylsiloxane), carboxylate ester, carboxamide, sulfide, sulfonate ester and amides (See for example: Carbohydrate Modifications in Antisense Research; Y.S. Sanghvi and P.D. Cook, Eds., ACS Symposium Series 580; Chapters 3 and 4, 40-65). Further neutral internucleoside linkages include nonionic linkages comprising mixed N, O, S and CH2 component parts.

In certain embodiments, oligonucleotides comprise modified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or modified internucleoside linkage motif. In certain embodiments, internucleoside linkages are arranged in a gapped motif. In such embodiments, the internucleoside linkages in each of two wing regions are different from the internucleoside linkages in the gap region. In certain embodiments the internucleoside linkages in the wings are phosphodiester and the internucleoside linkages in the gap are phosphorothioate. The nucleoside motif is independently selected, so such oligonucleotides having a gapped internucleoside linkage motif may or may not have a gapped nucleoside motif and if it does have a gapped nucleoside motif, the wing and gap lengths may or may not be the same.

In certain embodiments, oligonucleotides comprise a region having an alternating internucleoside linkage motif. In certain embodiments, oligonucleotides of the present invention comprise a region of uniformly modified internucleoside linkages. In certain such embodiments, the oligonucleotide comprises a region that is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide is uniformly linked by phosphorothioate. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate and at least one internucleoside linkage is phosphorothioate.

In certain embodiments, the oligonucleotide comprises at least 6 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 8 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 10 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 6 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 8 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 10 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least block of at least one 12 consecutive phosphorothioate internucleoside linkages. In certain such embodiments, at least one such block is located at the 3' end of the oligonucleotide. In certain such embodiments, at least one such block is located within 3 nucleosides of the 3' end of the oligonucleotide.

In certain embodiments, oligonucleotides comprise one or more methylphosponate linkages. In certain embodiments, oligonucleotides having a gapmer nucleoside motif comprise a linkage motif comprising all phosphorothioate linkages except for one or two methylphosponate linkages. In certain embodiments, one methylphosponate linkage is in the central gap of an oligonucleotide having a gapmer nucleoside motif.

In certain embodiments, it is desirable to arrange the number of phosphorothioate internucleoside linkages and phosphodiester internucleoside linkages to maintain nuclease resistance. In certain embodiments, it is desirable to arrange the number and position of phosphorothioate internucleoside linkages

and the number and position of phosphodiester internucleoside linkages to maintain nuclease resistance. In certain embodiments, the number of phosphorothioate internucleoside linkages may be decreased and the number of phosphodiester internucleoside linkages may be increased. In certain embodiments, the number of phosphorothioate internucleoside linkages may be decreased and the number of phosphodiester internucleoside linkages may be increased while still maintaining nuclease resistance. In certain embodiments it is desirable to decrease the number of phosphorothioate internucleoside linkages while retaining nuclease resistance. In certain embodiments it is desirable to increase the number of phosphodiester internucleoside linkages while retaining nuclease resistance.

Certain Motifs

In certain embodiments, compounds described herein comprise oligonucleotides. Oligonucleotides can have a motif, *e.g.* a pattern of unmodified and/or modified sugar moieties, nucleobases, and/or internucleoside linkages. In certain embodiments, modified oligonucleotides comprise one or more modified nucleoside comprising a modified sugar. In certain embodiments, modified oligonucleotides comprise one or more modified nucleosides comprising a modified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more modified internucleoside linkage. In such embodiments, the modified, unmodified, and differently modified sugar moieties, nucleobases, and/or internucleoside linkages of a modified oligonucleotide define a pattern or motif. In certain embodiments, the patterns of sugar moieties, nucleobases, and internucleoside linkages are each independent of one another. Thus, a modified oligonucleotide may be described by its sugar motif, nucleobase motif and/or internucleoside linkage motif (as used herein, nucleobase motif describes the modifications to the nucleobases independent of the sequence of nucleobases).

1. Certain Sugar Motifs

In certain embodiments, compounds described herein comprise oligonucleotides. In certain embodiments, oligonucleotides comprise one or more type of modified sugar and/or unmodified sugar moiety arranged along the oligonucleotide or region thereof in a defined pattern or sugar motif. In certain instances, such sugar motifs include but are not limited to any of the sugar modifications discussed herein.

In certain embodiments, modified oligonucleotides comprise or consist of a region having a gapmer motif, which comprises two external regions or "wings" and a central or internal region or "gap." The three regions of a gapmer motif (the 5'-wing, the gap, and the 3'-wing) form a contiguous sequence of nucleosides wherein at least some of the sugar moieties of the nucleosides of each of the wings differ from at least some of the sugar moieties of the nucleosides of the gap. Specifically, at least the sugar moieties of the nucleosides of each wing that are closest to the gap (the 3'-most nucleoside of the 5'-wing and the 5'-most nucleoside of the 3'-wing) differ from the sugar moiety of the neighboring gap nucleosides, thus defining the boundary between the wings and the gap (i.e., the wing/gap junction). In certain embodiments, the sugar moieties within the gap are the same as one another. In certain embodiments, the gap includes one or more nucleoside

having a sugar moiety that differs from the sugar moiety of one or more other nucleosides of the gap. In certain embodiments, the sugar motifs of the two wings are the same as one another (symmetric gapmer). In certain embodiments, the sugar motif of the 5'-wing differs from the sugar motif of the 3'-wing (asymmetric gapmer).

In certain embodiments, the wings of a gapmer comprise 1-5 nucleosides. In certain embodiments, the wings of a gapmer comprise 2-5 nucleosides. In certain embodiments, the wings of a gapmer comprise 3-5 nucleosides. In certain embodiments, the nucleosides of a gapmer are all modified nucleosides.

In certain embodiments, the gap of a gapmer comprises 7-12 nucleosides. In certain embodiments, the gap of a gapmer comprises 7-10 nucleosides. In certain embodiments, the gap of a gapmer comprises 8-10 nucleosides. In certain embodiments, the gap of a gapmer comprises 10 nucleosides. In certain embodiment, each nucleoside of the gap of a gapmer is an unmodified 2'-deoxy nucleoside.

In certain embodiments, the gapmer is a deoxy gapmer. In such embodiments, the nucleosides on the gap side of each wing/gap junction are unmodified 2'-deoxy nucleosides and the nucleosides on the wing sides of each wing/gap junction are modified nucleosides. In certain such embodiments, each nucleoside of the gap is an unmodified 2'-deoxy nucleoside. In certain such embodiments, each nucleoside of each wing is a modified nucleoside.

In certain embodiments, a modified oligonucleotide has a fully modified sugar motif wherein each nucleoside of the modified oligonucleotide comprises a modified sugar moiety. In certain embodiments, modified oligonucleotides comprise or consist of a region having a fully modified sugar motif wherein each nucleoside of the region comprises a modified sugar moiety. In certain embodiments, modified oligonucleotides comprise or consist of a region having a fully modified sugar motif, wherein each nucleoside within the fully modified region comprises the same modified sugar moiety, referred to herein as a uniformly modified sugar motif. In certain embodiments, a fully modified oligonucleotide is a uniformly modified oligonucleotide. In certain embodiments, each nucleoside of a uniformly modified comprises the same 2'-modification.

2. Certain Nucleobase Motifs

In certain embodiments, compounds described herein comprise oligonucleotides. In certain embodiments, oligonucleotides comprise modified and/or unmodified nucleobases arranged along the oligonucleotide or region thereof in a defined pattern or motif. In certain embodiments, each nucleobase is modified. In certain embodiments, none of the nucleobases are modified. In certain embodiments, each purine or each pyrimidine is modified. In certain embodiments, each adenine is modified. In certain embodiments, each guanine is modified. In certain embodiments, each thymine is modified. In certain embodiments, each uracil is modified. In certain embodiments, each cytosine is modified. In certain embodiments, some or all of the cytosine nucleobases in a modified oligonucleotide are 5-methylcytosines.

In certain embodiments, modified oligonucleotides comprise a block of modified nucleobases. In certain such embodiments, the block is at the 3'-end of the oligonucleotide. In certain embodiments the block

is within 3 nucleosides of the 3'-end of the oligonucleotide. In certain embodiments, the block is at the 5'-end of the oligonucleotide. In certain embodiments the block is within 3 nucleosides of the 5'-end of the oligonucleotide.

In certain embodiments, oligonucleotides having a gapmer motif comprise a nucleoside comprising a modified nucleobase. In certain such embodiments, one nucleoside comprising a modified nucleobase is in the central gap of an oligonucleotide having a gapmer motif. In certain such embodiments, the sugar moiety of said nucleoside is a 2'-deoxyribosyl moiety. In certain embodiments, the modified nucleobase is selected from: a 2-thiopyrimidine and a 5-propynepyrimidine.

3. Certain Internucleoside Linkage Motifs

In certain embodiments, compounds described herein comprise oligonucleotides. In certain embodiments, oligonucleotides comprise modified and/or unmodified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or motif. In certain embodiments, essentially each internucleoside linking group is a phosphate internucleoside linkage (P=O). In certain embodiments, each internucleoside linking group of a modified oligonucleotide is a phosphorothioate (P=S). In certain embodiments, each internucleoside linking group of a modified oligonucleotide is independently selected from a phosphorothioate and phosphate internucleoside linkage. In certain embodiments, the sugar motif of a modified oligonucleotide is a gapmer and the internucleoside linkages within the gap are all modified. In certain such embodiments, some or all of the internucleoside linkages in the wings are unmodified phosphate linkages. In certain embodiments, the terminal internucleoside linkages are modified.

Certain Modified Oligonucleotides

In certain embodiments, compounds described herein comprise modified oligonucleotides. In certain embodiments, the above modifications (sugar, nucleobase, internucleoside linkage) are incorporated into a modified oligonucleotide. In certain embodiments, modified oligonucleotides are characterized by their modification, motifs, and overall lengths. In certain embodiments, such parameters are each independent of one another. Thus, unless otherwise indicated, each internucleoside linkage of an oligonucleotide having a gapmer sugar motif may be modified or unmodified and may or may not follow the gapmer modification pattern of the sugar modifications. For example, the internucleoside linkages within the wing regions of a sugar gapmer may be the same or different from one another and may be the same or different from the internucleoside linkages of the gap region of the sugar motif. Likewise, such gapmer oligonucleotides may comprise one or more modified nucleobase independent of the gapmer pattern of the sugar modifications. Furthermore, in certain instances, an oligonucleotide is described by an overall length or range and by lengths or length ranges of two or more regions (e.g., a regions of nucleosides having specified sugar modifications), in such circumstances it may be possible to select numbers for each range that result in an oligonucleotide having an overall length falling outside the specified range. In such circumstances, both elements must be satisfied. For example, in certain embodiments, a modified oligonucleotide consists of 15-20 linked

nucleosides and has a sugar motif consisting of three regions, A, B, and C, wherein region A consists of 2-6 linked nucleosides having a specified sugar motif, region B consists of 6-10 linked nucleosides having a specified sugar motif, and region C consists of 2-6 linked nucleosides having a specified sugar motif. Such embodiments do not include modified oligonucleotides where A and C each consist of 6 linked nucleosides and B consists of 10 linked nucleosides (even though those numbers of nucleosides are permitted within the requirements for A, B, and C) because the overall length of such oligonucleotide is 22, which exceeds the upper limit of the overall length of the modified oligonucleotide (20). Herein, if a description of an oligonucleotide is silent with respect to one or more parameter, such parameter is not limited. Thus, a modified oligonucleotide described only as having a gapmer sugar motif without further description may have any length, internucleoside linkage motif, and nucleobase motif. Unless otherwise indicated, all modifications are independent of nucleobase sequence.

Certain Conjugated Compounds

In certain embodiments, the compounds described herein comprise or consist of an oligonucleotide (modified or unmodified) and optionally one or more conjugate groups and/or terminal groups. Conjugate groups consist of one or more conjugate moiety and a conjugate linker which links the conjugate moiety to the oligonucleotide. Conjugate groups may be attached to either or both ends of an oligonucleotide and/or at any internal position. In certain embodiments, conjugate groups are attached to the 2'-position of a nucleoside of a modified oligonucleotide. In certain embodiments, conjugate groups that are attached to either or both ends of an oligonucleotide are terminal groups. In certain such embodiments, conjugate groups or terminal groups are attached at the 3' and/or 5'-end of oligonucleotides. In certain such embodiments, conjugate groups (or terminal groups) are attached at the 3'-end of oligonucleotides. In certain embodiments, conjugate groups are attached near the 3'-end of oligonucleotides. In certain embodiments, conjugate groups (or terminal groups) are attached at the 5'-end of oligonucleotides. In certain embodiments, conjugate groups are attached near the 5'-end of oligonucleotides.

In certain embodiments, the oligonucleotide of a compound is modified. In certain embodiments, the oligonucleotide of a compound may have any nucleobase sequence. In certain embodiments, the oligonucleotide of a compound has a nucleobase sequence that is complementary to a target nucleic acid. In certain embodiments, oligonucleotides are complementary to a messenger RNA (mRNA). In certain embodiments, oligonucleotides are complementary to a sense transcript.

Examples of terminal groups include but are not limited to conjugate groups, capping groups, phosphate moieties, protecting groups, modified or unmodified nucleosides, and two or more nucleosides that are independently modified or unmodified.

A. Certain Conjugate Groups

In certain embodiments, oligonucleotides are covalently attached to one or more conjugate groups. In certain embodiments, conjugate groups modify one or more properties of the attached oligonucleotide,

including but not limited to pharmacodynamics, pharmacokinetics, stability, binding, absorption, tissue distribution, cellular distribution, cellular uptake, charge and clearance. In certain embodiments, conjugate groups impart a new property on the attached oligonucleotide, *e.g.*, fluorophores or reporter groups that enable detection of the oligonucleotide.

Certain conjugate groups and conjugate moieties have been described previously, for example: cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, *4*, 1053-1060), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, *660*, 306-309; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, *3*, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, *20*, 533-538), an aliphatic chain, *e.g.*, do-decan-diol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, *10*, 1111-1118; Kabanov et al., *FEBS Lett.*, 1990, *259*, 327-330; Svinarchuk et al., *Biochimie*, 1993, *75*, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, *36*, 3651-3654; Shea et al., *Nucl. Acids Res.*, 1990, *18*, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, *14*, 969-973), or adamantane acetic acid, a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, *1264*, 229-237), an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, *277*, 923-937), a tocopherol group (Nishina et al., *Molecular Therapy Nucleic Acids*, 2015, *4*, e220; doi:10.1038/mtna.2014.72 and Nishina et al., *Molecular Therapy*, 2008, *16*, 734-740), or a GalNAc cluster (*e.g.*, WO2014/179620).

1. Conjugate Moieties

Conjugate moieties include, without limitation, intercalators, reporter molecules, polyamines, polyamides, peptides, carbohydrates (e.g., GalNAc), vitamin moieties, polyethylene glycols, thioethers, polyethers, cholesterols, thiocholesterols, cholic acid moieties, folate, lipids, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins, fluorophores, and dyes.

In certain embodiments, a conjugate moiety comprises an active drug substance, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fen-bufen, ketoprofen, (*S*)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, fingolimod, flufenamic acid, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indo-methicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

2. Conjugate linkers

Conjugate moieties are attached to oligonucleotides through conjugate linkers. In certain compounds, a conjugate group is a single chemical bond (i.e. conjugate moiety is attached to an oligonucleotide via a conjugate linker through a single bond). In certain embodiments, the conjugate linker comprises a chain structure, such as a hydrocarbyl chain, or an oligomer of repeating units such as ethylene glycol, nucleosides, or amino acid units.

In certain embodiments, a conjugate linker comprises one or more groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether, and hydroxylamino. In certain such embodiments, the conjugate linker comprises groups selected from alkyl, amino, oxo, amide and ether groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and amide groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and ether groups. In certain embodiments, the conjugate linker comprises at least one phosphorus moiety. In certain embodiments, the conjugate linker comprises at least one phosphate group. In certain embodiments, the conjugate linker includes at least one neutral linking group.

In certain embodiments, conjugate linkers, including the conjugate linkers described above, are bifunctional linking moieties, *e.g.,* those known in the art to be useful for attaching conjugate groups to parent compounds, such as the oligonucleotides provided herein. In general, a bifunctional linking moiety comprises at least two functional groups. One of the functional groups is selected to bind to a particular site on a compound and the other is selected to bind to a conjugate group. Examples of functional groups used in a bifunctional linking moiety include but are not limited to electrophiles for reacting with nucleophilic groups and nucleophiles for reacting with electrophilic groups. In certain embodiments, bifunctional linking moieties comprise one or more groups selected from amino, hydroxyl, carboxylic acid, thiol, alkyl, alkenyl, and alkynyl.

Examples of conjugate linkers include but are not limited to pyrrolidine, 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and 6-aminohexanoic acid (AHEX or AHA). Other conjugate linkers include but are not limited to substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_2-C_{10} alkenyl or substituted or unsubstituted C_2-C_{10} alkynyl, wherein a nonlimiting list of preferred substituent groups includes hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

In certain embodiments, conjugate linkers comprise 1-10 linker-nucleosides. In certain embodiments, such linker-nucleosides are modified nucleosides. In certain embodiments such linker-nucleosides comprise a modified sugar moiety. In certain embodiments, linker-nucleosides are unmodified. In certain embodiments, linker-nucleosides comprise an optionally protected heterocyclic base selected from a purine, substituted purine, pyrimidine or substituted pyrimidine. In certain embodiments, a cleavable moiety is a nucleoside selected from uracil, thymine, cytosine, 4-N-benzoylcytosine, 5-methylcytosine, 4-N-benzoyl-5-methylcytosine, adenine, 6-N-benzoyladenine, guanine and 2-N-isobutyrylguanine. It is typically desirable for linker-nucleosides to be cleaved from the compound after it reaches a target tissue. Accordingly, linker-nucleosides are typically linked to one another and to the remainder of the compound through cleavable bonds. In certain embodiments, such cleavable bonds are phosphodiester bonds.

Herein, linker-nucleosides are not considered to be part of the oligonucleotide. Accordingly, in embodiments in which a compound comprises an oligonucleotide consisting of a specified number or range of linked nucleosides and/or a specified percent complementarity to a reference nucleic acid and the

compound also comprises a conjugate group comprising a conjugate linker comprising linker-nucleosides, those linker-nucleosides are not counted toward the length of the oligonucleotide and are not used in determining the percent complementarity of the oligonucleotide for the reference nucleic acid. For example, a compound may comprise (1) a modified oligonucleotide consisting of 8-30 nucleosides and (2) a conjugate group comprising 1-10 linker-nucleosides that are contiguous with the nucleosides of the modified oligonucleotide. The total number of contiguous linked nucleosides in such a compound is more than 30. Alternatively, an compound may comprise a modified oligonucleotide consisting of 8-30 nucleosides and no conjugate group. The total number of contiguous linked nucleosides in such a compound is no more than 30. Unless otherwise indicated conjugate linkers comprise no more than 10 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 5 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 3 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 2 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 1 linker-nucleoside.

In certain embodiments, it is desirable for a conjugate group to be cleaved from the oligonucleotide. For example, in certain circumstances compounds comprising a particular conjugate moiety are better taken up by a particular cell type, but once the compound has been taken up, it is desirable that the conjugate group be cleaved to release the unconjugated or parent oligonucleotide. Thus, certain conjugate may comprise one or more cleavable moieties, typically within the conjugate linker. In certain embodiments, a cleavable moiety is a cleavable bond. In certain embodiments, a cleavable moiety is a group of atoms comprising at least one cleavable bond. In certain embodiments, a cleavable moiety comprises a group of atoms having one, two, three, four, or more than four cleavable bonds. In certain embodiments, a cleavable moiety is selectively cleaved inside a cell or subcellular compartment, such as a lysosome. In certain embodiments, a cleavable moiety is selectively cleaved by endogenous enzymes, such as nucleases.

In certain embodiments, a cleavable bond is selected from among: an amide, an ester, an ether, one or both esters of a phosphodiester, a phosphate ester, a carbamate, or a disulfide. In certain embodiments, a cleavable bond is one or both of the esters of a phosphodiester. In certain embodiments, a cleavable moiety comprises a phosphate or phosphodiester. In certain embodiments, the cleavable moiety is a phosphate linkage between an oligonucleotide and a conjugate moiety or conjugate group.

In certain embodiments, a cleavable moiety comprises or consists of one or more linker-nucleosides. In certain such embodiments, one or more linker-nucleosides are linked to one another and/or to the remainder of the compound through cleavable bonds. In certain embodiments, such cleavable bonds are unmodified phosphodiester bonds. In certain embodiments, a cleavable moiety is 2'-deoxy nucleoside that is attached to either the 3' or 5'-terminal nucleoside of an oligonucleotide by a phosphate internucleoside linkage and covalently attached to the remainder of the conjugate linker or conjugate moiety by a phosphate or phosphorothioate linkage. In certain such embodiments, the cleavable moiety is 2'-deoxyadenosine.

Compositions and Methods for Formulating Pharmaceutical Compositions

Compounds described herein may be admixed with pharmaceutically acceptable active or inert substances 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.

In certain embodiments, the present invention provides pharmaceutical compositions comprising one or more compounds or a salt thereof. In certain embodiments, the compounds are antisense compounds or oligomeric compounds. In certain embodiments, the compounds comprise or consist of a modified oligonucleotide. In certain such embodiments, the pharmaceutical composition comprises a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutical composition comprises a sterile saline solution and one or more compound. In certain embodiments, such pharmaceutical composition consists of a sterile saline solution and one or more compound. In certain embodiments, the sterile saline is pharmaceutical grade saline. In certain embodiments, a pharmaceutical composition comprises one or more compound and sterile water. In certain embodiments, a pharmaceutical composition consists of one compound and sterile water. In certain embodiments, the sterile water is pharmaceutical grade water. In certain embodiments, a pharmaceutical composition comprises one or more compound and phosphate-buffered saline (PBS). In certain embodiments, a pharmaceutical composition consists of one or more compound and sterile PBS. In certain embodiments, the sterile PBS is pharmaceutical grade PBS. 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.

A compound described herein targeted to a KEAP1 nucleic acid can be utilized in pharmaceutical compositions by combining the compound with a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutically acceptable diluent is water, such as sterile water suitable for injection. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising a compound targeted to a KEAP1 nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is water. In certain embodiments, the compound comprises or consists of a modified oligonucleotide provided herein.

Pharmaceutical compositions comprising compounds provided herein 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. In certain embodiments, the compounds are antisense compounds or oligomeric compounds. In certain embodiments, the compound comprises or consists of a modified oligonucleotide. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of 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.

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

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

RNA-Sequencing

Total RNA was isolated either directly from cryosections of the tumor tissue or from organotypic epithelial cultures using TRIzol (Life Technologies) according to the manufacturer's instructions. For tissue sections, the tumors were embedded in OCT and cryosectioned. Sections from the middle of the tumor were stained using toluidine blue (Sigma) and assayed regarding the homogeneity of the section. Homogenous 30 μm sections comprising >90% malignant cells were immediately dispersed and homogenized in TRIzol. RNA quality was assayed by running an RNA 6000 Nano chip on a 2100 Bioanalyzer (Agilent). For high-throughput sequencing, RNA samples were required to have an RNA integrity number (RIN) ≥ 9. TruSeq (Illumina) libraries for polyA+ RNA-Seq were prepared from 0.5 – 1 μg RNA per sample. To ensure efficient cluster generation, an additional gel purification step of the libraries was applied. The libraries were multiplexed (4-6 libraries per lane) and sequenced paired-end 101 bp on the HiSeq2000 platform (Illumina), resulting in on average 40 Mio reads per library.

ADVANTAGES OF THE INVENTION

Provided herein are methods and compositions for the modulation of KEAP1 that can treat, prevent and/or ameliorate an oxidative stress related disease, disorder and/or condition such as metabolic disease (e.g., insulin resistance, metabolic syndrome), NASH and NAFLD. In a particular embodiment, provided are KEAP1 antisense oligonucleotides to treat, prevent and/or ameliorate a KEAP1 pathway related disease, disorder and/or condition or symptoms thereof as described herein.

KEAP1 functions as an important regulator of cellular antioxidant responses via its interactions with multiple substrates such as NRF2 and PGAM5. KEAP1 binding of its substrates sequesters the substrates in the cytoplasm and targets them for ubiquitination and degradation. In the presence of oxidative stress, KEAP1 releases its substrate which then can initiate multiple antioxidant response pathways.

Currently, commercially available therapies targeting various oxidative stress components (e.g., antioxidants) have been ineffective in completely inhibiting or blocking the oxidative stress pathways. The mechanism(s) for this ineffective inhibition has not been fully elucidated. An antisense oligonucleotide targeting KEAP1 may block multiple oxidative stress pathways due to its interactions with multiple substrates that respond to oxidative stress by different pathways. Accordingly, KEAP1 inhibition may not be susceptible to the mechanisms confounding commercially approved therapies for oxidative stress. Therefore, a KEAP1 antisense oligonucleotide may provide superior therapeutic efficacy over the current commercial therapeutics used to treat, prevent and/or ameliorate oxidative stress in a subject, especially those subject

suffering from fatty liver disease (e.g., hepatic steatosis, steatohepatitis, NASH, NAFLD, inflammation (e.g., asthma, COPD, airway hyperresponsiveness, renal inflammation, pulmonary inflammation, liver inflammation), fibrosis (e.g., pulmonary fibrosis, renal fibrosis, cystic fibrosis, liver fibrosis), cancer, age-related macular degeneration, atherosclerosis, hyperlipidemia, neurologic disorders, and/or a metabolic disease, disorder or condition such as metabolic syndrome, type 2 diabetes, diabetic nephropathy, diabetic cardiomyopathy, and insulin resistance.

Another advantage of KEAP1 ASO therapy is that it impacts the heart less than bardoxolone methyl treatment. Thus, KEAP1 ASO treatment may not have the adverse effects on the heart as seen after bardoxolone methyl treatment.

Another advantage of using a KEAP1 antisense oligonucleotide to combat oxidative stress is that it can be used in combination with commercially available antioxidants to provide an additive therapeutic effect.

Further, the antisense oligonucleotide targeting KEAP1 has been shown to localize to and decrease KEAP1 in multiple organs such as the lungs, liver and kidney. An ability to decrease KEAP1 in the liver tissue indicates that the oligonucleotide can target and inhibit the main source of KEAP1 production and also indicates that a KEAP1 antisense oligonucleotide can be useful in treating liver related diseases such as liver or hepatic fibrosis, cystic fibrosis, hepatic inflammation, steatohepatitis (e.g., NASH), hepatic fibrosis (e.g., NAFLD), hepatic cancer, and the like. An ability to decrease KEAP1 in the kidney tissue indicates that KEAP1 antisense oligonucleotide therapy can be useful in treating kidney related diseases such as nepthropathy (e.g., diabetic nephropathy), renal cancer, renal fibrosis,renal inflammation, cystic fibrosis and other renal diseases with an oxidative stress component. An ability to decrease KEAP1 in the lung tissue indicates that KEAP1 antisense oligonucleotide therapy can be useful in treating lung related diseases such as pulmonary inflammation, pulmonary fibrosis, cystic fibrosis, asthma, COPD, airway hyperresponsiveness and the like.

Reducing KEAP1, and therefore increasing multiple antioxidant pathways (e.g., via NRF2 and PGAM5), may be the most efficacious means of treating oxidative stress.

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 recited in the present application is incorporated herein by reference in its entirety.

Example 1: Antisense oligonucleotides targeting murine KEAP1

Newly designed chimeric antisense oligonucleotides (ASOs) targeting KEAP1 were designed as 16 nucleobase long oligonucleotides with a 3-10-3 cEt gapmer motif, wherein a gap segment consisting of ten linked deoxynucleosides is flanked by 5' and 3' wing segments each consisting of three linked nucleosides, wherein each nucleoside of each wing segment comprises a cEt sugar, wherein each internucleoside linkage is a phosphorothioate linkage and wherein each cytosine residue is a 5-methylcytosine. Three potent ASOs identified in an *in vitro* screen are listed in the table below: ISIS 645938, ISIS 645954 and ISIS 645958.

"Start site" indicates the 5'-most nucleoside to which the chimeric antisense oligonucleotide is targeted in the murine gene sequence. "Stop site" indicates the 3'-most nucleoside to which the chimeric antisense oligonucleotide is targeted in the murine gene sequence. Each ASO listed in the table below is targeted to murine KEAP1 mRNA, designated herein as SEQ ID NO: 5 (GENBANK Accession Number NM_016679.4) and/or the murine KEAP1 genomic sequence, designated herein as SEQ ID NO: 6 (the complement of GENBANK Accession Number NC_000075.6 truncated from nucleotide 21227001 to 21242000). In the table below, 'n/a' indicates that the ASO does not target that particular sequence with 100% complementarity.

Table 1

In vitro ASO inhibition of mouse KEAP1

ISIS NO	SEQ ID NO: 5 Start Site	SEQ ID NO: 5 Stop Site	SEQ ID NO: 6 Start Site	SEQ ID NO: 6 Stop Site	Sequence	Chemistry	IC50 uM	SEQ ID NO
645938	n/a	n/a	6587	6602	TATTCAGAATGCAGTC	kkk-d10-kkk	1.7	7
645954	1106	1121	3774	3789	ACATGTCAGATTCCTT	kkk-d10-kkk	2.0	8
645958	1353	1368	4021	4036	AATGATATCTAAGTGC	kkk-d10-kkk	1.5	9

Example 2: Dose-dependent ASO inhibition of KEAP1 in wild type mice

The three potent ASOs identified in by an *in vitro* screen above were assessed in a dose-dependent assay in C57 wildtype mice fed a normal chow diet (D12451 Research Diets, New Brunswick, New Jersey) to test their potency *in vivo*.

Treatment

C57BL/6 wildtype mice were divided into treatment groups consisting of six animals. Mice were injected intraperitoneally with 10 mg/kg, 25 mg/kg or 40 mg/kg of ASO as specified in the table below once a week for four weeks. 40 mg/kg of ISIS 549144 (a 3-10-3 cEt gapmer having the sequence GGCCAATACGCCGTCA, incorporated herein as SEQ ID NO: 10) was used as an antisense control, and

PBS alone was used as a control to which the ASO-treated groups were compared. Several clinical endpoints were measured over the course of the study. The body weights were measured weekly, tail bleeds were performed at baseline and weekly thereafter, as well as at the time of sacrifice. The mice were euthanized 24 hours after the last dose; organs and plasma were harvested for further analysis.

RNA analysis

At the end of the treatment period, RNA was extracted from liver, kidney and white adipose tissue (WAT) for quantitative real-time PCR analysis of RNA expression of KEAP1. Murine KEAP1 primer probe set RTS4398_MGB (forward sequence AAGGTCATGGAAAGGCTTATTGAGT, designated herein as SEQ ID NO: 12; reverse sequence GTTCATCACGTGCAGGACACA, designated herein as SEQ ID NO: 13; probe sequence TCTCCGTGGGCGAGAA, designated herein as SEQ ID NO: 14) was used to measure RNA levels. Results are presented in the table below as percent inhibition of KEAP1, relative to PBS control, normalized against Cyclophilin A. Treatment with the KEAP ASOs resulted in significant dose-dependent reductions of KEAP1 in the liver in comparison to the control ASO and the PBS control.

Table 2

ASO inhibition of KEAP1 RNA in organs of wild type mice

Treatment (ISIS NO)	Dose (mg/kg)	% inhibition		
		liver	kidney	WAT
saline	-	0	0	0
549144	40	0	0	0
645938	40	91	66	70
	25	82	66	61
	10	47	57	56
645954	40	86	66	62
	25	89	66	64
	10	69	58	49
645958	40	83	53	62
	25	67	51	58
	10	42	52	48

Body and organ weights

Body weights were measured weekly, and kidney, liver, spleen and white adipose tissue (WAT) weights were measured at the end of the fourth week of the study. Averages for each treatment group are presented in grams in the table below. Generally, ISIS 645938 and ISIS 645958 did not increase body weight or organ weight.

Table 3

Weights (g) of body and organs of wild type mice after ASO treatment

Treatment (ISIS NO)	Dose (mg/kg)	Body					liver	kidney	spleen	WAT
		wk 0	wk 1	wk 2	wk 3	wk 4				
saline	-	24	25	25	26	27	1.3	0.32	0.08	0.29
549144	40	23	24	25	25	26	1.4	0.32	0.08	0.33
645938	40	23	24	25	25	26	1.8	0.32	0.10	0.20
	25	24	25	26	26	27	1.8	0.32	0.11	0.16
	10	22	24	25	25	26	1.3	0.32	0.09	0.25
645954	40	23	24	24	25	25	2.6	0.32	0.08	0.16
	25	24	25	26	26	27	2.2	0.35	0.09	0.18
	10	24	25	26	27	27	1.6	0.35	0.09	0.23
645958	40	25	26	27	28	29	1.8	0.36	0.09	0.18
	25	24	25	27	27	28	1.6	0.36	0.10	0.19
	10	22	23	24	25	25	1.3	0.31	0.07	0.25

Plasma chemistry markers

To evaluate the effect of ASOs on plasma chemistry biomarkers, cardiac punctures were performed and plasma ALT, AST and other biomarkers were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The averages for each treatment group are presented in the table below. Generally, ISIS 645938 and ISIS 645958 were found to be tolerable in the mice and these ASOs were used in later studies.

Table 4

Plasma biomarkers in wild type mice after ASO treatment

Treatment (ISIS NO)	Dose (mg/kg)	ALT (U/L)	AST (U/L)	CHOL (mmol/L)	GLU (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TRIG (mmol/L)	NEFA	3HB
saline	-	31	46	81	266	66.0	18.1	101	0.27	218
549144	40	38	45	77	237	60.8	16.7	108	0.35	224
645938	40	36	61	88	255	66.3	23.7	89	0.36	141
	25	75	122	99	249	72.7	25.3	97	0.34	167
	10	42	69	79	255	62.4	16.9	78	0.39	165
645954	40	1305	972	125	190	71.2	52.8	163	0.46	193
	25	140	105	111	266	83.3	29.0	114	0.46	170
	10	47	101	96	263	76.7	19.9	78	0.44	163

645958	40	38	49	88	289	68.4	21.4	98	0.44	242
	25	73	68	92	260	71.0	23.4	95	0.49	167
	10	25	42	78	294	63.0	16.4	79	0.51	185

Protein analysis

Under basal conditions, KEAP1 represses Nrf2 through its molecular interaction. However, in response to oxidative and xenobiotic stress, KEAP1 releases Nrf2 from the KEAP1/Nrf2 complex, allowing nuclear translocation of Nrf2, binding of Nrf2 to Antioxidant Response Elements (AREs) and initiating transcription of a battery of cytoprotective detoxifying and antioxidant genes including glutathione S-transferase alpha 1 (GSTA1) and NAD(P)H: Quinone Oxidoreductase 1 (NQO1).

Murine GSTA1 mRNA levels in wild type mouse liver and WAT samples were quantified using quantitative real-time PCR. GSTA1 mRNA expression was assessed using mouse primer probe set: mGsta1_LTS34766 (Forward Sequence: CCCCTTTCCCTCTGCTGAAG, designated herein as SEQ ID NO: 15; Reverse Sequence: TGCAGCTTCACTGAATCTTGAAAG, designated herein as SEQ ID NO: 16; Probe Sequence: TTCCTTGCTTCTTGAATTTGTTTTGCATCCAT, designated herein as SEQ ID NO: 17).

The results are presented in the table below. The results indicate that treatment with ISIS 645938, 645954 or 645958 resulted in increased GSTA1 mRNA expression levels in both the liver and the WAT. The increase in GSTA1 suggests that ASO inhibition of KEAP1 expression increases Nrf2 activity and allows expression of ARE-containing genes such as GSTA1.

Table 5

Expression of GSTA1 in wildtype mice treated with ASOs

Treatment (ISIS NO)	Dose (mg/kg)	% saline	
		liver	WAT
saline	-	100	100
549144	40	104	222
645938	40	1989	3039
	25	1455	2122
	10	329	1054
645954	40	4454	8700
	25	2768	10250
	10	1326	1014
645958	40	910	2183
	25	493	1126
	10	342	154

NQO1 mRNA levels in wild type mouse liver, kidney and WAT were quantified using quantitative real-time PCR. NQO1 mRNA expression was assessed using mouse primer probe set: mNQO1_LTS00751 (Forward Sequence: CCTGGAAGGATGGAAGAAACG, designated herein as SEQ ID NO: 18; Reverse Sequence: CAGGCTGCTTGGAGCAAAAT, designated herein as SEQ ID NO: 19; Probe Sequence: AAACCGTCTGGGAGGAGACCCCACTX, designated herein as SEQ ID NO: 20, wherein X is a label).

Results are presented in the table below as percent saline. The results shown indicate that treatment with ISIS 645938, 645954 or 645958 resulted in dose dependent increases in NQO1 mRNA expression levels. The increase in NQO1 suggests that ASO inhibition of KEAP1 expression increases Nrf2 activity and allows expression of ARE-containing genes such as NQO1.

Table 6

Expression of NQO1 mRNA in wildtype mice treated with ASOs

Treatment (ISIS NO)	Dose (mg/kg)	% saline		
		liver	Kidney	WAT
saline	-	100	100	100
549144	40	119	108	105
645938	40	915	441	591
	25	594	396	545
	10	282	323	271
645954	40	2134	500	739
	25	1436	342	669
	10	490	325	379
645958	40	454	219	234
	25	303	231	204
	10	196	204	202

NQO1 Activity Assay

NQO1 activity was quantified in liver tissue homogenates using the Abcam NQO1 activity assay kit. Averages for each group are presented in the table below. The results show that NQO1 activity is increased after KEAP1 ASO treatment.

Table 7

Hepatic NQO1activity in wild type mice treated with 40 mg/kg/wk of ASO

Time (sec)	Treatment (ISIS NO)			
	saline	549144	645938	645958
0	0	0	0	0
20	0.003	0.001	0.014	0.008
40	0.014	-0.004	0.027	0.012
60	0.015	-0.006	0.043	0.020
80	0.012	0.000	0.046	0.025
100	0.012	0.008	0.061	0.031
120	0.011	0.001	0.067	0.040
140	0.013	0.002	0.080	0.046
160	0.015	0.002	0.088	0.054
180	0.016	0.004	0.103	0.063
200	0.017	0.005	0.110	0.068
220	0.018	0.006	0.128	0.077
240	0.019	0.007	0.135	0.086
260	0.019	0.007	0.148	0.092
280	0.020	0.008	0.161	0.101
300	0.023	0.011	0.171	0.108

In chow fed wildtype mice, dose titration of KEAP1 ASOs (40, 25, and 10 mg/kg/wk) for four weeks led to dose-dependent decreases in hepatic KEAP1, reaching 90% at the highest dose when compared to saline controls. This reduction in KEAP1 led to significant increases in the hepatic expression of the ARE-containing genes NQO1 and GSTA1, reaching 915% and 1989%, respectively, at the highest dose versus saline controls. Furthermore, administration of the KEAP1 ASO did not produce significant changes in body weight or liver transaminases relative to control, indicating the ASOs were well tolerated.

Example 3: ASO inhibition of KEAP1 in diet-induced obesity (DIO) mice

Frequently used in studies of diet-induced obesity (DIO), C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) are vulnerable to the development of obesity when fed a high fat diet. ISIS 645938, exhibiting significant *in vitro* inhibition of murine KEAP1 RNA, was tested in a DIO mouse model.

Six week old test mice were fed a 60 kcal% high-fat diet (HFD) (D12492 Research Diets) for fifteen weeks, while six week old mice in three control groups (eight animals per group) were fed a normal chow

diet, D12451 (a diet comprising 45 kcal% fat) or D12450B (a diet comprising 10 kcal% fat) for fifteen weeks.

Treatment

Treatment groups consisted of six to eight animals. Twenty-two week old male DIO mice ranging in weight from 36 g to 52 grams were continued on the high-fat diet for the duration of the study. ASOs were dissolved in 0.9% buffered saline (PBS) and sterilized by filtering through a 0.2 micron filter before injection in the mice.

The treatment groups were injected subcutaneously once a week for 6 weeks with the following:

1) 50 mg/kg of ISIS 645938 targeting murine KEAP1,

2) PBS solution, or

3) 50 mg/kg of control ASO ISIS 549144.

Several clinical endpoints were measured over the course of the study. The body and food weights were measured weekly, and tail bleeds were performed at baseline (after 15 weeks on HFD) and weekly thereafter, as well as at the time of sacrifice. The mice were euthanized 72 hours after the last dose (after 21-weeks on HFD) and after six-weeks of oligonucleotide treatment; organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of antisense oligonucleotides on several plasma chemistry markers (e.g. cholesterols, glucose, triglycerides and liver transaminases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), blood and plasma levels were measured at various time points. The results are presented in the table below. "BL" = baseline after four-hour fast. For the 3-week time point, a 16-hour-fasted tail bleed was taken 24-hours after the third dose (after 18 weeks on HFD). For the 6-week time point, a 12-hour-fasted tail bleed was taken 24-hours after the sixth dose (after 21 weeks on HFD). "N/A" = data not available. Treatment with ISIS 645938 was tolerable in the mice with a transitory increase in ALT and AST levels that subsided by the sixth week.

Table 8

Plasma liver transaminases in ASO-treated DIO mice

Diet and Treatment	ALT (U/L)			AST (U/L)		
	BL	3 wk.	6 wk.	BL	3 wk.	6 wk.
PBS normal chow	39	23	37	48	40	55

PBS 10% fat D12450B		N/A	23	N/A	N/A	50	N/A
PBS 45% fat D12451		N/A	49	N/A	N/A	70	N/A
HFD D12492	PBS	44	69	104	52	87	110
	ISIS 549144	51	76	152	62	100	90
	ISIS 645938	33	205	93	54	197	117

Plasma levels of glucose ("GLU"), triglycerides ("TG"), total cholesterol ("TC") and HDL were measured at baseline, 3-week and 6-week time points using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the table below.

Table 9

Plasma chemistry markers in ASO-treated DIO mice

Diet and Treatment		TC (mg/dL)			HDL (mg/dL)			GLU (mg/dL)			TG (mg/dL)		
		BL	3 wk	6 wk	BL	3 wk	6 wk	BL	3 wk	6 wk	BL	3 wk	6 wk
PBS normal chow		61	60	78	47	46	57	200	182	141	89	51	131
PBS 10% fat D12450B		N/A	121	N/A	N/A	96	N/A	N/A	148	N/A	N/A	84	N/A
PBS 45% fat D12451		N/A	168	N/A	N/A	130	N/A	N/A	185	N/A	N/A	87	N/A
HFD D12492	PBS	171	193	213	134	149	174	254	190	197	85	110	98
	549144	150	180	211	113	162	174	242	181	181	66	127	100
	645938	161	251	199	128	181	151	238	188	169	75	134	90

Glucose Tolerance Test (GTT)

At the fourth week of treatment, 24 hours after treatment with the fourth dose of ASO or PBS and the DIO mice having fasted for 16 hours, 2 grams of glucose per kg body weight was administered intraperitonally and blood was drawn at various time points using a One Touch II™ glucose monitor for a Glucose Tolerance Test (GTT). As shown in the table below, ISIS 645938 treated mice were able to clear glucose at a higher rate than control treated mice, indicating an improving trend in glucose tolerance with ISIS 645938 treatment.

Table 10

GTT: Blood glucose levels (mg/dL) in ASO treated DIO mice

Diet and Treatment		Time post glucose challenge (minutes)					
		0	15	30	60	90	120
PBS normal chow		80	264	259	183	167	113
PBS 10% fat D12450B		91	281	294	201	140	143
PBS 45% fat D12451		115	282	370	317	237	200
HFD D12492	PBS	162	344	362	298	247	217
	549144	157	283	345	264	231	199
	645938	157	299	287	210	190	168

Insulin Tolerance Test (ITT)

At the fifth week of treatment, 24 hours after treatment with the fifth dose of ASO or PBS and the DIO mice having fasted for four hours, 0.5U human insulin per kg body weight was administered intraperitonally and blood was drawn at various time points using a One Touch II™ glucose monitor for an insulin tolerance test (ITT). As shown in the table below, blood glucose levels are reduced in DIO mice treated with ISIS 645938 indicating that treatment with a KEAP1 ASO had a tendency to improve insulin sensitivity in the mice.

Table 11

ITT: Blood glucose levels (mg/dL) in ASO-treated DIO mice

Diet and Treatment			Time point (minutes)					
		0	15	30	45	60	90	120
PBS normal chow		135	113	99	118	141	167	170
HFD D12492	PBS	215	213	187	198	218	213	221
	549144	176	178	152	161	178	177	172
	645938	194	172	133	136	161	154	177

Body and organ weights

At the end of the study, 48 hours after treatment with the sixth dose of ASO or PBS, organs (liver, spleen, kidney) were harvested, animals were euthanized, and blood was drawn for measure of various clinical parameters.

Body weights of the mice were measured each week and at the time of sacrifice ("Sac"), and are presented in the table below. Treatment with ISIS 645938 induced a downward trend in body weight over the course of treatment.

Table 12

Body weights (g) of DIO mice after ASO treatment

ISIS NO	BL	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Sac
PBS	45	46	49	49	48	50	51	51
549144	44	45	47	48	50	50	51	51
645938	44	49	45	44	44	41	41	40

Kidney, liver and spleen weights were measured at the end of the study, and are presented as percent body weight (%BW) in the table below.

Table 13

Organ weights (g) of DIO mice after ASO treatment

Diet and Treatment		liver (%BW)	kidney (%BW)	spleen (%BW)
PBS normal chow		4.18	1.12	0.27
HFD D12492	PBS	3.88	0.76	0.24
	549144	4.68	0.91	0.29
	645938	6.93	1.13	0.43

ELISA assays

Insulin: An ALPCO Mouse Ultrasensitive Insulin ELISA kit (80-INSMSU-E01, E10) was used, without dilution, to measure plasma levels of insulin at baseline (BL) after a 4-hour fast, at three weeks after a 16-hour fast and at six weeks after a 16-hour fast, as shown in the table below. Treatment with ISIS 645938 decreased insulin and maintained similar glucose levels over the course of treatment when compared to control ASO indicating an improvement in insulin sensitivity.

Table 14

Plasma levels of insulin (ng/mL) in DIO mice after ASO treatment

Diet and Treatment	BL	3 wks	6 wks
PBS normal chow	1.63	0.32	1.07

HFD D12492	PBS	13.23	1.85	2.93
	549144	9.88	1.69	1.69
	645938	8.52	0.98	0.65

Leptin: A Quantikine Mouse/Rat Leptin ELISA kit (M0B00) was used at a 1:20 dilution to measure plasma Leptin levels, as shown in the table below. 48-hours after the sixth dose, leptin was measured. Leptin was decreased after treatment with ISIS 645938, indicating an improvement in leptin sensitivity. Generally, a reduction in leptin is associated with improvements in obesity and metabolic syndrome.

Adiponectin: A Quantikine Mouse Adiponectin/Acrp30 ELISA kit (MRP300) was used at a 1:2000 dilution, as shown in the table below. 48-hours after the sixth dose, adiponectin was measured. Adiponectin was not changed after treatment with ISIS 645938.

Table 15

Plasma Leptin and Adiponectin (ng/mL) in DIO mice after ASO treatment

Diet and Treatment		Leptin	Adiponectin
PBS normal chow		6	12
HFD D12492	PBS	103	13
	549144	76	12
	645938	27	14

KEAP1 Expression Analysis in the Organs:

Expression levels of KEAP1 in DIO mouse liver, fat, quadriceps muscle and pancreas were determined using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. RNA was isolated from each organ using standard protocols, and a murine KEAP1 primer probe set RTS4398_MGB was used to measure mRNA levels in the organs harvested from ASO-treated DIO mice. KEAP1 RNA levels were determined relative to total RNA using RIBOGREEN® prior to normalization to PBS-treated control. The results are presented in the table below as the average percent inhibition of KEAP1 RNA levels for each treatment group, relative to PBS-treated control and are denoted as "% inhibition." ISIS 645938 strongly inhibited KEAP1 levels in liver, fat and muscle.

Table 16

KEAP1 RNA levels in organs of DIO mice after ASO treatment

Diet and Treatment		% inhibition			
		Liver	fat	muscle	pancreas
PBS normal chow		4	10	0	1
HFD D12492	PBS	0	0	0	0
	549144	2	9	31	0
	645938	93	74	78	0

Liver triglyceride (TG) levels (mg/g of liver) were also assayed by biochemical quantitation and are presented in the table below. Liver TG levels are significantly reduced in DIO mice treated with ISIS 645938 indicating that a KEAP1 ASO may be effective in reducing or preventing hepatic steatosis, the initial pathological event in the development of NAFLD.

Table 17

Liver TG levels (mg/g of liver) of DIO mice after ASO treatment

Diet and Treatment		Liver TG
PBS normal chow		4.4
HFD D12492	PBS	95.5
	549144	82.5
	645938	14.2

To determine whether KEAP1 inhibition would improve metabolic syndrome in DIO mice, a KEAP1 ASO was administered to the mice. Treatment of mice with the KEAP1 ASO significantly reduced KEAP1 with concordant improvement in glucose tolerance and insulin signaling, as well as reductions in hepatic triglyceride accumulation indicating that a KEAP1 ASO may be beneficial in treating metabolic syndrome in an animal.

Example 4: ASO inhibition of KEAP1 in male Gubra mice

Lep^{ob}/Lep^{ob} mice fed a high fat/fructose/cholesterol diet is known herein as the "Gubra" mouse model (Gubra ApS, Horshom, Denmark). The Gubra mouse is an accelerated diet-induced obese mouse model for fatty liver disease including fatty liver, NASH, and fibrosis. The Gubra mouse exhibits elements of liver steatosis, ballooning degeneration of hepatocytes, inflammation and fibrosis and affects metabolic parameters including body weight, hyperinsulinemia, fasting hyperleptinemia and impaired glucose tolerance.

To develop the diet induced Gubra phenotype, five-week old male mice were fed a high fat/fructose/cholesterol diet (40% HFD, 18% fructose, 2% cholesterol) for 19 weeks prior to the start of the study. After 16-17 weeks on the diet, the mice were pre-screened and randomized into treatment groups after liver biopsy and histological assessment (e.g., scoring of fibrosis after staining with Sirius Red and steatosis after staining with H&E).

Treatment

ISIS 645938 was administered to Gubra mice to test its effects on the mice. After 19-weeks on the high fat/fructose/cholesterol diet, a group of ten mice were treated with subcutaneous weekly injections of 40 mg/kg ISIS 645938, 50 mg/kg 549144 or PBS control over the course of eight weeks.

At the end of the study (8-weeks), the mice were sacrificed and livers were removed at the time of sacrifice. Liver RNA was extracted, as well as liver TG and TC, and analyses of hepatic pathology including steatosis, fibrosis stage and NAFLD Activity Score (NAS) were assessed and compared to pre-study biopsies at baseline. RNA was extracted from liver for real-time PCR analysis of liver KEAP1 RNA levels.

Levels of KEAP1 in Gubra mouse liver were determined using real-time PCR and the RTS4398_MGB primer probe set to measure RNA levels. RNA levels were normalized to RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) and the results are presented as percent inhibition with respect to PBS-treated mice in the table below.

Table 18

ASO inhibition of KEAP1 in Gubra mouse liver

ISIS No	% inhibition
PBS	0
549144	3
645938	78

Body and organ weights

Body weights of the Gubra mice were measured every two days, and liver, left and right kidneys, and spleen weights were measured at week 8, at the end of the study. Also at the end of the study, EchoMRI scanning and terminal necropsy were performed, organs (liver, kidney, spleen, epididymal adipose tissue and quadriceps muscle) were harvested and liver, kidney and spleen weights were measured. Averages for each treatment group were calculated. Several weight points were chosen for presentation in the table below, along with weights of the organs relative to total body weight at the end of the study, and these data are presented (in grams) in the tables below.

Table 19

Weights (g) of body and organs of Gubra mice after antisense oligonucleotide treatment

Treatment (ISIS NO)	Body					Relative weight			
	day 0	day 16	day 28	day 44	day 54	Liver	L. Kidney	R. Kidney	Spleen
PBS	59	59	59	59	61	9.0	0.29	0.30	0.20
549144	59	60	60	61	63	10.8	0.28	0.29	0.21
645938	58	55	52	49	48	11.0	0.38	0.38	0.34

Over the course of the study, a significant reduction in body weight was observed as mice treated with ISIS 645938 were approximately 83% of their weight before treatment. As compared to control mice, the mice treated with ISIS 645938 exhibited slight increases in relative liver, kidney and spleen weights (expressed as a percentage of body weight) as compared to PBS and ISIS 549144 control.

Plasma chemistry markers

To evaluate the effect of ASOs on hepatic function, plasma concentrations of liver transaminases ALT and AST, as well as plasma lipids (TG and TC) were measured in Gubra mice at baseline and at the end of the 8-week study. The averages for each treatment group are presented in the table below. "BL" = baseline.

Table 20

Plasma chemistry markers in ASO treated Gubra mice

ISIS NO	ALT (U/L)		AST (U/L)		TC (mmol/L)		TG (mmol/L)	
	BL	8 wk	BL	8 wk	BL	8 wk	BL	8 wk
PBS	586	549	398	425	10	10	0.7	0.7
549144	702	636	445	462	11	11	0.7	0.6
645938	503	178	339	282	10	9	0.6	0.8

Overall, it was observed that the mice treated with ISIS 645938 exhibited an over 300 U/L reduction in plasma ALT as compared to baseline, a 57 U/L decrease in plasma AST as compared to baseline, a 1 mmol/L decrease in plasma TC as compared to baseline, and a 0.2 mmol/L increase in plasma TG over baseline. The data indicate that the KEAP1 ASO is decreasing liver damage as measured by ALT/AST.

An Oral Glucose Tolerance Test (OGTT) was performed. At week four of the eight week study, 4-hour fasted mice were subcutaneously injected with ISIS 645938 at time = 0 minus one hour, and blood glucose was measured. One hour later, at time 0, glucose (2 g/kg) was ingested by the mice and blood glucose was tested

again; thereafter, blood glucose was tested at 15 min, 30 min, 60 min and 120 min time points. Blood glucose area under the curve (AUC) was also calculated mmol/L x minute.

Table 21

OGTT: Blood glucose levels (mmol/L) in ASO treated Gubra mice

Treatment (ISIS NO)	Time post glucose challenge (minutes)					AUC mmol/L
	0	15	30	60	120	
PBS	8.5	15.2	15.8	10.3	7.6	1839
549144	7.9	13.4	13.5	8.9	7.2	1639
645938	7.2	15.2	14.2	7.5	6.9	1563

Liver TG and liver TC content (mg/g of liver) were also assayed by biochemical analysis, and are presented in the table below. Liver TG and TC levels are significantly reduced in Gubra mice treated with ISIS 645938 indicating that a KEAP1 ASO may be effective in reducing or preventing hepatic steatosis, the initial pathological event in the development of NAFLD.

Table 22

Liver TG and TC content (mg/g of liver) in Gubra mice after ASO treatment

Treatment	Liver TG	Liver TC
PBS	695	105
549144	739	103
645938	379	34

Histological assessment of NAFLD (steatosis, inflammation, damage/necrosis, and fibrosis), the current preclinical and clinical standard for assessment of NAFLD disease, was performed on sections both pre- and post-treatment using haematoxylin and eosin stain (H&E) stain, and fibrosis was assessed using Sirius Red stain. For liver steatosis, ISIS 645938 reduced steatosis by -37%, compared to no change with PBS treatment, which was in agreement with the biochemical analysis of liver triglyceride. ISIS 645938 treatment reduced ballooning degeneration by 100% versus no change with PBS treatment. Fibrosis was reduced by -16% with ISIS 645938 treatment versus a 73% increase with PBS treatment. Inflammation was increased to a similar extent across all treatment groups. Finally, the ISIS 645938 treatment reduced NAS (the sum of the scores for steatosis (0–3), hepatocellular ballooning (0–2) and lobular inflammation (0–3) by -21% versus a 5% increase with PBS treatment. Taken together, this indicates that a KEAP ASO treatment reduced and reversed NAFLD progression by decreasing steatosis, fibrosis, and degeneration.

Table 22

Liver histological assessment after KEAP1 ASO treatment

	Saline		ISIS 549144		ISIS 645938	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Steatosis	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	1.9 ± 0.1
Ballooning Degeneration	0.88 ± 0.13	0.88 ± 0.13	0.78 ± 0.15	0.89 ± 0.11	0.43 ± 0.2	0 ± 0
Fibrosis	1.5 ± 0.2	2.6 ± 0.2	1.8 ± 0.2	2.4 ± 0.2	1.9 ± 0.3	1.6 ± 0.2
Inflammation	1.6 ± 0.2	1.9 ± 0.13	1.7 ± 0.2	2.1 ± 0.1	1.4 ± 0.2	2.0 ± 0.3
NAS	5.5 ± 0.2	5.8 ± 0.2	5.4 ± 0.2	6.0 ± 0.2	4.9 ± 0.3	3.9 ± 0.3

Liver fibrosis markers hydroxyproline and collagen were assessed in the mice. Liver collagen mRNA levels were quantified using a Col1a2 assay (ThermoFisher Scientific assay ID # Mm00483888_m1). Liver hydroxyproline and collagen were reduced as shown in the table below indicating a reduction in liver fibrosis after treatment with KEAP1 ASO.

Table 23

Liver fibrosis markers in Gubra mice after ASO treatment

Treatment	Hepatic hydroxyproline (μg / mg liver)	Hepatic Col1a2 mRNA expression (% saline)
PBS	0.53	100
549144	0.50	101
645938	0.28	40

Overall, data from this Gubra mouse study indicated that an ASO targeting KEAP1 was active in liver, was well tolerated, decreased several biomarkers of metabolic and liver diseases, and KEAP1 is an important candidate for the treatment of obesity, type 2 diabetes and/or insulin sensitivity, hyperlipidemia, NASH, NAFLD and oxidative stress related diseases, disorders or conditions.

Example 5: ASO inhibition of KEAP1 in male ob/ob mice

ASOs described in the studies above will be evaluated for their ability to reduce murine KEAP1 RNA transcript in an 8-week ob/ob mice study.

Treatment

C57BL/6J-Lepr ob ("ob/ob") mice will be divided into treatment groups consisting of eight animals. Mice will be injected subcutaneously once a week for 8 weeks with either 25 or 10 mg/kg of ISIS 549144 control oligonucleotide, 25 or 10 mg/kg of ISIS 645938 or ISIS 645958, and one group of seven ob/ob mice

will be injected with PBS as a control to which the antisense oligonucleotide treated groups are compared. Several clinical endpoints will be measured over the course of the study. The body and food weights will be measured weekly, and tail bleeds will be performed at baseline and weekly thereafter, as well as at the time of sacrifice. The mice will be euthanized 72 hours after the last dose and after 8 weeks of ASO treatment organs and plasma will be harvested for further analysis.

RNA analysis

At the end of the treatment period, RNA will be extracted from liver, kidney, white adipose tissue (WAT) and pancreas for quantitative real-time PCR analysis of RNA expression of KEAP1. Murine KEAP1 primer probe set RTS4398_MGB will be used to measure RNA levels. Results will be presented as percent inhibition of KEAP1, relative to PBS control, normalized against Cyclophilin A. Both ISIS 645938 and ISIS 645958 will be shown as active inhibitors in multiple tissues with ISIS 645938 the more potent inhibitor.

Plasma chemistry markers

To evaluate the effect of treatment with ISIS oligonucleotides on plasma levels of various biomarkers of liver and kidney function, plasma levels of liver transaminases (ALT and AST), total cholesterol (CHOL), creatinine (CRE), glucose (GLU), HDL, LDL, triglycerides (TRIG), BUN, non-esterified fatty acids (NEFA), 3HB will be measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY).

Overall, the plasma chemistry results will indicate that both ISIS 645938 and ISIS 645958 are tolerable in ob/ob mice.

Protein analysis

The cytoprotective gene NQO1 is a downstream target gene of Nrf2 in the KEAP1 pathway. NQO1 protein levels in ob/ob mouse liver and kidney will be quantified using a commercially available ELISA kit according to the manufacturer described protocol. The results will indicate that treatment with ASOs targeting KEAP increase NQO1 expression levels. The result will suggest that ASO inhibition of KEAP1 decreases KEAP-mediated repression of Nrf2 activity and allows expression of Nrf2 downstream genes such as NQO1.

Kidney Injury Molecule-1 (Kim-1) and Neutrophil Gelatinase-Associated Lipocalin (NGAL) are biomarkers found in urine or plasma and are used to detect renal tubule injury. Kim-1 and NGAL protein levels in kidneys of ob/ob mice treated with ASOs will be quantified using a commercially available ELISA kit according to the manufacturer described protocol. The results will show a lack of significant increase in the biomarkers Kim-1 and NGAL and indicate that KEAP1 ASO treatment does not injure the kidney.

Body and organ weights

Body weights will be measured weekly, and kidney, liver, spleen and white adipose tissue (WAT) weights will be measured at the end of the study. Results showing a lack of significant increases in the body or organ weights in KEAP1 ASO treated mice will indicate that ASO treatment is tolerable for the mice.

Hepatic triglycerides

Hepatic triglyceride (TG) concentrations (□g/g of liver) will be assayed by ELISA. Liver TG levels will be significantly reduced in KEAP1 ASO treated ob/ob mice indicating that KEAP1 ASOs may be effective in reducing or preventing fatty liver diseases such as hepatic steatosis, NASH and/or NAFLD.

Glucose Tolerance Test (GTT)

At week four of the eight week study, a glucose tolerance test (GTT) will be performed. 24 hours after treatment with the fourth dose of ASO or PBS. 4-hour-fasted ob/ob mice will be intraperitonally administered 2 grams of glucose per kg body weight, and blood will be drawn at various timepoints using a One Touch II™ glucose monitor. KEAP1 ASO treated mice will be able to clear glucose at a higher rate than control treated mice, indicating an improvement in glucose tolerance with KEAP1 ASO treatment.

Example 6: ASO inhibition of KEAP1 in a House Dust Mite (HDM)-induced allergic asthma model

The House Dust Mite (HDM)-induced mouse model of severe asthma features many similarities to human allergic asthma, including the presence of eosinophilic lung inflammation and the release of inflammatory mediators and cytokines primarily associated with Th2-type inflammation.

As noted in the description above, the KEAP1/Nrf2 system is a major regulator of the intracellular antioxidant response to cellular oxidative and electrophile stress. Oxidative stress occurs when the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) exceeds the activity of intracellular antioxidant defense mechanisms. This imbalance causes an accumulation of ROS that leads to greater lipid peroxidation, damage to proteins, cell membranes and DNA and exacerbates the severity of several diseases. For example, oxidative stress plays an important role in various disease pathologies including diabetes, diabetic nephropathy, diabetic cardiomyopathy, insulin resistance, fibrosis (e.g. hepatic fibrosis or pulmonary fibrosis), NAFLD/NASH, cancer, chronic obstructive pulmonary disease (COPD), asthma, age related macular degeneration, atherosclerosis, and neurologic disorders.

In the basal condition, KEAP1 tethers Nrf2 and causes Nrf2 ubiquitination and degradation. Upon cellular stress assault, KEAP1 protein dissociates with Nrf2, allowing Nrf2 to translocate into the nucleus where it acts as a transcriptional activator to induce antioxidant genes including GSTs, GCLM, NQO1, NQO2, HO-1. Current evidence suggests that Nrf2 protein is downregulated in COPD, and activation of Nrf2 prevents COPD in cigarette smoke-exposed mice.

The pathology of chronic asthma shows prominent structural changes in the airway wall – specifically alteration in the extracellular matrix (ECM) and thickening of the airway smooth muscle (ASM). While not to be bound by theory, Nrf2 is currently believed to be an important determinant of smooth muscle function (An, S.S., *et al*., 2016, *J. Allergy Clin. Immunol*. pii: S0091-6749(16)00116-0. [Epub ahead of print]). In chronic asthma, the aforementioned structural changes may be attributable to defective Nrf2-directed regulation of oxidative stress, leading to abnormal ECM remodeling and increased contractility of the ASM cell, resulting in asthmatic airflow obstruction.

As noted in examples set forth in the present disclosure, it was observed that inhibition of KEAP1 results in increased transcription of antioxidant and detoxifying genes.

Treatment

8- to 10-week old female C57Bl6 mice, weighing approximately 20 g, were fed a normal chow diet and divided into treatment groups consisting of twelve animals. 10 mg/kg of ISIS 645938 was locally administered to the mouse lung by intratracheal administration twice a week starting two weeks before the first challenge with house dust mite (HDM) allergen at time 0. ISIS 549148 (a 3-10-3 cEt gapmer having the sequence GGCTACTACGCCGTCA, incorporated herein as SEQ ID NO: 11) was used as an ASO control, and saline alone served as a vehicle control to which the ASO-treated groups were compared. At time 0, the mice undergoing treatment with ASOs and the saline control group were then challenged with HDM allergen on days 0, 7, 14. Another group of "naïve" mice was not challenged with ASO or HDM allergen. At the end of study, lung function airway hyperresponsiveness (AHR) was measured for the various groups of mice. After lung function measurements, the mice were then sacrificed and lung tissue was harvested for further analysis.

Several endpoints were measured to examine the effects of targeting KEAP1. Target mRNA knockdown of KEAP1 was measured in addition to the effect on various mucus markers (mRNA). Differential cell counts of inflammatory cells in the lung were performed on the bronchoalveolar lavage (BAL) fluid to evaluate the effects of the ASOs targeting murine KEAP1 on cell recruitment to the airways. Lungs were then inflated and stained for mucus (PAS-stained).

RNA analysis

At the end of the treatment period, RNA was extracted from lung tissue for quantitative real-time PCR analysis of RNA expression for KEAP1. Murine KEAP1 primer probe set RTS4398_MGB (forward sequence AAGGTCATGGAAAGGCTTATTGAGT, designated herein as SEQ ID NO: 12; reverse sequence GTTCATCACGTGCAGGACACA, designated herein as SEQ ID NO: 13; probe sequence TCTCCGTGGGCGAGAA, designated herein as SEQ ID NO: 14) was used to measure RNA levels. Results are presented in the table below as percent inhibition of KEAP1, relative to PBS control, normalized against

RIBOGREEN®. Treatment with the KEAP1 ASOs resulted in a significant reduction of KEAP1 mRNA in the lung in comparison to the control groups.

Table 24

ASO inhibition of KEAP1 RNA in organs of HDM mice

Treatment (ISIS NO)		% inhibition
Naïve (no HDM)		0
HDM-induced	Saline	0
	645938	63
	549148	13

Mucus Marker RNA analysis

Murine markers of inflammation and mucus overproduction commonly increased during asthma were assessed including:

(1) inflammation marker macrophage-restricted F4/80 using primer probe set: F4/80, mEmr1_LTS00125 (Forward: GGCCATTGCCCAGATTTTC, designated herein as SEQ ID NO: 21; reverse: CGGTTGAGCAGACAGTGAATGA, designated herein as SEQ ID NO: 22; probe: CCAGATTGGCCCCTTGGCAAGCX, designated herein as SEQ ID NO: 23) to measure mRNA levels,

(2) mucus marker GOB5 (a member of the calcium-activated chloride channel family, a.k.a. chloride channel accessory 1, or CLCA1) primer probe set: Gob5, RTS1845 (Forward: CACTAAGGTGGCCTACCTCCAA, designated herein as SEQ ID NO: 24; reverse AGCTCGCTTGAATGCTGTATTTC, designated herein as SEQ ID NO: 25; probe: CCCAGGCACGGCTAAGGTTGGCX, designated herein as SEQ ID NO: 26) to measure mRNA levels,

(3) mucus marker SPDEF (SAM-pointed domain–containing ETS transcription factor) (primer probe set: SPDEF, RTS4444 (Forward: GCGAGGTCCTGAAAGATATTGAG, designated herein as SEQ ID NO: 27; reverse: GCCACTTCTGCACGTTACCA, designated herein as SEQ ID NO: 29; probe: CTTCTGAACATCACAGCAGACCCTGGG, designated herein as SEQ ID NO: 29) to measure mRNA levels, and

(4) mucus marker mucin 5 subtype B (MUC5B) (primer probe set: mMUC5B, RTS3745 (Forward: TGACTCCATATCCTCATCCACAAG, designated herein as SEQ ID NO: 30; reverse AGGTGTAAGGCGCTCATGCT, designated herein as SEQ ID NO: 31; probe: CACCTTCATCCCACCTATCACTGTCTTCCCX, designated herein as SEQ ID NO: 32) to measure mRNA levels.

The results are presented in the table below as % saline. The results indicate that treatment with ISIS 645938 resulted in decreased expression levels of the biomarkers of inflammation and mucus production.

Table 25

Expression of markers of inflammation in HDM-induced mice treated with ASOs

Treatment (ISIS NO)		% saline			
		Macrophage (F4/80)	GOB5	SPDEF	MUC5B
Naïve (no HDM)		41	8	79	77
HDM-induced	Saline	100	100	100	100
	645938	34	29	34	41
	549148	84	91	147	92

Bronchoalveolar lavage (BAL)

Differential cell counts of inflammatory cells in the lung were performed on the bronchoalveolar lavage (BAL) fluid to evaluate the effects of the ASOs targeting murine KEAP1. HDM greatly increases the number of eosinophils recruited to the airways. HDM-induced control mice treated with saline or ISIS 549148 showed an induction in eosinophils, whereas HDM-induced mice treated with ISIS 645938 targeting KEAP1 exhibited a shift in the percentage of BAL cells to more macrophages and fewer eosinophils.

Table 26

ASO inhibition of KEAP1 Reduces Eosinophils in HDM treated mice

Treatment (ISIS NO)		% BAL Eosinophils
Naïve (no HDM)		0
HDM-induced	PBS	100
	645938	35
	549148	78

Airway hyperresponsiveness (AHR)

To evaluate the effect of ASOs on airway hyperresponsiveness (AHR), 24 hours after the last HDM allergen challenge, anesthetized but awake mice were exposed to aerosolized PBS using an ultrasonic nebulizer to set a baseline value, and then increasing doses (0, 3, 6, 12 and 25 mg/ml) of aerosolized □-methacholine (Mch), a non-specific cholinergic agonist, were administered to induce bronchospasm and

airway obstruction. PenH (enhanced pause) values were measured for 2 minutes after each Mch aerosol dose, using a whole body plethysmograph (Buxco, Electronics, Troy, NY, USA). The average PenH values were expressed for the 25 mg/ml Mch concentration as the percentage of PBS control PenH value. KEAP1 ASO treatment moderately, but significantly reduced AHR as compared to PBS- or ASO-control treatment groups.

Table 27

ASO inhibition of KEAP1 Effects Lung Function (25mg/ml Mch)

Treatment (ISIS NO)		Penh % PBS Cont
Naïve (no HDM)		47
HDM-induced	PBS	100
	645938	72
	549148	87

Overall, KEAP1 ASO resulted in reduced target RNA in HDM-induced mouse lung tissue, reduced airway eosinophilia, reduced mucus markers, and had a slight effect on AHR, whereas the control ASO- and PBS-treated HDM-induced mice did not show these improvements.

Example 7: ASO inhibition of KEAP1 in male ob/ob mice

ASOs described in the studies above were evaluated for their ability to reduce murine KEAP1 RNA transcript in a 10-week ob/ob mice study.

Treatment

C57BL/6J-Lepr ob ("ob/ob") mice were divided into treatment groups consisting of eight animals. Mice were injected subcutaneously once a week for 10 weeks with 25 mg/kg of ISIS 549144 control oligonucleotide, or with 25 mg/kg or 10 mg/kg of ISIS 645938, and one group of eight ob/ob mice were injected with PBS as a control to which the antisense oligonucleotide treated groups are compared. Several clinical endpoints were measured over the course of the study. The body and food weights were measured weekly, and tail bleeds were performed at baseline and weekly thereafter, as well as at the time of sacrifice. The mice were euthanized 72 hours after the last dose and organs and plasma were harvested for further analysis.

KEAP1 RNA analysis

At the end of the treatment period, RNA was extracted from liver, kidney, heart, white adipose tissue (WAT), lung, pancreas, and spleen for quantitative real-time PCR analysis of mRNA expression of KEAP1.

Murine KEAP1 primer probe set RTS4398_MGB was used to measure mRNA levels. Results are presented as percent of KEAP1, relative to PBS control, normalized against Cyclophilin A. ISIS 645938 was shown as an active inhibitor in multiple tissues.

Table 28

Expression of KEAP1 in ob/ob mice treated with KEAP1 ASO

Treatment	Dose (mg/kg)	KEAP1 mRNA (% saline)						
		Liver	Kidney	Heart	WAT	Lung	Pancreas	Spleen
PBS	-	100	100	100	100	100	100	100
549144	25	88	93	86	110	106	99	109
645938	10	26	41	79	65	113	102	84
645938	25	14	27	59	47	66	95	81

NQO1 RNA analysis

At the end of the treatment period, RNA was extracted from heart, liver, kidney, white adipose tissue (WAT), lung, spleen, and pancreas for quantitative real-time PCR analysis of mRNA expression of NQO1. Murine NQO1 primer probe set mNQO1_LTS00751 was used to measure mRNA levels. Results are presented as percent of NQO1, relative to PBS control, normalized against Cyclophilin A. ISIS 645938 was shown to upregulate NQO1 expression in multiple tissues. The result shows that ASO inhibition of KEAP1 decreases KEAP-mediated repression of Nrf2 activity and allows expression of Nrf2 downstream genes such as NQO1.

Table 29

Expression of NQO1 in ob/ob mice treated with KEAP1 ASO

Treatment	Dose (mg/kg)	NQO1 mRNA (% saline)						
		Liver	Kidney	Heart	WAT	Lung	Pancreas	Spleen
PBS	-	100	100	100	100	100	100	100
549144	25	88	92	99	113	78	82	114
645938	10	304	332	100	220	198	194	99
645938	25	412	349	110	309	304	401	105

GSTA1 RNA analysis

At the end of the treatment period, RNA was extracted from heart, liver, kidney, white adipose tissue (WAT), lung, spleen, and pancreas for quantitative real-time PCR analysis of mRNA expression of GSTA1. Murine GSTA1 primer probe set mGsta1_LTS34766 was used to measure mRNA levels. Results are presented as percent of GSTA1, relative to PBS control, normalized against Cyclophilin A. ISIS 645938 was shown to upregulate GSTA1 expression.

Table 30

Expression of GSTA1 in ob/ob mice treated with KEAP1 ASO

Treatment	Dose (mg/kg)	GSTA1 mRNA (% saline)						
		Liver	Kidney	Heart	WAT	Lung	Pancreas	Spleen
PBS	-	100	100	100	100	100	100	100
549144	25	72	83	99	92	38	104	35
645938	10	250	928	253	141	65	354	110
645938	25	336	1054	219	226	117	87	108

Plasma chemistry markers

To evaluate the effect of treatment with ISIS oligonucleotides on plasma levels of various biomarkers of liver and kidney function, plasma levels of liver transaminases (ALT and AST), total cholesterol (CHOL), creatinine (CRE), HDL, LDL, triglycerides (TRIG), BUN, non-esterified fatty acids (NEFA), 3HB were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY).

Overall, the plasma chemistry results indicate that ISIS 645938 is tolerable in ob/ob mice.

Table 31

Plasma markers of ob/ob mice treated with ASOs

Treatment	Dose (mg/kg)	ALT (U/L)	AST (U/L)	CHOL (mmol/L)	CRE (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TRIG (mmol/L)	BUN (mg/dL)	NEFA (mEq/L)	3HB (mmol/L)
PBS	-	487.8	440.8	230.8	0.24	191.5	60.1	80.0	24.1	0.81	364.2
549144	25	342.4	309.5	224.5	0.21	190.6	48.8	71.4	23.6	0.85	292.0
645938	10	515.8	562.3	269.6	0.20	205.9	67.4	89.8	20.5	0.96	178.7
645938	25	451.5	632.0	269.1	0.18	200.7	63.3	101.3	19.5	0.95	153.6

Kidney biomarker analysis

Kidney Injury Molecule-1 (KIM-1) and Neutrophil Gelatinase-Associated Lipocalin (NGAL) are biomarkers used to detect renal tubule injury. At the end of the treatment period, RNA was extracted from kidney for quantitative real-time PCR analysis of mRNA expression of KIM-1 and NGAL. Murine KIM-1 primer probe set RTS1475 (Forward Sequence: TGGTTGCCTTCCGTGTCTCT, designated herein as SEQ ID NO: 33; Reverse Sequence: TCAGCTCGGGAATGCACAA, designated herein as SEQ ID NO: 34; Probe Sequence: AGATTGAAGCTTTGCAGAACGCAGCGX, designated herein as SEQ ID NO: 35, wherein X is a label) was used to measure KIM-1 mRNA levels. Murine NGAL (LCN2) primer probe set mLcn2_LTS00362 (Forward Sequence: GGCCTCAAGGACGACAACA, designated herein as SEQ ID NO: 36; Reverse Sequence: ACCACCCATTCAGTTGTCAATG, designated herein as SEQ ID NO: 37; Probe Sequence: CATCTTCTCTGTCCCCACCGACCAAX, designated herein as SEQ ID NO: 38, wherein X is a label) was used to measure NGAL mRNA levels. Results are presented as percent of KIM-1 or NGAL, relative to PBS control, normalized against Cyclophilin A.

The results show a lack of significant increase in the biomarkers KIM-1 and NGAL and indicate that KEAP1 ASO was well tolerated in the kidney.

Table 32

Kidney biomarkers in ob/ob mice treated with KEAP1 ASO

Treatment	Dose (mg/kg)	KIM-1 (% saline)	NGAL (% saline)
549144	25	105	83
645938	10	162	110
645938	25	233	118

Body and organ weights

Body weights were measured weekly. Kidney, liver, spleen and white adipose tissue (WAT) weights were measured at the end of the study and are presented as percent body weight (%BW) in the table below. Results showing a lack of significant increases in the body or organ weights in KEAP1 ASO treated mice indicate that ASO treatment is tolerable for the mice.

Table 33

Body weight of ob/ob mice treated with KEAP1 ASO

Treatment	Dose (mg/kg)	Body Weight (g)
PBS	-	59.3
549144	25	60
645938	10	52.8
645938	25	50.9

Table 34

Organ weights of ob/ob mice treated with KEAP1 ASO

Treatment	Dose (mg/kg)	Liver (%BW)	Kidney (%BW)	Spleen (%BW)	WAT (%BW)
PBS	-	7.29	0.70	0.23	5.60
549144	25	7.75	0.73	0.26	5.23
645938	10	9.51	0.82	0.19	4.55
645938	25	11.13	0.96	0.19	4.93

Glucose Tolerance Test (GTT)

An oral glucose tolerance test (OGTT) was performed at weeks 4 and 9 in the ob/ob mice. The mice were fasted for sixteen hours and were administered 1 gram of glucose per kg body weight, and blood was drawn at various timepoints using a One Touch II™ glucose monitor. At week 9, KEAP1 ASO treated mice were able to clear glucose at a higher rate than control treated mice, indicating an improvement in glucose tolerance with KEAP1 ASO treatment.

Table 35

Week 4 OGTT: Blood glucose levels (mg/dL) in ob/ob mice treated with KEAP1 ASO

Treatment	Dose (mg/kg)	Time post glucose challenge (minutes)				
		0 min	30 min	60 min	90 min	120 min
PBS	-	135	453	265	232	233
549144	25	137	391	236	231	227
645938	10	183	357	282	236	234
645938	25	171	408	244	221	211

Table 36

Week 9 OGTT: Blood glucose levels (mg/dL) in ob/ob mice treated with KEAP1 ASO

Treatment	Dose (mg/kg)	Time post glucose challenge (minutes)					
		0 min	15 min	30 min	60 min	90 min	120 min
PBS	-	138	451	400	242	222	194
549144	25	153	402	337	218	174	177
645938	10	130	387	287	198	181	156
645938	25	122	368	269	181	186	152

Glucose and insulin

After being fasted for 5-hours, a tail bleed was performed on the mice to measure glucose and insulin levels at weeks 5 and 8. KEAP1 ASO decreased both insulin and glucose as shown in the table below with the data suggesting an improvement in insulin sensitivity.

Table 37

Plasma levels of insulin (ng/mL) and glucose (mg/dL) in ob/ob mice treated with KEAP1 ASO

Treatment	Dose (mg/kg)	Insulin		Glucose	
		Week 5	Week 8	Week 5	Week 8
PBS	-	20.05	26.56	301	308
549144	25	33.46	26.01	236	240
645938	10	18.19	20.16	299	321
645938	25	21.65	12.41	201	211

Example 8: KEAP1 ASO comparison with bardoxolone methyl in wildtype mice

Bardoxolone methyl (RTA 402) is a small molecule inhibitor of the NF-κB pathway and an activator of the Nrf2 pathway. The drug was being tested in a Stage 3 clinical trial for kidney disease, however, the trial was ended early when a higher than expected incidence of cardiovascular adverse events was observed in the drug arm of the trial.

The effect of KEAP1 ASO treatment was compared to the effect of bardoxolone methyl treatment in a 6-week head-to-head study in C57 wildtype mice fed a normal chow diet (D12451 Research Diets, New Brunswick, New Jersey).

Treatment

C57BL/6 wildtype mice were divided into treatment groups consisting of four animals. Antisense oligonucleotide treated groups were injected subcutaneously once a week for 6 weeks with either 25 or 40 mg/kg of ISIS 549144 control oligonucleotide, 25 or 40 mg/kg of ISIS 645938, 25 or 40 mg/kg of ISIS 645958, and one group of four mice was injected with PBS as a control to which the antisense oligonucleotide treated groups are compared. Bardoxolone methyl (RTA 402) treated groups were given 2.5 or 10 mg/kg by oral gavage 5 days per week for 6 weeks, and one group of four mice was given a vehicle control (Cremophor EL:DMSO:PBS (1:1:8) mixture) by oral gavage as a control to which the bardoxolone methyl treated groups are compared. The mice were euthanized 24 hours after the last dose; liver and heart were harvested for further analysis.

KEAP1 RNA analysis

At the end of the treatment period, RNA was extracted from liver and heart for quantitative real-time PCR analysis of mRNA expression of KEAP1. Murine KEAP1 primer probe set RTS4398_MGB (forward sequence AAGGTCATGGAAAGGCTTATTGAGT, designated herein as SEQ ID NO: 12; reverse sequence GTTCATCACGTGCAGGACACA, designated herein as SEQ ID NO: 13; probe sequence TCTCCGTGGGCGAGAA, designated herein as SEQ ID NO: 14) was used to measure mRNA levels. Results are presented in the table below as percent of KEAP1 mRNA, relative to PBS or vehicle control, normalized against Cyclophilin A.

Treatment with KEAP1 ASO resulted in significant dose-dependent reductions of KEAP1 mRNA expression in the liver and heart compared to the ASO control (ISIS 54944) and PBS control as shown in the table below. Treatment with bardoxolone methyl resulted in an increase of KEAP1 mRNA expression in the liver and a decrease in the heart compared to the vehicle control as shown in the table below.

Table 38

Expression of KEAP1 in wildtype mice treated with KEAP1 ASO or bardoxolone methyl

Treatment	Dose (mg/kg)	Liver	Heart
549144 (% PBS)	25	108.7	80.2
549144 (% PBS)	40	95.5	68.9
645938 (% PBS)	25	17.8	64.0
645938 (% PBS)	40	8.9	53.2
645958 (% PBS)	25	31.4	78.4
645958 (% PBS)	40	21.3	63.6
RTA 402 (% vehicle)	2.5	156.9	97.9
RTA 402 (% vehicle)	10	135.6	91.5

NQO1 RNA analysis

At the end of the treatment period, RNA was extracted from liver and heart for quantitative real-time PCR analysis of mRNA expression of NQO1. Murine NQO1 primer probe set mNQO1_LTS00751 (forward sequence CCTGGAAGGATGGAAGAAACG, designated herein as SEQ ID NO: 18; reverse sequence CAGGCTGCTTGGAGCAAAAT, designated herein as SEQ ID NO: 19; probe sequence AAACCGTCTGGGAGGAGACCCCACTX, designated herein as SEQ ID NO: 20, wherein X is a label) was used to measure mRNA levels. Results are presented in the table below as percent of NQO1, relative to PBS control, normalized against Cyclophilin A.

Treatment with both the KEAP1 ASO and bardoxolone methyl resulted in significant dose-dependent increases in NQO1 mRNA expression in the liver in comparison to the control ASO, the vehicle control or PBS.

Treatment with bardoxolone methyl, but not with the KEAP1 ASO, resulted in significant dose-dependent increases in NQO1 mRNA expression in the heart in comparison to the control ASO, the vehicle control or PBS.

Table 39

Expression of NQO1 in wildtype mice treated with KEAP1 ASO or bardoxolone methyl

Treatment	Dose (mg/kg)	Liver	Heart
549144 (% PBS)	25	98.1	71.3
549144 (% PBS)	40	66.7	62.9
645938 (% PBS)	25	537.8	79.3
645938 (% PBS)	40	912.2	83.6
645958 (% PBS)	25	220	99.3
645958 (% PBS)	40	442.63	76.1
RTA 402 (% vehicle)	2.5	830.9	200.4
RTA 402 (% vehicle)	10	1067.8	271.3

GSTA1 RNA analysis

At the end of the treatment period, RNA was extracted from liver and heart for quantitative real-time PCR analysis of mRNA expression of GSTA1. Murine GSTA1 primer probe set mGsta1_LTS34766 (forward sequence CCCCTTTCCCTCTGCTGAAG, designated herein as SEQ ID NO: 15; reverse sequence TGCAGCTTCACTGAATCTTGAAAG, designated herein as SEQ ID NO: 16; probe sequence TTCCTTGCTTCTTGAATTTGTTTTGCATCCAT, designated herein as SEQ ID NO: 17) was used to measure mRNA levels. Results are presented in the table below as percent of GSTA1, relative to PBS or vehicle control, normalized against Cyclophilin A.

Treatment with both the KEAP1 ASO and bardoxolone methyl resulted in significant dose-dependent increases in GSTA1 mRNA expression in the liver in comparison to the control ASO, the vehicle control or PBS.

Treatment with bardoxolone methyl, but not with the KEAP1 ASO, resulted in significant dose-dependent increases in GSTA1 mRNA expression in the heart in comparison to the control ASO, the vehicle control or PBS.

Table 40

Expression of GSTA1 in wildtype mice treated with KEAP1 ASO or bardoxolone methyl

Treatment	Dose (mg/kg)	Liver	Heart
549144 (% PBS)	25	84	71.8
549144 (% PBS)	40	49.3	63.1
645938 (% PBS)	25	1560.8	71.8
645938 (% PBS)	40	2883.9	78.1
645958 (% PBS)	25	636.4	79.2
645958 (% PBS)	40	1892.2	112.9
RTA 402 (% vehicle)	2.5	1087.1	483.6
RTA 402 (% vehicle)	10	1817.3	615.0

As the data show, KEAP1 ASO treatment impacts the heart less than bardoxolone methyl treatment. Thus, KEAP1 ASO treatment may not have adverse effects on the heart such as those seen after bardoxolone methyl treatment.

Example 9: ASO inhibition of KEAP1 in a bleomycin-induced lung fibrosis model

Pulmonary fibrosis is a component of many interstitial lung diseases, including idiopathic pulmonary fibrosis, a chronic, progressive disease for which there is currently no effective therapy. Bleomycin has been widely used in rodents to model pulmonary fibrosis for the study of mechanisms involved in fibrogenesis and for evaluation of potential therapies (*Curr. Protoc. Pharmacol.* 40:5.46.1-5.46.17. © 2008 by John Wiley & Sons, Inc.).

Treatment

8- to 10-week old male C57Bl6 mice, weighing approximately 20 g, were fed a normal chow diet and divided into treatment groups consisting of twenty animals. 10 mg/kg of ISIS 645938 was locally administered to the mouse lung by intratracheal administration twice a week starting two weeks before the first challenge with bleomycin. ISIS 549148 was used as an ASO control, and saline alone served as a vehicle control to which the ASO-treated groups were compared. At the end of two weeks, the mice undergoing treatment with ASOs and the saline control group received a single intratracheal administration of bleomycin at 5U/kg in 50 μl of PBS. Another group of 16 "naïve" mice was not challenged with ASO or bleomycin. The groups are further defined in the table below.

Table 41

Groups of mice

Group#	ASO	Bleomycin
1	No	No
2	No	Yes
3	645938 (Keap1)	Yes
4	549148 (control)	Yes

Body weights and survivial rates of the mice were regularly monitored. The assays are described below and demonstrate the tolerability of treatment of the mice with antisense oligonucleotides targeting KEAP1.

Body weights

Body weights of the mice were regularly measured. Results are presented in the table below. Treatment with the KEAP1 ASOs did not result in any adverse change in weight in comparison to the naive control group.

Table 42

Body weights

Group#	ASO	Bleomycin	Day 7	Day 17	Day 30
1	No	No	27	29	29
2	No	Yes	26	25	22
3	645938 (Keap1)	Yes	26	25	26
4	549148 (control)	Yes	26	25	26

Survival Rate

The survival rates of the mice were regularly monitored. Results are presented in the table below. Treatment with the KEAP1 ASOs did not result in any adverse effect on survival compared to the naive control group.

Table 43

Survival rate (% of naïve control)

Group#	ASO	Bleomycin	Day 17	Day 24	Day 30
1	No	No	100	100	100
2	No	Yes	100	100	95
3	645938 (Keap1)	Yes	100	100	100
4	549148 (control)	Yes	100	94	88

Several endpoints were measured in the lungs to examine the effects of targeting KEAP1. Mice were perfused with 10 mL of cold PBS through the right ventricle. After the lung was cleared of blood, 1 mL of cold William E buffer (ThermoFisher Scientific) with 1 mg/mL Pronase (Roche) was injected into trachea until the lung was inflated. After 30 min of incubation at 37 °C, the lung was removed, minced, and incubated in William E buffer with 0.2 mg/mL DNase I (Roche), 2 mg/mL collagenase (Roche), 0.1 U/mL elastase (Roche) for 10 min. After a slow speed spin (2,000 rpm for 10 min), the cell pellet was washed twice with Wash buffer (cold PBS, pH 7.2 with 0.2 mg/mL DNase I) and resuspended in 2.3 mL Wash buffer. The cell suspension was layered on top of 7% (w/v) OptiPrep gradient (Sigma-Aldrich) and centrifuged at 3,000 rpm for 25 min using slow acceleration and deceleration modes. Cell fractions at 7%, 10%, 16%, and 40% (w/v) were collected and diluted into cold Wash buffer. After centrifugation at 2,000 rpm for 10 min, the cell pellet was dissolved in RLT buffer (Qiagen) with 1% 2-mercaptoethanol (Sigma-Aldrich), and RNA was purified. Target mRNA knockdown of KEAP1 was measured in addition to the effect on various fibrosis markers (mRNA). Differential cell counts of inflammatory cells in the lung were performed on the bronchoalveolar lavage (BAL) fluid to evaluate the effects of the ASOs targeting murine KEAP1 on cell recruitment to the airways.

RNA analysis

At the end of the treatment period, RNA was extracted from lung tissue for quantitative real-time PCR analysis of RNA expression for KEAP1. Murine KEAP1 primer probe set RTS4398_MGB was used to measure RNA levels. Results are presented in the table below as percent inhibition of KEAP1, relative to PBS control, normalized against RIBOGREEN®. There was a 75% reduction of KEAP1 mRNA in the lungs of the mice group treated with KEAP1 ASO in comparison to the control group.

Markers for fibrosis, such as collagen-1, SMA, Timp1, and TGFβ, were also measured. The results are presented below and demonstrate reduction in several markers of fibrosis aftrer treatment with KEAP1 ASO.

Table 44

Levels of fibrosis markers (% of naïve control)

Group#	ASO	Bleomycin	Collagen-1	SMA	Timp1	TGFβ
1	No	No	100	100	100	100
2	No	Yes	561	190	5012	5012
3	645938 (Keap1)	Yes	167	201	701	701
4	549148 (control)	Yes	276	173	1293	1293

Example 10: ASO inhibition of KEAP1 in a western diet model

ASOs described in the studies above were evaluated for their effect on C57BL/6 mice fed with a high fat/high cholesterol diet.

Treatment

C57BL/6 mice were fed a high fat/high cholesterol diet (Diet 88137; 42% Kcal from fat) for 5 months prior to dosing. The mice were then divided into treatment groups consisting of 6-9 animals each based on body weight, plasma ALT, insulin, glucose, leptin, and albumin. Mice were injected subcutaneously once a week for 12 weeks with 25 mg/kg of ISIS 549144 control oligonucleotide, or with 25 mg/kg or 40 mg/kg of ISIS 645938, and one group of were injected with PBS as a control to which the antisense oligonucleotide treated groups are compared. Several clinical endpoints were measured over the course of the study.

KEAP1 RNA analysis

At the end of the treatment period, RNA was extracted from liver, kidney, heart, white adipose tissue (WAT), lung, muscle, and pancreas for quantitative real-time PCR analysis of mRNA expression of KEAP1. Results are presented as percent of KEAP1, relative to PBS control, normalized against Cyclophilin A. ISIS 645938 significantly reduced Keap1 expression in the multiple tissues in a dose responsive manner.

Table 45

% inhibition (compared to control) of Keap1 mRNA expression

Treatment	Dose (mg/kg)	KEAP1 mRNA (% saline)						
		Liver	Kidney	Heart	WAT	Lung	Muscle	Pancreas
549144	25	0	0	0	0	0	0	0
645938	25	91	73	66	68	61	71	3
645938	40	94	78	77	70	72	81	10

NQO1 RNA analysis

At the end of the treatment period, RNA was extracted from heart, liver, kidney, white adipose tissue (WAT), lung, and muscle for quantitative real-time PCR analysis of mRNA expression of NQO1. Results are presented as percent of NQO1, relative to PBS control, normalized against Cyclophilin A. ISIS 645938 was shown to upregulate NQO1 expression in multiple tissues. The result shows that ASO inhibition of KEAP1 decreases KEAP-mediated repression of Nrf2 activity and allows expression of Nrf2 downstream genes such as NQO1.

Table 46

% increase in expression of NQO1

Treatment	Dose (mg/kg)	KEAP1 mRNA (% saline)						
		Liver	Kidney	Heart	WAT	Lung	Muscle	Pancreas
549144	25	0	0	0	0	0	0	0
645938	25	504	442	6	222	165	46	134
645938	40	492	431	-8	364	202	24	219

GSTA1 RNA analysis

At the end of the treatment period, RNA was extracted from liver, kidney, heart, white adipose tissue (WAT), lung, muscle, and pancreas for quantitative real-time PCR analysis of mRNA expression of GSTA1. Results are presented as percent of GSTA1, relative to PBS control, normalized against Cyclophilin A. ISIS 645938 was shown to upregulate GSTA1 expression.

Table 47

% increase in expression of GSTA1

Treatment	Dose (mg/kg)	KEAP1 mRNA (% saline)						
		Liver	Kidney	Heart	WAT	Lung	Muscle	Pancreas
549144	25	0	0	0	0	0	0	0
645938	25	641	958	30	282	17	542	23
645938	40	463	1142	22	647	32	382	29

Body and organ weights

Body weights were measured weekly. Kidney, liver, spleen and white adipose tissue (WAT) weights were measured at the end of the study and are presented as percent body weight (%BW) in the table below. Results indicate that treatment with Keap1 antisense oligonucleotide reduced the body weight in the mice.

Table 48

Body weight (g) of mice fed a western diet

Treatment	Dose (mg/kg)	Week 1	Week 5	Week 9	Week 12
549144	25	50	49	53	52
645938	25	50	45	47	46
645938	40	48	42	43	40

Table 49

Organ weights of mice fed a western diet

Treatment	Dose (mg/kg)	Liver (%BW)	Kidney (%BW)	Spleen (%BW)	WAT (%BW)
549144	25	9.2	0.9	0.3	4.2
645938	25	10.3	1.1	0.6	4.9
645938	40	10.2	1.3	0.8	3.6
Non-treated	-	8.9	0.9	0.4	3.9

Plasma chemistry markers

To evaluate the effect of treatment with ISIS oligonucleotides on plasma levels of various biomarkers of liver and kidney function, plasma levels of liver transaminases (ALT and AST), albumin, and bilirubin were measured at 12 weeks using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). 'n.d.' indicates no data for that mice group.

Overall, the plasma chemistry results indicate that ISIS 645938 reduced plasma ALT and albumin levels, and was tolerable in the mice.

Table 50

Plasma markers mice fed a western diet

Treatment	Dose (mg/kg)	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)	Total bilirubin (mg/dL)
549144	25	303	209	2.7	0.18
645938	25	129	221	2.4	0.09
645938	40	113	240	2.1	0.07
Non-treated	-	454	368	2.9	n.d.

Intraperitoneal Glucose tolerance test (GTT)

To assess insulin sensitivity in the mice, IPGTT was performed to measure the clearance of an intraperitoneally injected glucose load from the body. Mice were fasted for approximately 16 hours and fasted blood glucose levels were measured before intraperitoneal injection of a solution of glucose. Blood glucose levels were measured at different time points for 2 hours. The test was performed at week 5 and week 10. In both instances, KEAP1 ASO treated mice were able to clear glucose at a higher rate than control treated mice, indicating an improvement in insulin sensitivity with KEAP1 ASO treatment. The Area Under the Curve (AUC) for both weeks is also presented and demonstrates improvement in insulin sensitivity in this model.

Table 51

Blood glucose levels (mg/dL) at week 10 in mice fed a western diet

Treatment	Dose (mg/kg)	0 min	15 min	30 min	60 min	90 min	120 min
549144	25	144	288	291	280	271	264
645938	25	163	284	288	238	186	166
645938	40	142	282	273	223	174	158

Table 52

IPGTT AUC in mice fed a western diet

Treatment	Dose (mg/kg)	5 week	10 week
549144	25	25448	32429
645938	25	19803	27151
645938	40	21480	28180

Hepatic lipid Accumulation

To assess the effect on lipid accumulation in the liver of the mice, levels of triglycerides, free fatty acids, cholesterol ester, and free cholesterol in the liver were measured. The data is presented in the table below. KEAP1 ASO treated mice had significantly reduced levels of liver lipid accumulation.

Table 53

Liver lipid levels (mg/g liver) in mice fed a western diet

Treatment	Dose (mg/kg)	Triglycerides	Free cholesterol	Free Fatty Acids	Cholesterol Ester
549144	25	191	2.23	9.32	31
645938	25	75	1.55	2.45	7
645938	40	27	1.23	0.56	2

Hepatic lipogenic gene expression

To assess the effect on lipogenic gene expression in the liver of the mice, gene expression levels of FAS, SREBP1c, and ACC1 in the liver were measured. The data is presented in the table below. KEAP1 ASO treated mice had significantly reduced mRNA expression levels of all these lipogenic genes, indicating reduced lipid generation.

Table 54

% inhibition of lipogenic gene expression (compared to control) in the liver

Treatment	Dose (mg/kg)	FAS	SREBP1c	ACC1
549144	25	0	0	0
645938	25	58	50	44
645938	40	69	66	64

Expression levels of hepatic fibrosis markers

To assess the effect on fibrosis in the liver of the mice, gene expression levels of COL1a1, COL1a2, αSMA, TGFβ, TIMP1, and TIMP2 in the liver were measured. Protein levels of fibrosis markers, TIMP1, glutathione, and hydroxyproline were also measured. The data is presented in the tables below. KEAP1 ASO treated mice had significantly reduced mRNA levels of all the fibrosis markers indicating reduction in fibrosis in the liver. The levels of glutathione, an important antioxidant, are increased in these mice, while TIMP1 and hydroxyproline levels were reduced, confirming that antisense inhibition of KEAP1 reduced hepatic fibrosis in this model.

Table 55

% inhibition of gene expression of fibrosis markers (compared to control) in the liver

Treatment	Dose (mg/kg)	COL1a1	COL1a2	αSMA	TGFβ	TIMP1	TIMP2
549144	25	0	0	0	0	0	0
645938	25	63	55	18	41	65	63
645938	40	74	70	18	50	74	70

Table 56

Protein expression of fibrosis markers in the liver

Treatment	Dose (mg/kg)	TIMP1 (pg/mg liver protein)	Glutathione (μmol/g protein)	Hydroxyproline (μmol/g protein)
549144	25	49.7	11.2	1.9
645938	25	27.9	30.6	1.3
645938	40	23.1	28.0	1.0

What is claimed is:

1. A method of reducing a disease, disorder, or condition related to oxidative stress in an animal comprising administering a therapeutically effective amount of a compound comprising a KEAP1 specific inhibitor to the animal, thereby reducing oxidative stress in the animal.
2. A method of treating, preventing, delaying or ameliorating a disease, disorder or condition related to oxidative stress in an animal comprising administering a therapeutically effective amount of a compound comprising a KEAP1 specific inhibitor to the animal, thereby preventing, delaying or ameliorating the disease, disorder or condition related to oxidative stress in the animal.
3. A method of treating an animal at risk for a disease, disorder or condition related to oxidative stress, comprising administering to said animal a therapeutically effective amount of a compound comprising a KEAP1 specific inhibitor, wherein the animal at risk for the disease, disorder or condition related to oxidative stress is treated.
4. The method of any of claims 1, 2, or 3, wherein the disease, disorder or condition is a metabolic disorder.
5. The method of claim 4, wherein the metabolic disorder is metabolic syndrome, type 2 diabetes, diabetic nephropathy, diabetic cardiopathy, or insulin resistance.
6. The method of any of claims 1, 2, or 3, wherein the disease, disorder or condition is a fatty liver disease, disorder, or condition.
7. The method of claim 6, wherein the fatty liver disease, disorder, or condition is hepatic steatosis, NASH, or NAFLD.
8. The method of any of claims 1, 2, or 3, wherein the disease, disorder or condition is an inflammatory disease.
9. The method of claim 8, wherein the inflammatory disease is asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, or airway hyperreponsiveness.
10. The method of any of claims 1, 2, or 3, wherein the disease, disorder or condition is an allergic disease.
11. The method of claim 10, wherein the allergic disease is asthma.
12. The method of any of claims 1, 2, or 3, wherein the disease, disorder or condition is a fibrotic disease.
13. The method of claim 12, wherein the fibrotic disease is pulmonary fibrosis, renal fibrosis, cystic fibrosis, or hepatic fibrosis.
14. The method of inhibiting expression or activity of KEAP1 in a cell comprising contacting the cell with a compound comprising a KEAP1 specific inhibitor, thereby inhibiting expression or activity of KEAP1 in the cell.
15. The method of claim 14, wherein the cell is in an individual.
16. The methof of claim 15, wherein the individual is human.

17. The method of any preceding claim, wherein the KEAP1 specific inhibitor is selected from any of a nucleic acid, a polypeptide, an antibody, and a small molecule.
18. The method of any preceding claim, wherein the compound comprises a modified oligonucleotide targeting KEAP1.
19. The method of claim 18, wherein the compound is single stranded.
20. The method of claim 18, wherein the compound is double stranded.
21. The method of any one of claims 18-20, wherein the modified oligonucleotide is 12 to 30 linked nucleosides in length.
22. The method of any one of claims 18-21, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage, at least one modified sugar moiety, or at least one modified nucleobase.
23. The method of claim 22, wherein the at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage, the at least one modified sugar is a bicyclic sugar or 2'-O-methoxyethyl and the at least one modified nucleobase is a 5-methylcytosine.
24. The method of claim 22 or 23, wherein at least one modified sugar comprises a 4'- $CH(CH_3)$-O-2' bridge or a 4'- $(CH_2)_n$-O-2' bridge, wherein n is 1 or 2.
25. The method of any one of claims 18-24, wherein the modified oligonucleotide comprises:

    a gap segment consisting of linked deoxynucleosides;

    a 5' wing segment consisting of linked nucleosides;

    a 3' wing segment consisting linked nucleosides;

    wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.
26. Use of a compound comprising a KEAP-1 specific inhibitor for the manufacture or preparation of a medicament for treating a disease, disorder or condition related to oxidative stress.
27. Use of a compound comprising a KEAP1 specific inhibitor for the treatment of a disease, disorder or condition related to oxidative stress.
28. The use of claim 26 or 27, wherein the disease, disorder or condition related to oxidative stress is a metabolic disorder, a fatty liver disease, disorder or condition, an inflammatory disease, an allergic disease, or a fibrotic disease.
29. The use of claim 28, wherein the metabolic disorder is metabolic syndrome, type 2 diabetes, diabetic nephropathy, diabetic cardiopathy, or insulin resistance.
30. The use of claim 28, wherein the fatty liver disease, disorder, or condition is hepatic steatosis, NASH, or NAFLD.
31. The use of claim 28, wherein the inflammatory disease is asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, or airway hyperreponsiveness.
32. The use of claim 28, wherein the allergic disease is asthma.

33. The use of claim 28, wherein the fibrotic disease is pulmonary fibrosis, renal fibrosis, cystic fibrosis, or hepatic fibrosis.
34. The use of any of claims 26-33, wherein the KEAP1 specific inhibitor is is selected from a nucleic acid, a polypeptide, an antibody, and a small molecule.
35. The use of any of claims 26-33, wherein the compound comprises a modified oligonucleotide targeted to KEAP1.
36. The use of claim 35, wherein the compound is single-stranded.
37. The use of claim 35, wherein the compound is double-stranded.
38. The use of any one of claims 35-37, wherein the modified oligonucleotide is 12 to 30 linked nucleosides in length.
39. The use of any one of claims 35-38, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage, at least one modified sugar moiety, or at least one modified nucleobase.
40. The use of claim 39, wherein the at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage, the at least one modified sugar is a bicyclic sugar or 2'-O-methyoxyethyl, and the at least one modified nucleobase is a 5-methylcytosine.
41. The use of claim 39 or 40, wherein at least one modified sugar comprises a 4'- $CH(CH_3)$-O-2' bridge or a 4'- $(CH_2)_n$-O-2' bridge, wherein n is 1 or 2.
42. The use of any one of claims 35-41, wherein the modified oligonucleotide comprises:

    a gap segment consisting of linked deoxynucleosides;

    a 5' wing segment consisting of linked nucleosides;

    a 3' wing segment consisting linked nucleosides;

    wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/022788

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61P 9/10; A61P 11/06; A61P 25/00; A61P 25/28; C07H 21/02 (2017.01)

CPC - A61K 31/353; A61K 36/16; A61K 45/06; A61K 48/00; C12N 9/0083; C12N 15/8509; G01N 2500/10 (2017.05)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 424/752; 514/296; 514/456 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2014/0256767 A1 (RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY et al) 11 September 2014 (11.09.2014) entire document	1, 4, 26 --- 5-7, 10, 11
X -- Y	US 2015/0080462 A1 (THE JOHNS HOPKINS UNIVERSITY) 19 March 2015 (19.03.2015) entire document	2, 3, 8, 9, 12-16, 27, 28, 31, 33 --- 29, 30, 32
Y	WO 2013/170068 A2 (KARYOPHARM THERAPEUTICS, INC.) 14 November 2013 (14.11.2013) entire document	5, 10, 11, 29, 32
Y	CHAMBEL et al. "The Dual Role of Nrf2 in Nonalcoholic Fatty Liver Disease: Regulation of Antioxidant Defenses and Hepatic Lipid Metabolism," 08 May 2015 (08.05.2015) Biomed Res. Int., Vol. 2015, Pgs. 1-11. entire document	6, 7, 30
A	US 2013/0298263 A1 (IWAWAKI et al) 07 November 2013 (07.11.2013) entire document	1-16, 26-33

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 May 2017	05 JUN 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/022788

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

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

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

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

3. ☒ Claims Nos.: 17-25, 34-42
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.