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(54) **Title:** MODULATION OF COPPER RELATED DISEASES BY CTR1

(57) **Abstract:** Disclosed herein are antisense compounds and methods for modulating CTR1 and modulating a copper related dis-
ease, disorder or condition in an individual in need thereof. Copper related diseases in an individual such as Wilson's disease can be
treated, ameliorated prevented with the administration of antisense compounds targeted to CTR1.



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MODULATION OF COPPER RELATED DISEASES BY CTR1

SEQUENCE LISTING

The present application is being filed along with a Sequence Listing in electronic format.

- 5 The Sequence Listing is provided as a file entitled BIOL0209WOSEQ.txt created August 29, 2013, which is 160 kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

- 10 The present invention provides methods, compounds, and compositions for modulating a copper related disease by administering a CTR1 modulator to an animal. The present invention also provides methods, compounds, and compositions for modulating Wilson's disease and symptoms thereof, by administering a CTR1 specific inhibitor to an animal.

15 BACKGROUND OF THE INVENTION

- Copper is a trace element that acts as an electron transfer agent because of its ability to donate or accept electrons through redox reactions. Thus, copper is an essential cofactor for enzymes that perform critical functions in vital physiological processes, including energy generation, detoxification of superoxide anions, iron metabolism, and neurotransmitter biosynthesis. Copper also functions in a variety of other biological processes, such as immune response, angiogenesis, cardiovascular function and signaling. Although copper is an essential micronutrient, it is highly toxic when accumulated in excess (Heejeong Kim et al., Am J Physiol Gastrointest Liver Physiol 296: G356–G364, 2009; Uauy et al., Am J Clin Nutr 67: 952S-959S, 1998). In complex organisms such as mammals, the balance between copper necessity and toxicity is achieved at both the cellular and organ level. Copper homeostasis is thought to be maintained by adjusting copper absorption in the duodenum and copper excretion in hepatic biliary processing. Copper absorption in the enterocyte is thought to be controlled by the plasma membrane copper transporter 1 (also known as CTR1, hCTR1 or SLC31A1) and the copper efflux transporter ATP7A, which moves from the Golgi to the basal lateral membrane when transferring copper for export. An imbalance of copper may lead to disease in an animal.
- 20
- 25
- 30

Various diseases related to copper imbalance have been identified. Copper overload disorders include idiopathic copper toxicosis (ICT). ICT disorders encompass hepatic copper overload disorders such as Indian childhood cirrhosis (OMIM 215600), endemic Tyrolean infantile cirrhosis (OMIM 215600), and sporadic cases occurring worldwide. ICT is characterized by liver fibrosis and eventual cirrhosis resulting from copper accumulation. Different population studies suggest that ICT displays an autosomal recessive inherited pattern (Kuo et al., 2006, J Nutr 136(1):21-26; Vonk et al., Am J Clin Nutr 2008, 88(suppl):840S-5S). In addition to a genetic predisposition, environmental factors such as high copper exposure seem to be important for the manifestation of ICT. In a variety of patients, several candidate genes, including *ATP7B*, have been excluded, but the causative genes have not yet been identified (Kuo et al., 2006, J Nutr 136(1):21-26). ICT patients, suffering from hepatic overload of copper, could benefit from a reduction in their levels of copper.

Another copper related disease is Wilson's disease (WD). WD is an autosomal recessive human genetic disease associated with toxic accumulation of copper in the liver attributable to defects of a copper-transporting P-type ATPase. In patients with WD, copper accumulates in the liver, brain, blood, kidney and cornea. The Wilson's disease gene, *ATP7B*, encodes a transmembrane protein ATPase which functions as a copper-dependent P-type ATPase responsible for incorporation of copper to ceruloplasmin and secreting access of copper to the bile. Clinical manifestations of WD include hepatic disease ranging from fibrosis, mild hepatitis, acute liver failure, cirrhosis and/or neurological symptoms. WD is fatal if untreated and early recognition by means of clinical, biochemical or genetic examination and initiation of therapy with copper chelators, zinc salts or even liver transplantation in cases of acute and chronic liver failure are essential for favorable outcome. The current treatments are lifelong with severe side effects such as neurological deterioration, hypersensitivity syndrome and bone marrow depression (Uauy et al., Am J Clin Nutr 67: 952S-959S, 1998; Subramanian et al., *Curr Neurol Neurosci Rep* 2002, 2:317-323). Like ICT patients, WD patients suffering from overload of copper could benefit from a reduction in copper accumulation.

Modulation of CTR1 may affect the amount of copper accumulated in a patient as the CTR1 family of proteins plays a critical role for copper uptake across the plasma membrane in eukaryotes ranging from yeast to humans. Characterizations of CTR1 mRNA levels in mice and humans showed ubiquitous expression with high levels in the liver and kidney. Mice completely

deleted of the CTR1 gene exhibit profound growth and developmental defects and die in utero in midgestation, demonstrating the important roles for CTR1 in embryo development. Mice in which CTR1 is deleted specifically in the liver show reduction in hepatic copper concentrations and the activities of copper-requiring enzymes are reduced (Subramanian et al., Curr Neurol Neurosci Rep 2002, 2:317-323). The human gene encoding CTR1 was identified by complementation of a yeast mutant that is defective in high-affinity copper uptake and is required for copper uptake in eukaryotic cells (Zhou and Gitschier, PNAS 94:781-7486, 1997). Two CTR1 members (CTR1 and CTR2) have been identified in the human and mouse genomes.

As CTR1 plays a critical role in copper acquisition in the liver, it may be a target for antisense therapy to treat patients with an imbalance of copper. Patients with an overload of copper, such as ICT or WD patients, could benefit from a reduction in copper accumulation induced by CTR1 targeted antisense inhibition.

SUMMARY OF THE INVENTION

Provided herein are methods, compounds, and compositions for modulating levels of CTR1 mRNA and/or protein in an animal. Provided herein are methods, compounds, and compositions for modulating levels of CTR1 mRNA and/or protein in an animal in order to modulate a copper related disease, disorder and/or condition in the animal. Also provided herein are methods, compounds, and compositions for identifying an animal having, or at risk of having, a copper related disease, disorder and/or condition and administering a therapeutically effective amount of a compound targeting CTR1 to the animal for: ameliorating the copper related disease, disorder and/or condition in the animal; treating the animal at risk for copper related disease, disorder and/or condition; inhibiting CTR1 expression in the animal suffering from the copper related disease, disorder and/or condition; reducing the risk of the copper related disease, disorder and/or condition in the animal; reducing copper accumulation in a cell, tissue and/or organ of an animal suffering from a copper related disease. In certain embodiments, the CTR1 modulator or compound targeting CTR1 is a CTR1 specific inhibitor.

In certain embodiments, CTR1 specific inhibitors decrease levels of CTR1 mRNA and/or protein. In certain embodiments, CTR1 specific inhibitors are nucleic acids, proteins, or small molecules. In certain embodiments, the nucleic acid is an antisense compound. In certain embodiments, the antisense compound is an antisense oligonucleotide. In certain embodiments,

the antisense oligonucleotide is a modified antisense oligonucleotide. In certain embodiments, the antisense oligonucleotide is complementary to a CTR1 nucleic acid as shown in any of SEQ ID NOs: 1-8.

Certain embodiments provide a method for inhibiting CTR1 expression in an animal having, or at risk of having, a copper related disease, disorder and/or condition comprising selecting the animal having, or at risk of having, the copper related disease, disorder or condition, and administering a compound comprising a CTR1 specific inhibitor to the animal, wherein the compound administered to the animal inhibits CTR1 expression in the animal having, or at risk of having, the copper related disease, disorder and/or condition.

Certain embodiments provide a method for treating an animal having, or at risk of having, a copper related disease, disorder and/or condition comprising selecting the animal having, or at risk of having, the copper related disease, disorder or condition, and administering a therapeutically effective amount of a compound comprising a CTR1 specific inhibitor to the animal, wherein the compound administered to the animal treats the animal having, or at risk of having, the copper related disease, disorder and/or condition.

In certain embodiments, an animal having or at risk for a copper related disease, disorder and/or condition is treated by selecting the animal having, or at risk of having, the copper related disease, disorder and/or condition and administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides. The modified oligonucleotide can be complementary to a CTR1 nucleic acid as shown in any of SEQ ID NOs: 1-8.

In certain embodiments, an animal having or at risk for a copper related disease, disorder and/or condition is treated by selecting the animal having or at risk for the copper related disease, disorder and/or condition and administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to a target segment or target region of SEQ ID NOs: 1-8 as described herein.

In certain embodiments, the CTR1 modulation occurs in a cell, tissue, organ or organism. In certain embodiments, the cell, tissue or organ is in an animal. In certain embodiments the cell or tissue is in the liver, brain, blood, kidney and/or cornea. In certain embodiments, the organ is a liver, brain, blood, kidney and/or cornea. In certain embodiments, the animal is a human. In

certain embodiments, CTR1 mRNA levels are reduced. In certain embodiments, CTR1 protein levels are reduced. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

Also provided are methods, compounds, and compositions useful for preventing, treating, and ameliorating copper related diseases, disorders, and conditions.

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 invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including” as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

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

Definitions

Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis. Where permitted, all patents, applications, published applications and other publications, GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout the disclosure herein are incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

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

“2’-O-methoxyethyl” (also 2’-MOE and 2’-O(CH₂)₂-OCH₃) refers to an O-methoxy-ethyl modification of the 2’ position of a furosyl ring. A 2’-O-methoxyethyl modified sugar is a modified sugar.

5 “2’-O-methoxyethyl nucleotide” means a nucleotide comprising a 2’-O-methoxyethyl modified sugar moiety.

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

10 “About” means within $\pm 10\%$ of a value. For example, if it is stated “the compounds inhibited CTR1 by at least about 70%”, it is implied that the CTR1 levels are inhibited within a range of 63% to 77%.

15 “Active pharmaceutical agent” or “pharmaceutical agent” means the substance or substances in a pharmaceutical composition that provide a therapeutic benefit when administered to an individual. For example, in certain embodiments an antisense oligonucleotide targeted to CTR1 is an active pharmaceutical agent.

“Active target region” or “target region” means a region to which one or more active antisense compounds is targeted.

“Active antisense compounds” means antisense compounds that reduce target nucleic acid levels or protein levels.

20 “Administered concomitantly” refers to the co-administration of two agents in any manner in which the pharmacological effects of both are manifest in the patient at the same time. Concomitant administration does not require that both agents be administered in a single pharmaceutical composition, in the same dosage form, or by the same route of administration. The effects of both agents need not manifest themselves at the same time. The effects need only
25 be overlapping for a period of time and need not be co-extensive.

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

30 “Amelioration” refers to a 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 of one or more indicators of a condition or disease. The severity of indicators may be determined by subjective or objective measures, which are known to those

skilled in the art. For example, amelioration of a copper related disease can be assessed by measuring the reduction of copper accumulated in a cell, tissue or organ.

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

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

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

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

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

“Alzheimer’s Disease” or “AD” is a neurodegenerative disease resulting in loss of cognitive function in a subject. Copper toxicity has been postulated to have a causative role in AD formation (Squitti, *J Trace Elem Med Biol.* 2012 June, 26(2-3):93-6; Brewer, *J Trace Elem Med Biol.* 2012 June, 26(2-3):89-92). In a rabbit model of AD, trace amounts of copper in the drinking water of the rabbits induce β -amyloid (A β) accumulation (Sparks and Schreurs, 2003, *PNAS*, 100(19):11065-11069). Curcumin analogues that chelate metals such as iron and copper have been shown to decrease metal-induced A β aggregation in vitro (Chen et al., 2011, *Bioorganic & Medicinal Chemistry* 19(18):5596-604).

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

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

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

5 “Chimeric antisense compound” means an antisense compound that has at least two chemically distinct regions.

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

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

15 “Constrained ethyl” or “cEt” refers to a bicyclic nucleoside having a furanosyl sugar that comprises a methyl(methyleneoxy) (4'-CH(CH₃)-O-2') bridge between the 4' and the 2' carbon atoms.

“Contiguous nucleobases” means nucleobases immediately adjacent to each other.

“Copper accumulation”, “increased copper” or “excess copper” means an increased level
20 of copper present in a cell, tissue and/or organ of an animal. Copper accumulation can be assessed by comparing the level of copper present in the cells, tissues and/or organs of an animal with a copper accumulation disease to the level of copper in an animal without the copper accumulation disease. Examples of copper accumulation diseases include Wilson's disease and the like.

25 “Copper accumulation inhibitor” refers to any drug that prevents copper accumulation, slows copper accumulation or reduces copper accumulation in a cell, tissue or organ. Examples of copper accumulation inhibitors include CTR1 inhibitor, metal chelator, copper absorption inhibitor, antioxidant, cholinesterase inhibitor, copper binding protein or a drug that induces production of the copper binding protein.

30 “Copper related disease, disorder and/or condition” refers to any disease, disorder or condition related to copper system in an animal. Examples of copper related diseases, disorders

and/or conditions include Wilson's disease, Menkes disease, copper toxicosis, neurological diseases (e.g., Alzheimer's Disease, Parkinson's Disease, amyotrophic lateral sclerosis (ALS) or Lou Gehrig's Disease, dementia, Huntington's Disease, schizophrenia), amyloid related diseases (e.g., Alzheimer's Disease, Parkinson's Disease, atherosclerosis) anemias, inflammatory diseases (e.g., myositis, cholangitis, hepatitis), cardiovascular diseases (e.g., atherosclerosis, stroke, peripheral vascular disease), fibrosis and cirrhosis (e.g., Indian childhood cirrhosis, endemic Tyrolean infantile cirrhosis). Some copper related diseases, disorders and/or conditions, such as Wilson's disease, may be caused by copper accumulation in the cells, tissues and/or organs of an animal.

“Copper transporter 1” and “CTR1” are used interchangeably herein. CTR1 is also known “solute carrier family 31 (copper transporters), member 1” and “SLC31A1”.

“Copper transporter 1 nucleic acid” or “CTR1 nucleic acid” means any nucleic acid encoding CTR1. For example, in certain embodiments, a CTR1 nucleic acid includes a DNA sequence encoding CTR1, a RNA sequence transcribed from DNA encoding CTR1 (including genomic DNA comprising introns and exons), and a mRNA sequence encoding CTR1. “CTR1 mRNA” means a mRNA encoding a CTR1 protein.

“CTR1 specific inhibitor” refers to any agent capable of specifically inhibiting the expression of CTR1 mRNA and/or CTR1 protein at the molecular level. For example, CTR1 specific inhibitors include nucleic acids (including antisense compounds such as RNaseH, siRNA and blockmer antisense compounds), peptides, antibodies, small molecules, and other agents capable of specifically inhibiting the expression of CTR1 mRNA and/or CTR1 protein. In certain embodiments, by specifically modulating CTR1 mRNA level and/or CTR1 protein expression, CTR1 specific inhibitors can affect components of the copper transport pathway. In certain embodiments, by specifically modulating CTR1 mRNA level and/or CTR1 protein expression, CTR1 specific inhibitors can affect copper related diseases, disorders and/or conditions such as Wilson's disease. Similarly, in certain embodiments, CTR1 specific inhibitors can affect other molecular processes in an animal.

“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 may be a liquid, e.g. saline solution.

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

“Effective amount” means the amount of active pharmaceutical agent sufficient to effectuate a desired physiological outcome in an individual in need of the agent. The effective amount 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. In an example, an effective amount of a CTR1 antisense oligonucleotide decreases copper accumulation and/or ameliorates organ damage due to copper accumulation.

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

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

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

“Identifying an animal having, or at risk for having, a copper related disease, disorder and/or condition” means identifying an animal having been diagnosed with a copper related disease, disorder and/or condition or identifying an animal predisposed to develop a copper related disease, disorder and/or condition. Individuals predisposed to develop a copper related

disease, disorder and/or condition include, for example, individuals predisposed to develop Wilson's disease. Such identification may be accomplished by any method including evaluating an individual's medical history and standard clinical tests or assessments.

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

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

“Internucleoside linkage” refers to the chemical bond between nucleosides.

“Linked nucleosides” means adjacent nucleosides which are bonded together.

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

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

“Modified nucleotide” means a nucleotide having, independently, a modified sugar moiety, modified internucleoside linkage and/or modified nucleobase. A “modified nucleoside” means a nucleoside having a modified sugar moiety and/or modified nucleobase.

“Modified oligonucleotide” means an oligonucleotide comprising a modified internucleoside linkage, a modified sugar and/or a modified nucleobase.

“Modified sugar” refers to a substitution or change from a natural sugar.

“Modulating” refers to changing or adjusting a feature in a cell, tissue, organ or organism. For example, modulating CTR1 mRNA can mean to increase or decrease the level of CTR1 mRNA and/or CTR1 protein in a cell, tissue, organ or organism. Modulating CTR1 mRNA and/or protein can lead to an increase or decrease in a copper related disease, disorder and/or condition in a cell, tissue, organ or organism. A “modulator” effects the change in the cell, tissue, organ or organism. For example, a CTR1 antisense compound can be a modulator that increases or decreases the amount of CTR1 mRNA and/or CTR1 protein in a cell, tissue, organ or organism.

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

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

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

“Nucleic acid” refers to molecules composed of monomeric nucleotides. A nucleic acid includes ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, double-stranded nucleic acids, small interfering ribonucleic acids (siRNA), and microRNAs (miRNA).

5 “Nucleobase” means a heterocyclic moiety capable of pairing with a base of another nucleic acid.

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

“Nucleoside” means a nucleobase linked to a sugar.

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

“Oligomeric compound” or “oligomer” means a polymer of linked monomeric subunits which is capable of hybridizing to at least a region of a nucleic acid molecule.

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

“Parenteral administration” means administration through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g. intrathecal or intracerebroventricular administration.

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

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

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

30 “Portion” means a defined number of contiguous (i.e. linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a

target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an antisense compound.

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

“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 oligonucleotide” means an oligonucleotide which is not hybridized to a complementary strand.

“Specifically hybridizable” refers to an antisense compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, i.e. under physiological conditions in the case of *in vivo* assays and therapeutic treatments. In an example, an antisense compound is specifically hybridizable to a target when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a loss of activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and under conditions in which assays are performed in the case of *in vitro* assays.

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

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

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

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

“Treat” refers to administering a pharmaceutical composition to an animal in order to effect an alteration or improvement of a disease, disorder, or condition in the animal. In certain
5 embodiments, one or more pharmaceutical compositions can be administered to the animal.

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

10 “Wilson disease”, “Wilson’s Disease” or “WD” is an autosomal recessive human genetic disease associated with toxic accumulation of copper in the liver attributable to defects of a copper-transporting P-type ATPase. In patients with WD, copper accumulates in the liver, brain, blood, kidney and cornea. The Wilson’s disease gene, ATP7B, encodes a transmembrane protein ATPase which functions as a copper-dependent P-type ATPase responsible for incorporation of
15 copper to ceruloplasmin and secreting access of copper to the bile. Clinical manifestations of WD include hepatic disease ranging from fibrosis, mild hepatitis, acute liver failure, cirrhosis and/or neurological symptoms. WD is fatal if untreated and early recognition by means of clinical, biochemical or genetic examination and initiation of therapy with copper chelators, zinc salts or even liver transplantation in cases of acute and chronic liver failure are essential for
20 favorable outcome.

Certain Embodiments

In certain embodiments, provided are compounds specifically modulating CTR1. In certain embodiments, the CTR1 specific modulators are CTR1 specific inhibitors for use in
25 treating, preventing, or ameliorating a copper related disease, disorder and/or condition. In certain embodiments, CTR1 specific inhibitors are nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of CTR1 mRNA and/or CTR1 protein. In certain embodiments, the CTR1 specific inhibitors are antisense compounds. In certain embodiments, the antisense compounds are
30 antisense oligonucleotides. In certain embodiments, the antisense oligonucleotides are modified antisense oligonucleotides.

In certain embodiments, the compounds target a CTR1 nucleic acid. In certain embodiments, the CTR1 nucleic acid is any of the human sequences set forth in GENBANK Accession No. NM_001859.3 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. BC061924.1 (incorporated herein as SEQ ID NO: 2), and nucleotides 45147500 to 45194000 of GENBANK Accession No. NT_008470.19 (incorporated herein as SEQ ID NO: 3). In certain embodiments, the CTR1 nucleic acid is any of the mouse sequences set forth in GENBANK Accession NM_175090.4 (incorporated herein as SEQ ID NO: 4), GENBANK Accession BC065147.1 (incorporated herein as SEQ ID NO: 5), and nucleotides 1648350 to 1683400 of GENBANK Accession No. NT_039260.7 (incorporated herein as SEQ ID NO: 6). In certain embodiments, the CTR1 nucleic acid is any of the rat sequences set forth in GENBANK Accession NM_133600.1 (incorporated herein as SEQ ID NO: 7) and nucleotides 16321050 to 16349620 of GENBANK Accession NW_047713.2 (incorporated herein as SEQ ID NO: 8).

In certain embodiments, the compounds targeting CTR1 described herein are for use in modulating CTR1 expression. In certain embodiments, the CTR1 modulators are CTR1 specific inhibitors. In certain embodiments, the CTR1 specific inhibitors decrease CTR1 expression and/or copper levels in a cell, tissue or organ. In certain embodiments, the compounds targeting CTR1 inhibit CTR1 expression by at least 70%, 75%, 80%, 85%, 90%, 95% or 100%.

Certain embodiments provide a method for inhibiting CTR1 expression in an animal having, or at risk of having, a copper related disease, disorder and/or condition comprising selecting the animal having, or at risk of having, the copper related disease, disorder or condition, and administering a compound targeting CTR1 to the animal, wherein the compound administered to the animal inhibits CTR1 expression in the animal having, or at risk of having, the copper related disease, disorder and/or condition. In certain embodiments, the compound comprises a CTR1 specific inhibitor.

Certain embodiments provide a method for treating an animal having, or at risk of having, a CTR1 related disease, disorder and/or condition comprising selecting the animal having, or at risk of having, a CTR1 disease, disorder and/or condition, and administering a therapeutically effective amount of a compound targeting CTR1 to the animal, wherein the compound administered to the animal treats the animal having, or at risk of having, the CTR1 disease, disorder and/or condition. In certain embodiments, the compound comprises a CTR1 specific inhibitor.

Certain embodiments provide a method for treating, preventing and/or ameliorating cell, tissue and/or organ damage in an animal having, or at risk of having, a copper related disease, disorder and/or condition comprising selecting the animal having, or at risk of having, a copper disease, disorder and/or condition, and administering a therapeutically effective amount of a compound targeting CTR1 to the animal, wherein the compound administered to the animal treats, prevents and/or ameliorates damage in the cell, tissue and/or organ of the animal having, or at risk of having, the copper disease, disorder and/or condition. In certain embodiments, the compound comprises a CTR1 specific inhibitor. In certain embodiments, the cell or tissue includes, but is not limited to, cell or tissue derived from liver, brain, blood, kidney and/or cornea. In certain embodiments, the organ includes, but is not limited to, liver, brain, blood, kidney and/or cornea.

In certain embodiments, the cell, tissue and/or organ damage is prevented or reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or 50%.

Certain embodiments provide a method for treating an animal having, or at risk of having, a copper related disease, disorder and/or condition comprising selecting the animal having, or at risk of having, the copper related disease, disorder or condition, and administering a therapeutically effective amount of a compound targeting CTR1 to the animal, wherein the compound administered to the animal treats the animal having, or at risk of having, the copper related disease, disorder and/or condition. In certain embodiments, the compound comprises a CTR1 specific inhibitor.

In certain embodiments, the copper related disease, disorder and/or condition is copper accumulation in cells, tissues and/or organs of the animal. In certain embodiments, the CTR1 specific inhibitor reduces copper levels in the cells, tissues and/or organs by at least 10%, 15%, 20%, 25% or 30%. In certain embodiments, the copper related disease, disorder or condition is Wilson's Disease, copper toxicosis, neurological diseases (e.g., Alzheimer's Disease, Parkinson's Disease, amyotrophic lateral sclerosis (ALS) or Lou Gehrig's Disease, dementia, Huntington's Disease, schizophrenia), amyloid related diseases (e.g., Alzheimer's Disease, Parkinson's Disease), anemias, inflammatory diseases (e.g., myositis, cholangitis, hepatitis), cardiovascular diseases (e.g., atherosclerosis, stroke, peripheral vascular disease), fibrosis and cirrhosis (e.g., Indian childhood cirrhosis, endemic Tyrolean infantile cirrhosis) and other copper related diseases, disorders and/or conditions or symptoms thereof.

In certain embodiments, a marker of the copper related disease, disorder and/or condition is selected from one or more of copper accumulation in a cell, tissue or organ of an animal and conditions or symptoms thereof. In certain embodiments, the copper accumulation occurs in, among other cells, tissues or organs, the liver, brain, blood, kidney and/or cornea of an animal.

5 In certain embodiments, the compound described herein is for use in a method for treating an animal having, or at risk for having, a copper related disease comprising selecting the animal having, or at risk for having, a copper related disease, and administering to the animal a therapeutically effective amount of the compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified antisense oligonucleotide is
10 complementary to a CTR1 nucleic acid as shown in any of SEQ ID NOs: 1-8, and wherein the compound administered to the animal treats the animal having, or at risk for having, the copper related disease. In certain embodiments, the compound comprises a CTR1 specific inhibitor.

Certain embodiments provide a method for reducing copper accumulation in a cell, organ or tissue of an animal comprising administering a compound comprising a CTR1 specific
15 inhibitor to the animal. In certain embodiments, the organ includes, but is not limited to, liver, brain, blood, kidney and cornea. In certain embodiments, the cell or tissue includes, but is not limited to, liver, brain, blood, kidney and cornea. In certain embodiments, the CTR1 specific inhibitor is an antisense oligonucleotide targeting CTR1.

Certain embodiments provide a method for reducing ALT and/or AST levels in an animal
20 comprising administering a compound comprising a CTR1 specific inhibitor to the animal. In certain embodiments, the CTR1 specific inhibitor is an antisense oligonucleotide targeting CTR1.

In certain embodiments, the compound for use in the methods comprises an antisense oligonucleotide comprising a nucleobase sequence at least 70%, at least 75%, at least 80%, at
25 least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an equal length portion of a nucleobase sequence recited in any of SEQ ID NOs: 1-8. In certain embodiments, the compound may comprise a modified oligonucleotide comprising a nucleobase sequence 100% complementary to an equal length portion of SEQ ID NOs: 1-8.

30 In certain embodiments, the compounds for use in the methods comprise an antisense oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence

comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19 or at least 20 contiguous nucleobases of a nucleobase sequence complementary to any of the sequences recited in SEQ ID NOs: 1-8.

5 In certain embodiments, the compounds for use in the methods comprise an antisense oligonucleotide consisting of 12 to 30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 linked nucleosides.

In certain embodiments, the compounds for use in the methods consist of a single-stranded modified oligonucleotide.

10 In certain embodiments, the compounds for use in the methods comprise at least one modified internucleoside linkage. In certain embodiments, the modified internucleoside linkage is a phosphorothioate internucleoside linkage. In certain embodiments, each modified internucleoside linkage is a phosphorothioate internucleoside linkage.

15 In certain embodiments, the compounds for use in the methods comprise at least one nucleoside comprising a modified sugar. In certain embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the modified sugar comprises a 2'-O-methoxyethyl (2'MOE).

In certain embodiments, the compounds for use in the methods comprise at least one nucleoside comprising a modified nucleobase. In certain embodiments, the modified nucleobase
20 is a 5-methylcytosine.

In certain embodiments, the compounds for use in the methods comprise a modified antisense oligonucleotide comprising: (i) a gap segment consisting of linked deoxynucleosides; (ii) a 5' wing segment consisting of linked nucleosides; (iii) a 3' wing segment consisting of linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between
25 the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

In certain embodiments, the compounds for use in the methods comprise a modified antisense oligonucleotide comprising: (i) a gap segment consisting of eight to sixteen linked deoxynucleosides; (ii) a 5' wing segment consisting of two to six linked nucleosides; (iii) a 3'
30 wing segment consisting of two to six linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment, wherein

each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

In certain embodiments, the compounds for use in the methods comprise a modified antisense oligonucleotide comprising: (i) a gap segment consisting of ten linked
5 deoxynucleosides; (ii) a 5' wing segment consisting of five linked nucleosides; (iii) a 3' wing segment consisting of five linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

10 In certain embodiments, the animal is a human.

In certain embodiments, administration to an animal is by a parenteral route. In certain embodiments, the parenteral administration is any of subcutaneous or intravenous administration. In certain embodiments, the compound described herein is administered to the animal once or more a day, once or more a week, once or more every two weeks, once or more every month,
15 once or more a quarter, once or more every half year, once or more every year, once or more every five years or once or more every ten years.

In certain embodiments, the compound described herein is co-administered with one or more second agent(s). The compound of the invention and one or more second agent can be administered concomitantly or sequentially.

20 In certain embodiments, the second agent can be a CTR1 specific inhibitor, metal chelator, copper absorption inhibitor, antioxidant, cholinesterase inhibitor, copper binding protein or a drug that induces production of the copper binding protein. Examples of CTR1 specific inhibitors include, but are not limited to, nucleic acids (including antisense compounds such as RNaseH, siRNA, antisense oligonucleotides and blockmer antisense compounds),
25 peptides, antibodies, small molecules, and other agents capable of specifically inhibiting the expression of CTR1 mRNA and/or CTR1 protein. Examples of metal chelators include, but are not limited to any of dimercaprol, penicillamine, trientine, phytic acid and the like. Examples of copper absorption inhibitors include tetrathiomolybdate and zinc salts. Examples of zinc salts include zinc acetate, zinc carbonate, zinc sulfate, zinc gluconate, zinc oxide, zinc chloride and
30 zinc stearate.

In certain embodiments, the compound or oligonucleotide is in salt form.

In certain embodiments, the compounds or compositions are formulated with a pharmaceutically acceptable carrier or diluent.

In certain embodiments, provided is use of a compound targeting CTR1 as described herein in the manufacture of a medicament. In certain embodiments, provided is use of a compound targeting CTR1 as described herein for treating, preventing, or ameliorating a copper related disease, disorder and/or condition as described herein. In certain embodiments, the compound is a CTR1 specific inhibitor, for use in treating, preventing, or ameliorating a copper related disease, disorder and/or condition. In certain embodiments, the CTR1 specific inhibitor is a nucleic acid (including antisense compound), peptide, antibody, small molecule, or other agent capable of inhibiting the expression of CTR1 mRNA and/or CTR1 protein. In certain embodiments, the CTR1 specific inhibitor is an antisense compound. In certain embodiments, the antisense compound is a modified antisense oligonucleotide. In certain embodiments, the antisense oligonucleotide is a modified antisense oligonucleotide. In certain embodiments, the CTR1 has a sequence as shown in any of SEQ ID NOs: 1-8.

In certain embodiments, the CTR1 specific inhibitor is used to reduce CTR1 expression. The CTR1 compound can be used in combination therapy with one or more additional agent or therapy as described herein. Agents or therapies can be administered concomitantly or sequentially to an animal.

In certain embodiments, provided is a kit for treating, preventing, or ameliorating a copper related disease and/or condition, disease, disorder or condition, wherein the kit comprises: (i) a CTR1 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 a copper related disease, disorder or condition as described herein.

Antisense Compounds

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

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

In certain embodiments, an antisense compound targeted to CTR1 nucleic acid is 10 to 30 nucleotides in length. In other words, antisense compounds are from 10 to 30 linked nucleobases. In other embodiments, the antisense compound comprises a modified oligonucleotide consisting of 8 to 80, 10 to 80, 12 to 50, 15 to 30, 18 to 24, 19 to 22, or 20 linked nucleobases. In certain such embodiments, the antisense compound comprises a modified oligonucleotide consisting of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked nucleobases in length, or a range defined by any two of the above values. In some embodiments, the antisense compound is an antisense oligonucleotide.

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

In certain embodiments, the antisense compound comprises a lengthened or long modified oligonucleotide. When a single additional nucleoside is present in a lengthened oligonucleotide, the additional nucleoside can be located at the 5' end, 3' end or central portion of the oligonucleotide. When two or more additional nucleosides are present, the added nucleosides can be adjacent to each other, for example, in an oligonucleotide having two nucleosides added to the 5' end (5' addition), to the 3' end (3' addition) or the central portion, of

the oligonucleotide. Alternatively, the added nucleoside can be dispersed throughout the antisense compound, for example, in an oligonucleotide having one or more nucleoside added to the 5' end, one or more nucleoside added to the 3' end, and/or one or more nucleoside added to the central portion.

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

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

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

25 *Antisense Compound Motifs*

In certain embodiments, antisense compounds targeted to a CTR1 nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as enhanced the inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases.

30 Chimeric antisense compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding

affinity for the target nucleic acid, and/or increased inhibitory activity. A second region of a chimeric antisense compound can optionally serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

Antisense compounds having a gapmer motif are considered chimeric antisense

5 compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain
10 embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer can in some embodiments include β -D-ribonucleosides, β -D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides can include 2'-MOE, and 2'-O-CH₃, among others), and bicyclic sugar modified nucleosides (such bicyclic
15 sugar modified nucleosides can include those having a 4'-(CH₂)_n-O-2' bridge, where n=1 or n=2). Each distinct region comprises uniform sugar moieties or comprises different types of sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5' wing region, "Y" represents the length of the gap region, and "Z" represents the length of the 3' wing region. As used herein, a gapmer described as "X-Y-Z" has
20 a configuration such that the gap segment is positioned immediately adjacent each of the 5' wing segment and the 3' wing segment. Thus, no intervening nucleotides exist between the 5' wing segment and gap segment, or the gap segment and the 3' wing segment. Any of the antisense compounds described herein can have a gapmer motif. In some embodiments, X and Z are the same, in other embodiments they are different. In some embodiments, the sugar moieties in the X
25 wing segment are the same. In some embodiments, the types of sugar moieties in the X wing segment are the different. In some embodiments, the sugar moieties in the Z wing segment are the same. In some embodiments, the types of sugar moieties in the Z wing segment are the different.

In a preferred embodiment, Y is between 8 and 15 nucleotides. X, Y or Z can be any of
30 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more nucleotides. Thus, gapmers include, but are not limited to, for example 5-10-5, 4-8-

4, 4-12-3, 4-12-4, 3-14-3, 2-13-5, 2-16-2, 1-18-1, 3-10-3, 2-10-2, 1-10-1, 2-8-2, 6-8-6, 5-8-5, 1-8-1, 2-6-2, 6-8-6, 5-8-5, 1-8-1, 2-6-2, 2-13-2, 1-8-2, 2-8-3, 3-10-2, 1-18-2, or 2-18-2.

In certain embodiments, the antisense compound as a “wingmer” motif, having a wing-gap or gap-wing configuration, i.e. an X-Y or Y-Z configuration as described above for the gapmer configuration. Thus, wingmer configurations include, but are not limited to, for example 5-10, 8-4, 4-12, 12-4, 3-14, 16-2, 18-1, 10-3, 2-10, 1-10, 8-2, 2-13, or 5-13.

In certain embodiments, antisense compounds targeted to a CTR1 nucleic acid possess a 5-10-5 gapmer motif. In certain embodiments, antisense compounds targeted to a CTR1 nucleic acid possess a 3-14-3 gapmer motif. In certain embodiments, antisense compounds targeted to a CTR1 nucleic acid possess a 2-13-5 gapmer motif.

In certain embodiments, an antisense compound targeted to a CTR1 nucleic acid has a gap-widened motif.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode human CTR1 include, without limitation, the following: GENBANK Accession No. NM_001859.3 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. BC061924.1 (incorporated herein as SEQ ID NO: 2), and nucleotides 45147500 to 45194000 of GENBANK Accession No. NT_008470.19 (incorporated herein as SEQ ID NO: 3).

Nucleotide sequences that encode mouse CTR1 include, without limitation, the following: GENBANK Accession NM_175090.4 (incorporated herein as SEQ ID NO: 4), GENBANK Accession BC065147.1 (incorporated herein as SEQ ID NO: 5), and nucleotides 1648350 to 1683400 of GENBANK Accession No. NT_039260.7 (incorporated herein as SEQ ID NO: 6).

Nucleotide sequences that encode rat CTR1 include, without limitation, the following: GENBANK Accession NM_133600.1 (incorporated herein as SEQ ID NO: 7) and nucleotides 16321050 to 16349620 of GENBANK Accession NW_047713.2 (incorporated herein as SEQ ID NO: 8).

It is understood that the sequence set forth in each SEQ ID NO in the examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, antisense compounds defined by a SEQ ID NO may comprise,

independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Antisense compounds described by Isis Number (Isis No) indicate a combination of nucleobase sequence and motif.

5 In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for CTR1 can be obtained by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In certain embodiments, a target region may
10 encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the target region.

In certain embodiments, a "target segment" is a smaller, sub-portion of a target region within a nucleic acid. For example, a target segment can be the sequence of nucleotides of a target nucleic acid to which one or more antisense compound is targeted. "5' target site" refers
15 to the 5'-most nucleotide of a target segment. "3' target site" refers to the 3'-most nucleotide of a target segment.

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

A target region may contain one or more target segments. Multiple target segments within a target region may be overlapping. Alternatively, they may be non-overlapping. In certain embodiments, target segments within a target region are separated by no more than about
25 300 nucleotides. In certain emodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more than, is no more than about, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid, or is a range defined by any two of the preceeding values. In certain embodiments, target segments within a target region are separated by no more than, or no more than about, 5 nucleotides on the target
30 nucleic acid. In certain embodiments, target segments are contiguous. Contemplated are target

regions defined by a range having a starting nucleic acid that is any of the 5' target sites or 3' target sites listed herein.

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

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

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within an active target region. In certain embodiments, reductions in CTR1 mRNA levels are indicative of inhibition of CTR1 expression. Reductions in levels of a CTR1 protein are also indicative of inhibition of target mRNA levels. Further, phenotypic changes are indicative of inhibition of CTR1 expression. For example, a decrease in copper levels can be indicative of inhibition of CTR1 expression.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a CTR1 nucleic acid. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

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

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art (Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, 3rd Ed., 2001). In certain embodiments, the antisense compounds provided herein are specifically hybridizable with a CTR1 nucleic acid.

Complementarity

An antisense compound and a target nucleic acid are complementary to each other when a sufficient number of nucleobases of the antisense compound can hydrogen bond with the
5 corresponding nucleobases of the target nucleic acid, such that a desired effect will occur (e.g., antisense inhibition of a target nucleic acid, such as a CTR1 nucleic acid).

Non-complementary nucleobases between an antisense compound and a CTR1 nucleic acid may be tolerated provided that the antisense compound remains able to specifically hybridize to a target nucleic acid. Moreover, an antisense compound may hybridize over one or
10 more segments of a CTR1 nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a CTR1 nucleic acid, a target region,
15 target segment, or specified portion thereof. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods. For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be
20 clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having 4 (four) noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present
25 invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul et al., J. Mol. Biol., 1990, 215, 403 410; Zhang and Madden, Genome Res., 1997, 7, 649 656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence
30 Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv.

Appl. Math., 1981, 2, 482-489).

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, antisense compound may be fully complementary to a CTR1 nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, “fully complementary” means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be “fully complementary” to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

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

In certain embodiments, antisense compounds that are, or are up to, 12, 13, 14, 15, 16, 17, 18, 19, or 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 CTR1 nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more

than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a CTR1 nucleic acid, or specified portion thereof.

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

Identity

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

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

Modifications

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

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

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

Modified Internucleoside Linkages

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

Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, and

phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

In certain embodiments, antisense compounds targeted to a CTR1 nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

Modified Sugar Moieties

Antisense compounds can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise chemically modified ribofuranose ring moieties. Examples of chemically modified ribofuranose rings include without limitation, addition of substituent groups (including 5' and 2' substituent groups, bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA), replacement of the ribosyl ring oxygen atom with S, N(R), or C(R₁)(R₂) (R, R₁ and R₂ are each independently H, C₁-C₁₂ alkyl or a protecting group) and combinations thereof. Examples of chemically modified sugars include 2'-F-5'-methyl substituted nucleoside (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5',2'-bis substituted nucleosides) or replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a BNA (see PCT International Application WO 2007/134181 Published on 11/22/07 wherein LNA is substituted with for example a 5'-methyl or a 5'-vinyl group).

Examples of nucleosides having modified sugar moieties include without limitation nucleosides comprising 5'-vinyl, 5'-methyl (*R* or *S*), 4'-S, 2'-F, 2'-OCH₃, 2'-OCH₂CH₃, 2'-OCH₂CH₂F and 2'-O(CH₂)₂OCH₃ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C₁-C₁₀ alkyl, OCF₃, OCH₂F, O(CH₂)₂SCH₃, O(CH₂)₂-O-N(R_m)(R_n), O-CH₂-C(=O)-N(R_m)(R_n), and O-CH₂-C(=O)-N(R₁)-(CH₂)₂-N(R_m)(R_n), where each R₁, R_m and R_n is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl.

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include without limitation nucleosides

comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to one of the formulae: 4'-(CH₂)-O-2' (LNA); 4'-(CH₂)-S-2'; 4'-(CH₂)₂-O-2' (ENA); 4'-CH(CH₃)-O-2' (also referred to as constrained ethyl or cEt) and 4'-CH(CH₂OCH₃)-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH₃)(CH₃)-O-2' (and analogs thereof see published International Application WO/2009/006478, published January 8, 2009); 4'-CH₂-N(OCH₃)-2' (and analogs thereof see published International Application WO/2008/150729, published December 11, 2008); 4'-CH₂-O-N(CH₃)-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH₂-N(R)-O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH₂-C(H)(CH₃)-2' (see Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂-C(=CH₂)-2' (and analogs thereof see published International Application WO 2008/154401, published on December 8, 2008).

Further reports related to bicyclic nucleosides can also be found in published literature (see for example: Singh *et al.*, *Chem. Commun.*, 1998, 4, 455-456; Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630; Wahlestedt *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 5633-5638; Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222; Singh *et al.*, *J. Org. Chem.*, 1998, 63, 10035-10039; Srivastava *et al.*, *J. Am. Chem. Soc.*, 2007, 129(26) 8362-8379; Elayadi *et al.*, *Curr. Opinion Invest. Drugs*, 2001, 2, 558-561; Braasch *et al.*, *Chem. Biol.*, 2001, 8, 1-7; and Orum *et al.*, *Curr. Opinion Mol. Ther.*, 2001, 3, 239-243; U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 7,034,133; 7,053,207; 7,399,845; 7,547,684; and 7,696,345; U.S. Patent Publication No. US2008-0039618; US2009-0012281; U.S. Patent Serial Nos. 60/989,574; 61/026,995; 61/026,998; 61/056,564; 61/086,231; 61/097,787; and 61/099,844; Published PCT International applications WO 1994/014226; WO 2004/106356; WO 2005/021570; WO 2007/134181; WO 2008/150729; WO 2008/154401; and WO 2009/006478. Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α -L-ribofuranose and β -D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

In certain embodiments, bicyclic sugar moieties of BNA nucleosides include, but are not limited to, compounds having at least one bridge between the 4' and the 2' position of the

pentofuranosyl sugar moiety wherein such bridges independently comprises 1 or from 2 to 4 linked groups independently selected from $-[C(R_a)(R_b)]_n-$, $-C(R_a)=C(R_b)-$, $-C(R_a)=N-$, $-C(=O)-$, $-C(=NR_a)-$, $-C(=S)-$, $-O-$, $-Si(R_a)_2-$, $-S(=O)_x-$, and $-N(R_a)-$;

wherein:

5 x is 0, 1, or 2;

n is 1, 2, 3, or 4;

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

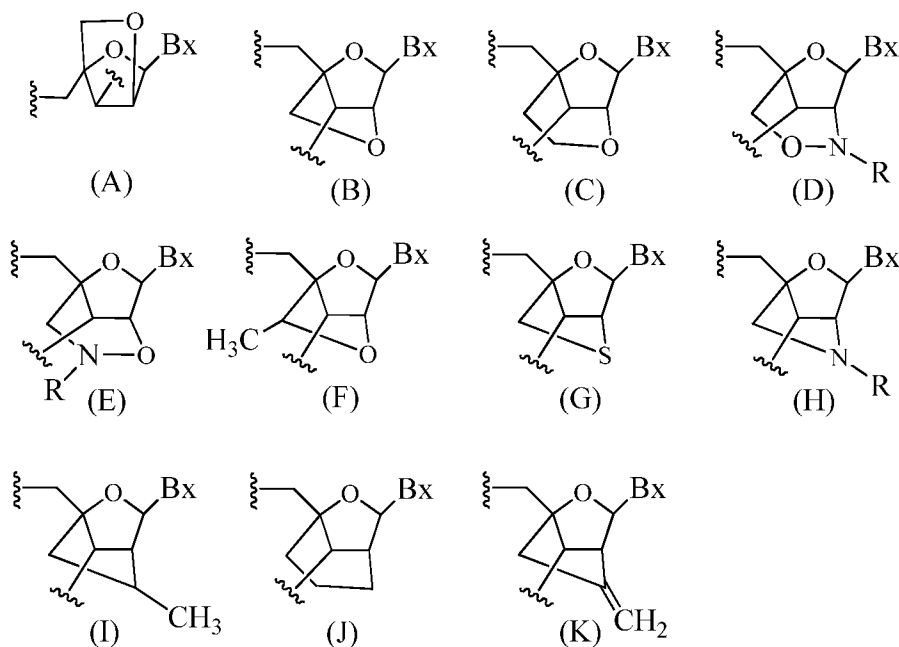
each J_1 and J_2 is, independently, H, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, acyl ($C(=O)-H$), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1 - C_{12} aminoalkyl, substituted C_1 - C_{12} aminoalkyl or a protecting group.

In certain embodiments, the bridge of a bicyclic sugar moiety is $-[C(R_a)(R_b)]_n-$, $-[C(R_a)(R_b)]_n-O-$, $-C(R_aR_b)-N(R)-O-$ or $-C(R_aR_b)-O-N(R)-$. In certain embodiments, the bridge is 4'- CH_2 -2', 4'-(CH_2)₂-2', 4'-(CH_2)₃-2', 4'- CH_2 -O-2', 4'-(CH_2)₂-O-2', 4'- CH_2 -O-N(R)-2' and 4'- CH_2 -N(R)-O-2' wherein each R is, independently, H, a protecting group or C_1 - C_{12} alkyl.

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

In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α -L-methyleneoxy (4'- CH_2 -O-2') BNA, (B) β -D-methyleneoxy (4'- CH_2 -O-2') BNA, (C) ethyleneoxy (4'-(CH_2)₂-O-2') BNA, (D) aminoxy (4'- CH_2 -O-N(R)-2') BNA, (E) oxyamino (4'- CH_2 -N(R)-O-2') BNA, and (F) methyl(methyleneoxy) (4'-CH(CH_3)-O-2') BNA, (G) methylene-thio (4'- CH_2 -S-2') BNA, (H) methylene-amino (4'- CH_2 -N(R)-2') BNA, (I) methyl

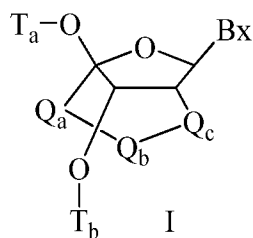
carbocyclic (4'-CH₂-CH(CH₃)-2') BNA, (J) propylene carbocyclic (4'-(CH₂)₃-2') BNA and (K) vinyl BNA as depicted below.



5

wherein Bx is the base moiety and R is independently H, a protecting group, C₁-C₁₂ alkyl or C₁-C₁₂ alkoxy.

In certain embodiments, bicyclic nucleosides are provided having Formula I:



wherein:

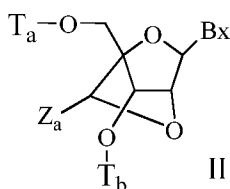
Bx is a heterocyclic base moiety;

-Q_a-Q_b-Q_c- is -CH₂-N(R_c)-CH₂-, -C(=O)-N(R_c)-CH₂-, -CH₂-O-N(R_c)-, -CH₂-N(R_c)-O- or -N(R_c)-O-CH₂;

R_c is C₁-C₁₂ alkyl or an amino protecting group; and

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

In certain embodiments, bicyclic nucleosides are provided having Formula II:



wherein:

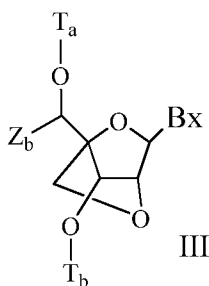
Bx is a heterocyclic base moiety;

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

Z_a is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, substituted C₁-C₆ alkyl, substituted C₂-C₆ alkenyl, substituted C₂-C₆ alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thio.

In one embodiment, each of the substituted groups is, independently, mono or poly substituted with substituent groups independently selected from halogen, oxo, hydroxyl, OJ_c, NJ_cJ_d, SJ_c, N₃, OC(=X)J_e, and NJ_eC(=X)NJ_cJ_d, wherein each J_c, J_d and J_e is, independently, H, C₁-C₆ alkyl, or substituted C₁-C₆ alkyl and X is O or NJ_c.

In certain embodiments, bicyclic nucleosides are provided having Formula III:



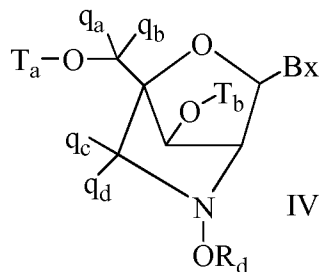
wherein:

Bx is a heterocyclic base moiety;

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

Z_b is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, substituted C₁-C₆ alkyl, substituted C₂-C₆ alkenyl, substituted C₂-C₆ alkynyl or substituted acyl (C(=O)-).

In certain embodiments, bicyclic nucleosides are provided having Formula IV:



wherein:

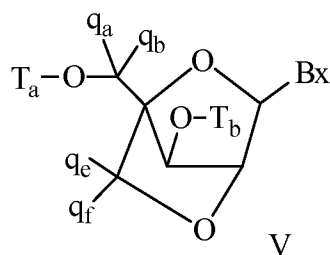
10 Bx is a heterocyclic base moiety;

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

R_d is C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl;

15 each q_a, q_b, q_c and q_d is, independently, H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, acyl, substituted acyl, C₁-C₆ aminoalkyl or substituted C₁-C₆ aminoalkyl;

In certain embodiments, bicyclic nucleosides are provided having Formula V:



wherein:

Bx is a heterocyclic base moiety;

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

q_a, q_b, q_e and q_f are each, independently, hydrogen, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy, substituted C₁-C₁₂ alkoxy, OJ_j, SJ_j, SOJ_j, SO₂J_j, NJ_jJ_k, N₃, CN, C(=O)OJ_j, C(=O)NJ_jJ_k, C(=O)J_j, O-C(=O)NJ_jJ_k, N(H)C(=NH)NJ_jJ_k, N(H)C(=O)NJ_jJ_k or N(H)C(=S)NJ_jJ_k;

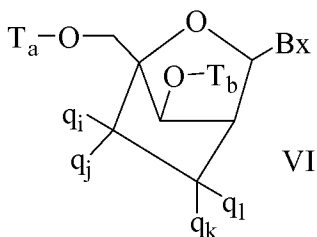
or q_e and q_f together are =C(q_g)(q_h);

q_g and q_h are each, independently, H, halogen, C₁-C₁₂ alkyl or substituted C₁-C₁₂ alkyl.

The synthesis and preparation of the methyleneoxy (4'-CH₂-O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630). BNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

Analogues of methyleneoxy (4'-CH₂-O-2') BNA and 2'-thio-BNAs, have also been prepared (Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymecopperes has also been described (Wengel *et al.*, WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel conformationally restricted high-affinity oligonucleotide analog has been described in the art (Singh *et al.*, *J. Org. Chem.*, 1998, 63, 10035-10039). In addition, 2'-amino- and 2'-methyldamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

In certain embodiments, bicyclic nucleosides are provided having Formula VI:



wherein:

Bx is a heterocyclic base moiety;

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

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

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

One carbocyclic bicyclic nucleoside having a 4'-(CH_2)₃-2' bridge and the alkenyl analog bridge 4'-CH=CH-CH₂-2' have been described (Freier *et al.*, *Nucleic Acids Research*, 1997, 25(22), 4429-4443 and Alback *et al.*, *J. Org. Chem.*, 2006, 71, 7731-7740). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (Srivastava *et al.*, *J. Am. Chem. Soc.*, 2007, 129(26), 8362-8379).

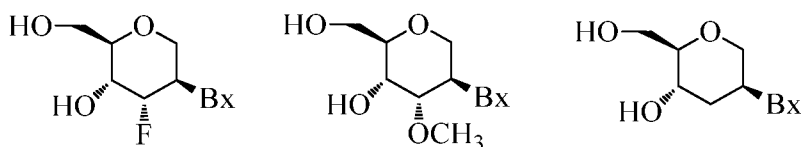
As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting two carbon atoms of the furanose ring connects the 2' carbon atom and the 4' carbon atom of the sugar ring.

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

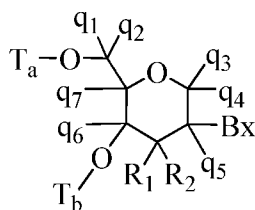
As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nF$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2'- substituent groups can also be selected from: C_1 - C_{12} alkyl, substituted alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, F, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino,

substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving pharmacokinetic properties, or a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modified nucleosides comprise a 2'-MOE side chain (Baker *et al.*, *J. Biol. Chem.*, 1997, 272, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'- *O*-methyl, *O*-propyl, and *O*-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (Martin, *Helv. Chim. Acta*, 1995, 78, 486-504; Altmann *et al.*, *Chimia*, 1996, 50, 168-176; Altmann *et al.*, *Biochem. Soc. Trans.*, 1996, 24, 630-637; and Altmann *et al.*, *Nucleosides Nucleotides*, 1997, 16, 917-926).

As used herein, a “modified tetrahydropyran nucleoside” or “modified THP nucleoside” means a nucleoside having a six-membered tetrahydropyran “sugar” substituted in for the pentofuranosyl residue in normal nucleosides (a sugar surrogate). Modified THP nucleosides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (see Leumann, *Bioorg. Med. Chem.*, 2002, 10, 841-854) or fluoro HNA (F-HNA) having a tetrahydropyran ring system as illustrated below:



In certain embodiments, sugar surrogates are selected having Formula VII:



VII

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

Bx is a heterocyclic base moiety;

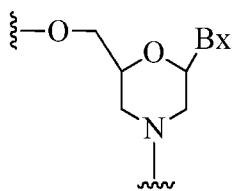
T_a and T_b are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound or one of T_a and T_b is an

internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound and the other of T_a and T_b is H, a hydroxyl protecting group, a linked conjugate group or a 5' or 3'-terminal group;

q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl; and each of R₁ and R₂ is selected from hydrogen, hydroxyl, halogen, substituted or unsubstituted alkoxy, NJ₁J₂, SJ₁, N₃, OC(=X)J₁, OC(=X)NJ₁J₂, NJ₃C(=X)NJ₁J₂ and CN, wherein X is O, S or NJ₁ and each J₁, J₂ and J₃ is, independently, H or C₁-C₆ alkyl.

In certain embodiments, the modified THP nucleosides of Formula VII are provided wherein q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each H. In certain embodiments, at least one of q₁, q₂, q₃, q₄, q₅, q₆ and q₇ is other than H. In certain embodiments, at least one of q₁, q₂, q₃, q₄, q₅, q₆ and q₇ is methyl. In certain embodiments, THP nucleosides of Formula VII are provided wherein one of R₁ and R₂ is fluoro. In certain embodiments, R₁ is fluoro and R₂ is H; R₁ is methoxy and R₂ is H, and R₁ is methoxyethoxy and R₂ 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 oligomeric compounds has been reported (see for example: Braasch *et al.*, *Biochemistry*, 2002, 41, 4503-4510; and U.S. Patents 5,698,685; 5,166,315; 5,185,444; and 5,034,506). As used here, the term “morpholino” means a sugar surrogate having the following formula:

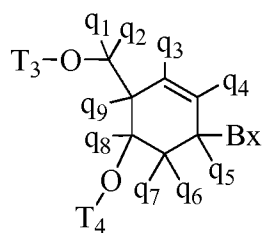


In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are referred to herein as “modified morpholinos.”

Combinations of modifications are also provided without limitation, such as 2'-F-5'-methyl substituted nucleosides (see PCT International Application WO 2008/101157 published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and further substitution at the 2'-position (see published U.S. Patent

Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a bicyclic nucleic acid (see PCT International Application WO 2007/134181, published on 11/22/07 wherein a 4'-CH₂-O-2' bicyclic nucleoside is further substituted at the 5' position with a 5'-methyl or a 5'-vinyl group). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (*see, e.g.*, Srivastava *et al.*, *J. Am. Chem. Soc.* 2007, 129(26), 8362-8379).

In certain embodiments, antisense compounds comprise one or more modified cyclohexenyl nucleosides, which is a nucleoside having a six-membered cyclohexenyl in place of the pentofuranosyl residue in naturally occurring nucleosides. Modified cyclohexenyl nucleosides include, but are not limited to those described in the art (see for example commonly owned, published PCT Application WO 2010/036696, published on April 10, 2010, Robeyns *et al.*, *J. Am. Chem. Soc.*, 2008, 130(6), 1979-1984; Horváth *et al.*, *Tetrahedron Letters*, 2007, 48, 3621-3623; Nauwelaerts *et al.*, *J. Am. Chem. Soc.*, 2007, 129(30), 9340-9348; Gu *et al.*, *Nucleosides, Nucleotides & Nucleic Acids*, 2005, 24(5-7), 993-998; Nauwelaerts *et al.*, *Nucleic Acids Research*, 2005, 33(8), 2452-2463; Robeyns *et al.*, *Acta Crystallographica, Section F: Structural Biology and Crystallization Communications*, 2005, F61(6), 585-586; Gu *et al.*, *Tetrahedron*, 2004, 60(9), 2111-2123; Gu *et al.*, *Oligonucleotides*, 2003, 13(6), 479-489; Wang *et al.*, *J. Org. Chem.*, 2003, 68, 4499-4505; Verbeure *et al.*, *Nucleic Acids Research*, 2001, 29(24), 4941-4947; Wang *et al.*, *J. Org. Chem.*, 2001, 66, 8478-82; Wang *et al.*, *Nucleosides, Nucleotides & Nucleic Acids*, 2001, 20(4-7), 785-788; Wang *et al.*, *J. Am. Chem.*, 2000, 122, 8595-8602; Published PCT application, WO 06/047842; and Published PCT Application WO 01/049687; the text of each is incorporated by reference herein, in their entirety). Certain modified cyclohexenyl nucleosides have Formula X.



X

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

Bx is a heterocyclic base moiety;

T₃ and T₄ are each, independently, an internucleoside linking group linking the cyclohexenyl nucleoside analog to an antisense compound or one of T₃ and T₄ is an internucleoside linking group linking the tetrahydropyran nucleoside analog to an antisense compound and the other of T₃ and T₄ is H, a hydroxyl protecting group, a linked conjugate group, or a 5'-or 3'-terminal group; and

q₁, q₂, q₃, q₄, q₅, q₆, q₇, q₈ and q₉ are each, independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, substituted C₂-C₆ alkynyl or other sugar substituent group.

As used herein, "2'-modified" or "2'-substituted" refers to a nucleoside comprising a sugar comprising a substituent at the 2' position other than H or OH. 2'-modified nucleosides, include, but are not limited to, bicyclic nucleosides wherein the bridge connecting two carbon atoms of the sugar ring connects the 2' carbon and another carbon of the sugar ring; and nucleosides with non-bridging 2'substituents, such as allyl, amino, azido, thio, O-allyl, O-C₁-C₁₀ alkyl, -OCF₃, O-(CH₂)₂-O-CH₃, 2'-O(CH₂)₂SCH₃, O-(CH₂)₂-O-N(R_m)(R_n), or O-CH₂-C(=O)-N(R_m)(R_n), where each R_m and R_n is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl. 2'-modified nucleosides may further comprise other modifications, for example at other positions of the sugar and/or at the nucleobase.

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

As used herein, "2'-OMe" or "2'-OCH₃" or "2'-O-methyl" each refers to a nucleoside comprising a sugar comprising an -OCH₃ group at the 2' position of the sugar ring.

As used herein, "MOE" or "2'-MOE" or "2'-OCH₂CH₂OCH₃" or "2'-O-methoxyethyl" each refers to a nucleoside comprising a sugar comprising a -OCH₂CH₂OCH₃ group at the 2' position of the sugar ring.

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

Many other bicyclo and tricyclo sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds (see for

example review article: Leumann, *Bioorg. Med. Chem.*, 2002, 10, 841-854). Such ring systems can undergo various additional substitutions to enhance activity.

Methods for the preparations of modified sugars are well known to those skilled in the art. Some representative U.S. patents that teach the preparation of such modified sugars include without limitation, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,670,633; 5,700,920; 5,792,847 and 6,600,032 and International Application PCT/US2005/019219, filed June 2, 2005 and published as WO 2005/121371 on December 22, 2005, and each of which is herein incorporated by reference in its entirety.

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

In certain embodiments, antisense compounds comprise one or more nucleosides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleosides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a bicyclic nucleoside having a (4'-CH(CH₃)-O-2') bridging group. In certain embodiments, the (4'-CH(CH₃)-O-2') modified nucleosides are arranged throughout the wings of a gapmer motif.

Modified Nucleobases

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

Y.S., Crooke, S.T. and Lebleu, B., eds., *Antisense Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278).

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

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

In certain embodiments, antisense compounds targeted to a CTR1 nucleic acid comprise one or more modified nucleobases. In certain embodiments, gap-widened antisense oligonucleotides targeted to a CTR1 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.

25 *Compositions and Methods for Formulating Pharmaceutical Compositions*

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

An antisense compound targeted to a CTR1 nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier.

In certain embodiments, the “pharmaceutical carrier” or “excipient” is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and can be selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc.).

Pharmaceutically acceptable organic or inorganic excipients, which do not deleteriously react with nucleic acids, suitable for parenteral or non-parenteral administration can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to a CTR1 nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or

indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

5 In certain embodiments, a pharmaceutical composition is prepared for administration by injection (e.g., intravenous, subcutaneous, intramuscular, etc.). In certain of such embodiments, a pharmaceutical composition comprises a carrier and is formulated in aqueous solution, such as water or physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer (e.g., PBS). In certain embodiments, other ingredients are included
10 (e.g., ingredients that aid in solubility or serve as preservatives). In certain embodiments, injectable suspensions are prepared using appropriate liquid carriers, suspending agents and the like. Certain pharmaceutical compositions for injection are presented in unit dosage form, e.g., in ampoules or in multi-dose containers.

15 *Dosing*

 In certain embodiments, pharmaceutical compositions are administered according to a dosing regimen (e.g., dose, dose frequency, and duration) wherein the dosing regimen can be selected to achieve a desired effect. The desired effect can be, for example, reduction of CTR1 or the prevention, reduction, amelioration or slowing the progression of a disease, disorder or
20 condition associated with CTR1 or a copper related disease, disorder and/or condition.

 In certain embodiments, the variables of the dosing regimen are adjusted to result in a desired concentration of pharmaceutical composition in a subject. "Concentration of pharmaceutical composition" as used with regard to dose regimen can refer to the compound, oligonucleotide, or active ingredient of the pharmaceutical composition. For example, in certain
25 embodiments, dose and dose frequency are adjusted to provide a tissue concentration or plasma concentration of a pharmaceutical composition at an amount sufficient to achieve a desired effect.

 Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is
30 effected or a diminution of the disease state is achieved. Dosing is also dependent on drug potency and metabolism. In certain embodiments, dosage is from 0.01 µg to 100mg per kg of

body weight, or within a range of 0.001mg to 1000mg dosing, and may be given once or more daily, weekly, monthly, quarterly or yearly, or even once every 2 to 20 years. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μ g to 100mg per kg of body weight, once or more daily, once or more weekly, once or more monthly, once or more quarterly, once or more yearly, to once every 20 years or ranging from 0.001mg to 1000mg dosing. In certain embodiments, it may be desirable to administer the oligonucleotide from at most once daily, once weekly, once monthly, once quarterly, once yearly, once every two years, once every three years, once every four years, once every five years, once every ten year, to once every 20 years.

Administration

The compounds or pharmaceutical compositions of the present invention can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be oral, inhaled or parenteral.

In certain embodiments, the compounds and compositions as described herein are administered parenterally. Parenteral administration includes intravenous, intra-arterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular (e.g., intracerebroventricular), administration.

In certain embodiments, parenteral administration is by infusion. Infusion can be chronic or continuous or short or intermittent. In certain embodiments, infused pharmaceutical agents are delivered with a pump.

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

In certain embodiments, formulations for parenteral, intrathecal or intraventricular administration can include sterile aqueous solutions which can also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

In certain embodiments, formulations for oral administration of the compounds or compositions can include, but is not limited to, pharmaceutical carriers, excipients, powders or

granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitables. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In certain embodiments, oral formulations are those in which compounds provided herein are administered in conjunction with one or more penetration enhancers, surfactants and chelators.

Conjugated Antisense Compounds

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

In certain embodiments, antisense compounds can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of antisense compounds to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect the antisense compound having terminal nucleic acid from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap), or at the 3'-terminus (3'-cap), or can be present on both termini. Cap structures are well known in the art and include, for example, inverted deoxy abasic caps. Further 3' and 5'-stabilizing groups that can be used to cap one or both ends of an antisense compound to impart nuclease stability include those disclosed in WO 03/004602 published on January 16, 2003.

Cell culture and antisense compounds treatment

The effects of antisense compounds on the level, activity or expression of CTR1 nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commercial vendors (*e.g.* American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and cells are cultured according to the vendor's instructions using commercially available reagents (*e.g.*

Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, HepG2 cells, Hep3B cells, and primary hepatocytes.

In vitro testing of antisense oligonucleotides

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

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

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

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

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

25 Another reagent used to introduce antisense oligonucleotides into cultured cells includes Oligofectamine™ (Invitrogen Life Technologies, Carlsbad, CA). Antisense oligonucleotide is mixed with Oligofectamine™ in Opti-MEM™-1 reduced serum medium (Invitrogen Life Technologies, Carlsbad, CA) to achieve the desired concentration of oligonucleotide with an Oligofectamine™ to oligonucleotide ratio of approximately 0.2 to 0.8 µL per 100 nM.

30 Another reagent used to introduce antisense oligonucleotides into cultured cells includes FuGENE 6 (Roche Diagnostics Corp., Indianapolis, IN). Antisense oligomeric compound was

mixed with FuGENE 6 in 1 mL of serum-free RPMI to achieve the desired concentration of oligonucleotide with a FuGENE 6 to oligomeric compound ratio of 1 to 4 μ L of FuGENE 6 per 100 nM.

Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation (Sambrook and Russell in *Molecular Cloning. A Laboratory Manual*. Third Edition. Cold Spring Harbor laboratory Press, Cold Spring Harbor, New York. 2001).

Cells are treated with antisense oligonucleotides by routine methods. Cells are typically harvested 16-24 hours after antisense oligonucleotide treatment, at which time RNA or protein levels of target nucleic acids are measured by methods known in the art and described herein (Sambrook and Russell in *Molecular Cloning. A Laboratory Manual*. Third Edition. Cold Spring Harbor laboratory Press, Cold Spring Harbor, New York. 2001). In general, when treatments are performed in multiple replicates, the data are presented as the average of the replicate treatments.

The concentration of antisense oligonucleotide used varies from cell line to cell line. Methods to determine the optimal antisense oligonucleotide concentration for a particular cell line are well known in the art (Sambrook and Russell in *Molecular Cloning. A Laboratory Manual*. Third Edition. Cold Spring Harbor laboratory Press, Cold Spring Harbor, New York. 2001). Antisense oligonucleotides are typically used at concentrations ranging from 1 nM to 300 nM when transfected with LIPOFECTAMINE2000®, Lipofectin or Cytofectin. Antisense oligonucleotides are used at higher concentrations ranging from 625 to 20,000 nM when transfected using electroporation.

RNA Isolation

RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art (Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, 3rd Ed., 2001). RNA is prepared using methods well known in the art, for example, using the TRIZOL® Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommended protocols.

Analysis of inhibition of target levels or expression

Inhibition of levels or expression of a CTR1 nucleic acid can be assayed in a variety of ways known in the art (Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, 3rd

Ed., 2001). For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymeccoppere chain reaction (PCR), or quantitaive real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time
5 PCR can be conveniently accomplished using the commercially available ABI PRISM® 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

Quantitative Real-Time PCR Analysis of Target RNA Levels

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

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

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

30 Probes and primers are designed to hybridize to a CTR1 nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as PRIMER EXPRESS® Software (Applied Biosystems, Foster City, CA).

The PCR probes can have JOE or FAM covalently linked to the 5' end and TAMRA or MGB covalently linked to the 3' end, where JOE or FAM is the fluorescent reporter dye and TAMRA or MGB is the quencher dye. In some cell types, primers and probe designed to a sequence from a different species are used to measure expression. For example, a human GAPDH primer and probe set can be used to measure GAPDH expression in monkey-derived cells and cell lines.

Gene target quantities obtained by RT, real-time PCR can be normalized using either the expression level of GAPDH, a gene whose expression is constant, or by quantifying total RNA using RiboGreenTM (Molecular Probes, Inc. Eugene, OR). GAPDH expression can be quantified by RT, real-time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA can be quantified using RiboGreenTM RNA quantification reagent (Molecular Probes, Inc. Eugene, OR).

Analysis of Protein Levels

Antisense inhibition of CTR1 nucleic acids can be assessed by measuring CTR1 protein levels. Protein levels of CTR1 can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS) (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed., 2001). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art. Antibodies useful for the detection of human and rat CTR1 are commercially available.

In Vivo Testing of Antisense Compounds

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of CTR1 and/or a copper related disease, disorder and/or condition and produce phenotypic changes such as a decrease in one or more symptoms or markers of the copper related disease. Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are

formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration, such as intraperitoneal, intravenous, and subcutaneous. Calculation of antisense oligonucleotide dosage and dosing frequency depends upon factors such as route of administration and animal body weight. In one
5 embodiment, following a period of treatment with antisense oligonucleotides, RNA is isolated from liver tissue and changes in CTR1 nucleic acid expression are measured. Changes in CTR1 protein levels can also be measured. Changes in CTR1 expression can also be measured by determining the level of copper present in a cell, tissue or organ. Copper related diseases, disorders and/or conditions may be used as markers for determining the level of CTR1
10 inhibition.

Certain Indications

In certain embodiments, the invention provides methods of treating an individual comprising administering one or more pharmaceutical compositions of the present invention. In
15 certain embodiments, the individual has, or is at risk for, a copper related disease, disorder or condition. In certain embodiments the invention provides methods for prophylactically reducing CTR1 expression in an individual. Certain embodiments include treating an individual in need thereof by administering to the individual a therapeutically effective amount of CTR1 specific inhibitor. In certain embodiments, the CTR1 specific inhibitor is an antisense compound targeted
20 to a CTR1 nucleic acid.

In certain embodiments, administration of a therapeutically effective amount of a CTR1 specific inhibitor is accompanied by monitoring of CTR1 levels in the serum, cell, tissue or organ of an individual, to determine an individual's response to administration of the CTR1 specific inhibitor. In certain embodiments, administration of a therapeutically effective amount
25 of a CTR1 specific inhibitor is accompanied by monitoring of copper levels in the serum, cell, tissue or organ of an individual, to determine an individual's response to administration of the CTR1 specific inhibitor. An individual's response to administration of the CTR1 specific inhibitor is used by a physician to determine the amount and duration of therapeutic intervention.

In certain embodiments, administration of a CTR1 specific inhibitor such as an antisense
30 compound targeted to a CTR1 nucleic acid results in reduction of CTR1 expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99% or 100% or a range defined

by any two of these values. In certain embodiments, administration of a CTR1 specific inhibitor such as an antisense compound targeted to a CTR1 nucleic acid results in a reduction of copper levels by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99% or 100% or a range defined by any two of these values. In certain embodiments, administration of a CTR1 specific inhibitor such as an antisense compound targeted to a CTR1 nucleic acid results in a change to a copper related disease, disorder, condition, symptom or marker (e.g., copper accumulation in a cell, tissue or organ). In certain embodiments, administration of a CTR1 specific inhibitor increases or decreases the copper related disease, disorder, condition, symptom or marker by at least 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% or a range defined by any two of these values.

In certain embodiments, pharmaceutical compositions comprising a CTR1 specific inhibitor such as an antisense compound targeted to CTR1 are used for the preparation of a medicament for treating a patient suffering or susceptible to a copper related disease, disorder or condition.

In certain embodiments, the methods described herein include administering a compound comprising an antisense oligonucleotide having an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobase portion complementary to CTR1.

Certain Combination Therapies

In certain embodiments, a first agent comprising a CTR1 specific inhibitor such as an antisense compound provided herein is co-administered with one or more secondary agents. In certain embodiments, the antisense compound is an antisense oligonucleotide. In certain embodiments, the antisense oligonucleotide is a modified oligonucleotide.

In certain embodiments, such second agents are designed to treat the same copper related disease, disorder or condition as the first agent described herein. In certain embodiments, such second agents are designed to treat a different disease, disorder, or condition as the first agent described herein. In certain embodiments, such second agents are designed to treat an undesired side effect of one or more pharmaceutical compositions as described herein. In certain embodiments, such first agents are designed to treat an undesired side effect of a second agent. In certain embodiments, second agents are co-administered with the first agent to treat an undesired effect of the first agent. In certain embodiments, second agents are co-administered with the first

agent to produce a combinational or additive effect. In certain embodiments, second agents are co-administered with the first agent to produce a synergistic effect.

In certain embodiments, the co-administration of the first and second agents permits use of lower dosages than would be required to achieve a therapeutic or prophylactic effect if the agents were administered as independent therapy. In certain embodiments the dose of a co-administered second agent is the same as the dose that would be administered if the second agent was administered alone. In certain embodiments the dose of a co-administered second agent is greater than the dose that would be administered if the second agent was administered alone.

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

In certain embodiments, second agents include, but are not limited to, CTR1 specific inhibitor, metal chelator, copper absorption inhibitor, antioxidant, cholinesterase inhibitor, life-style or environmental changes, copper binding protein or a drug that induces production of the copper binding protein. Examples of CTR1 specific inhibitors include, but are not limited to, nucleic acids (including antisense compounds such as RNasH, siRNA, antisense oligonucleotides and blockmer antisense compounds), peptides, antibodies, small molecules, and other agents capable of specifically inhibiting the expression of CTR1 mRNA and/or CTR1 protein. Examples of metal chelators include, but are not limited to, any of dimercaprol, penicillamine, trientine and the like. Examples of copper absorption inhibitors include, but are not limited to, any of tetrathiomolybdate and zinc salts. Examples of zinc salts include, but are not limited to, any of zinc acetate, zinc carbonate, zinc sulfate, zinc gluconate, zinc oxide, zinc chloride and zinc stearate. Examples of life-style or environmental changes include a change in diet to decrease intake of copper rich foods and monitoring of drinking water sources for copper.

The second agents can be used in combination with the therapeutic compounds described herein to decrease a copper related disease, disorder and/or condition such as Wilson's disease or Alzheimer's Disease or any copper accumulation disease, disorder or condition in the animal.

ADVANTAGES OF THE INVENTION

Provided herein are methods and compositions for the modulation of CTR1 that can treat, prevent and/or ameliorate a copper related disease, disorder and/or condition such as Wilson's disease or Alzheimer's Disease. In a particular embodiment, provided are CTR1 specific inhibitors to treat, prevent and/or ameliorate a copper related disease, disorder and/or condition or symptoms thereof as described herein. The CTR1 specific inhibitors can be antisense compounds targeting CTR1 as disclosed herein. The antisense compounds can be antisense oligonucleotides targeting a nucleic acid encoding CTR1 protein.

Currently, commercially available therapies treating copper accumulation diseases may have serious side effects (chelators) or work slowly (zinc). Chelators such as penicillamine and trientine may exacerbate neurological symptoms caused by copper accumulation or in some cases induce lupus or myasthenia in the treated subject. Zinc removes copper slowly from a subject's body, therefore, zinc is not used as a first line therapy for patients who already have symptoms when diagnosed with a copper accumulation disease. Zinc is more often used in a maintenance regimen once copper levels are reduced in a subject.

Accordingly, there is a need for additional therapeutics to decrease copper accumulation expeditiously. As shown herein, antisense compounds targeting CTR1 decrease copper levels in an animal within four weeks of administration. Further, inhibition of CTR1 reduces copper levels in multiple tissues such as liver, brain and blood. Reducing copper in the brain may ameliorate diseases that affect brain tissue such as Alzheimer's Disease. Additionally, inhibition of hepatic CTR1 can decrease liver uptake of copper from the circulation, or other source of copper, and thereby reduce liver damage. Inhibition of intestinal CTR1 can slow the progression of cell, tissue or organ damage by reducing copper intake from the diet and reducing the level of copper in the cell, tissue or organ. Accordingly, CTR1 specific inhibitors such as antisense compounds may provide superior therapeutic efficacy over the current commercial therapeutics used to treat, prevent and/or ameliorate a copper accumulation disease, disorder or condition in a subject, especially those subject suffering from Wilson's disease or Alzheimer's Disease. Also, CTR1 specific inhibitors such as antisense compounds can be used in combination with commercially available medications to treat copper related diseases in order to provide an additional therapeutic effect.

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: Selection of antisense oligonucleotides targeting solute carrier family 31 (copper transporters) member 1 (CTR1) in a multiple dose assay

Antisense oligonucleotides were targeted to a murine CTR1 nucleic acid and were tested for potency in mouse primary hepatocytes. Cells were plated at a density of 40,000 cells per well and transfected using electroporation with 437.5 nM, 875 nM, 1,750 nM, 3,500 nM, and 7,000 nM concentrations of antisense oligonucleotide. After a treatment period of approximately 16 hours, RNA was isolated from the cells and CTR1 mRNA levels were measured by quantitative real-time PCR.

ISIS 526814 (GTCTTAAATTGATTGTCCTG; SEQ ID NO: 9), which was one of the antisense oligonucleotides tested in the assay, was designed as a 5-10-5 MOE gapmer, and is 20 nucleosides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 5 nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout the gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout the gapmer are 5-methylcytosines. ISIS 526814 is targeted to nucleobases 1436 to 1455 of mouse CTR1 (GENBANK Accession No. NM_175090.4, SEQ ID NO: 4). ISIS 526814 had an IC₅₀ of 0.49 μ M in the assay.

ISIS 526887 (CCTAAGTGCCATCAATCCTG; SEQ ID NO: 10), which was another of the antisense oligonucleotides tested in the assay, was designed as a 5-10-5 MOE gapmer, and is 20 nucleosides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 5 nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing

segment has a 2'-MOE modification. The internucleoside linkages throughout the gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout the gapmer are 5-methylcytosines. ISIS 526887 is targeted to nucleobases 1179 to 1198 of mouse CTR1 (GENBANK Accession No. NM_175090.4, SEQ ID NO: 4). ISIS 526887 had an IC₅₀ of 0.52 μ M in the assay.

Example 2: *In vivo* antisense inhibition of murine CTR1

The potency of ISIS 526887 was tested in vivo in C3H wild-type (WT) mice.

Treatment

Groups of 4 C3H WT mice each were injected with 25 mg/kg/week, 50 mg/kg/week, or 100 mg/kg/week of ISIS 552887 or ISIS 526814 administered for 4 weeks. A group of mice was injected with 100 mg/kg/week of control oligonucleotide, ISIS 141923 (CCTTCCCTGAAGGTTCTCC (SEQ ID NO: 11), 5-10-5 MOE gapmer with no known murine target) administered weekly for 4 weeks. A control group of mice was injected with phosphate buffered saline (PBS) administered weekly for 4 weeks. The mice were sacrificed 48 hours after the last dose.

CTR1 RNA Analysis

After treatment with ISIS 526887, RNA was extracted from liver, kidney, and small intestinal tissues for real-time PCR analysis of CTR1, using primer probe set RTS3444 (forward sequence TCTCAAGTCAGCATTCGCTACAA, designated herein as SEQ ID NO: 12; reverse sequence CATCTGCTGCCCAACAGTTTT, designated herein as SEQ ID NO: 13; probe sequence ATGCCTGTCCCAGGACCAAATGGAACX, designated herein as SEQ ID NO: 14). As shown in Table 1, treatment with ISIS 526887 achieved significant dose-dependent reduction of murine CTR1 mRNA over the PBS control. Results are presented as percent inhibition of CTR1, relative to control. The control oligonucleotide had no effect on CTR1 levels, as expected.

RNA was extracted from liver after treatment with ISIS 526814 and also analyzed for CTR1. As shown in Table 1, treatment with ISIS 526814 also achieved significant dose-dependent reduction of murine CTR1 mRNA over the PBS control.

Table 1
Percent inhibition of murine CTR1 mRNA in C3H WT mice

Dose (mg/kg/wk)	ISIS 526887 treated Liver	ISIS 526887 treated Kidney	ISIS 526887 treated Small Intestine	ISIS 526814 treated Liver
25	58	45	63	70
50	83	51	63	85
100	94	54	75	94

Analysis of metal levels

5 The levels of copper in the liver were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) by EXOVA method (<http://www.exova.com/industry-sectors/pharmaceuticals/pharma-technical-articles/254-heavy-metals-analysis-by-icpms>; Exova, United Kingdom). As shown in Table 2, treatment with ISIS 526887 achieved significant reduction ($p < 0.005$) in copper levels compared to the PBS control.

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Table 2
Levels of liver copper ($\mu\text{g/g}$ dry weight) in C3H WT mice

Metal	PBS control	ISIS 141923	ISIS 552887
Copper	13	14	9

Example 3: Effect of antisense inhibition of murine CTR1 in a Toxic milk (Tx) mouse model

15 The potency and efficacy of ISIS 526887 was tested *in vivo* in C3HeB/FeJ-*Atp7b*^{tx-j}/J mice (also known as the Tx model). The toxic milk mouse is a commonly used mouse model for WD characterization. Tx mice have a missense mutation in the ATP7b gene, similar to the human phenotype, suffer from copper accumulation in the liver and brain, decrease in serum copper and have increased urinary copper excretion, similar to the human condition (British
20 Med. Bull. 1999 55:544; Lancet Sem. 2007 369: 397; Mamm. Genome 2001 12: 793; Biochem. 2000 352: 565).

Study 1*Treatment*

Groups of nine-week old Tx mice were injected with 100 mg/kg/week of ISIS 552887 or ISIS 141923 administered for 6 weeks. A control group of mice was injected with phosphate buffered saline (PBS) administered weekly for 6 weeks. The mice were sacrificed 48 hours after the last dose.

CTR1 RNA Analysis

RNA was extracted from liver, kidney, and intestinal tissues for real-time PCR analysis of CTR1, using primer probe set RTS3444. The levels of CTR1 mRNA expression the liver of WT mice and Tx mice treated with PBS are shown in Table 3, normalized to RIBOGREEN®. As shown in Table 4, treatment with ISIS 526887 achieved significant reduction of murine CTR1 mRNA over the PBS control. The control oligonucleotide had no effect on CTR1 levels, as expected.

Table 3
CTR1 mRNA/RIBOGREEN® in liver

	levels
WT mice	0.00017
Tx mice	0.00012

Table 4
Percent inhibition of murine CTR1 mRNA in Tx mice

ISIS No	Liver	Kidney	Small Intestine
526887	47	54	46
141923	0	4	9

Analysis of metal levels

The levels of copper in the liver were measured using ICP MS by the EXOVA method. As shown in Table 5, treatment with ISIS 526887 achieved significant reduction in copper levels compared to the PBS control.

Table 5
Levels of liver copper ($\mu\text{g/g}$ dry weight) in Tx mice

Metal	PBS control	ISIS 141923	ISIS 552887
Copper	1045	825	328

Effect on liver histopathology

5 Liver samples were embedded in paraffin, sectioned and stained with H&E (Sheehan and Hrapchak, Theory and Practice of Histotechnology, 2nd Edition; Battelle Memorial Institute, Columbus, OH, 1987; Thompson, Samuel W. Selected Histochemical and Histopathological Methods, Charles C. Thomas, Springfield, IL, 1966). The sections were reviewed by microscope. The liver section from mice treated with PBS displayed increased heterogeneity of hepatocyte
10 karyomegaly, glycogenated nuclei, and microvesicular change. The liver section from mice treated with ISIS 141923 showed a similar morphology. In comparison, the liver section from mice treated with ISIS 526887 showed noticeable improvement of uniformity and nuclear morphology. Accordingly, treatment with an antisense oligonucleotide targeting CTR1 significantly improved the liver pathology.

15

Study 2

Treatment

Groups of ten 10-week old Tx mice were injected with 100 mg/kg/week of ISIS 552887, ISIS 552814 or ISIS 141923 administered for 17 weeks. A control group of ten mice was
20 injected with phosphate buffered saline (PBS) administered weekly for 6 weeks. The mice were sacrificed 48 hours after the last dose.

CTR1 RNA Analysis

RNA was extracted from liver and kidney tissues for real-time PCR analysis of CTR1, using primer probe set RTS3444. The levels of CTR1 mRNA expression the liver of WT mice
25 and Tx mice treated with PBS are shown in Table 6, normalized to RIBOGREEN®. As shown in Table 6, treatment with either ISIS 526814 or ISIS 526887 achieved significant reduction of

murine CTR1 mRNA over the PBS control. The control oligonucleotide had no effect on CTR1 levels, as expected.

Table 6
Percent inhibition of murine CTR1 mRNA in Tx mice

ISIS No	Liver	Kidney
526814	90	62
526887	90	59
141923	0	0

5 *Analysis of metal levels*

The levels of copper in the liver, brain, and blood were measured using ICP-MS by the EXOVA method. As shown in Tables 7, 8, and 9, antisense inhibition of CTR1 achieved significant reduction ($p < 0.05$) in copper levels compared to the PBS control. 'n.d.' indicates that there was no data for that particular ASO treatment.

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Table 7
Levels of copper in old Tx mice

	PBS control	ISIS 526814	ISIS 526887	ISIS 141923
Liver ($\mu\text{g/g}$)	618	23	32	495
Brain ($\mu\text{g/g}$)	21	8	n.d.	12
Blood ($\mu\text{g/mL}$)	7	3	3	6

Effect on liver histopathology

Liver samples were embedded in paraffin, sectioned and stained with H&E. The sections were reviewed by microscope. In the liver sections of mice treated with PBS or ISIS 141923, hepatocytes showed marked hepatocellular polyploidy and hepatocellular hypertrophy (megakaryocytosis) with marked nuclear pleomorphism, often with both cytoplasmic and nuclear hypereosinophilia and foamy cytoplasm. Hepatocellular nuclei were often swollen, containing up to five large nucleoli and occasional mitotic figures and inclusions. There were frequent signs of Kupffer liver lipofuscin or hemosiderin pigment accumulation. There was multifocal karyopyknosis and karyorrhexis (hepatocellular necrosis) with associated infiltration of neutrophils and macrophages. There was swelling and proliferation of endothelial cells and fibroblasts. In contrast, liver sections from mice treated with ISIS 552814 or ISIS 552887 showed complete normalization of liver pathology.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY) (Nyblom, H. et al., Alcohol & Alcoholism 39: 336-339, 2004; Tietz NW (Ed): Clinical Guide to Laboratory Tests, 3rd ed. W. B. Saunders, Philadelphia, PA, 1995). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 8 expressed in IU/L.

Due to liver disease, Tx milk mice exhibit increased levels of ALT and AST. Treatment with both ISIS 526814 and ISIS 526887 significantly reduced ($p < 0.05$) the ALT level of the mice compared to the PBS control. The levels of ALT and AST in treated mice were comparable to that observed in standard WT mice.

Table 8
Transaminase levels (IU/L) of Tx mice

	ALT	AST
PBS control	127	142
ISIS 141923	141	178
ISIS 526814	57	117
ISIS 526887	57	124

Example 4: Effect of combination therapy of antisense inhibition of murine CTR1 and treatment with penicillamine in a Toxic milk (Tx) mouse model

Penicillamine administered to Tx mice increased free copper in various parts of the brain and increased evidence of oxidative stress (Chen, D.-B., et al., PLoS ONE 75: e37709, 2012). The effects of CTR1 ASO inhibition in combination with penicillamine was tested in vivo in Tx mice.

Treatment

A group of 18-24 week old Tx mice was injected with 50 mg/kg of ISIS 526814 administered twice weekly for 15 days (100 mg/kg/week dosage). A second group of 18-24 week old Tx mice was injected with 50 mg/kg of ISIS 526814 administered twice weekly, and were

also administered 100 mg/kg of penicillamine twice a day (200 mg/kg/day dosage) by oral gavage, for 15 days. A control group of 12 week old mice was injected with phosphate buffered saline (PBS) administered twice weekly for 15 days (PBS control group). A second control group of 12 week old mice was injected with phosphate buffered saline (PBS) administered twice weekly, and were also administered 100 mg/kg of penicillamine twice a day (200 mg/kg/day dosage) by oral gavage, for 15 days (Penicillamine control group). The mice were sacrificed 48 hours after the last dose of ISIS 526814 or 14 hours after the last dose of penicillamine.

CTR1 RNA Analysis

RNA was extracted from liver and kidney tissues for real-time PCR analysis of CTR1, using primer probe set RTS3444. The levels of CTR1 mRNA expression are shown in Table 9, normalized to RIBOGREEN®. The results indicate that treatment with ISIS 526814 achieved reduction of murine CTR1 mRNA in the liver and kidney over the PBS control group.

Table 9

Percent change of murine CTR1 mRNA compared to the PBS control in Tx mice

Treatment	Liver	Kidney
Penicillamine control	+31	+1
ISIS 526814	-61	-28
ISIS 526814+ Penicillamine	-73	-41

Analysis of metal levels

The levels of copper and zinc in the liver and brain were measured by the Michigan State University Diagnostic Center for Population and Animal Health (DCPAH) (<http://www.animalhealth.msu.edu/>) according to their methods. As shown in Tables 10 and 11, antisense inhibition of CTR1 achieved a downward trend in liver copper accumulation at the end of the two week treatment. Treatment with penicillamine along with ISIS 526814 produced a marked synergy in the liver, significantly reducing liver copper accumulation. Similarly, significant reduction in liver zinc accumulation was observed in the animal group treated with a combination of penicillamine along with ISIS 526814.

Table 10
Levels of copper (ppm) in Tx mice

Treatment	Liver	Cerebellum
PBS control	562	44
Penicillamine control	519	45
ISIS 526814	507	44
ISIS 526814+ Penicillamine	230	45

Table 11
Levels of zinc (ppm) in Tx mice

Treatment	Liver	Cerebellum
PBS control	159	89
Penicillamine control	179	64
ISIS 526814	160	67
ISIS 526814+ Penicillamine	124	72

CLAIMS

1. A method for inhibiting CTR1 expression in an animal having, or at risk of having, a copper related disease, disorder and/or condition comprising
 - (a) selecting the animal having, or at risk of having, the copper related disease, disorder or condition, and
 - (b) administering a compound comprising a CTR1 specific inhibitor to the animal, wherein the compound administered to the animal inhibits CTR1 expression in the animal having, or at risk of having, the copper related disease, disorder and/or condition.
2. A method for treating an animal having, or at risk of having, a CTR1 related disease, disorder and/or condition comprising
 - (a) selecting the animal having, or at risk of having, a CTR1 disease, disorder and/or condition, and
 - (b) administering a therapeutically effective amount of a compound comprising a CTR1 specific inhibitor to the animal, wherein the compound administered to the animal treats the animal having, or at risk of having, the CTR1 disease, disorder and/or condition.
3. A method for treating an animal having, or at risk of having, a copper related disease, disorder and/or condition comprising
 - (a) selecting the animal having, or at risk of having, the copper related disease, disorder or condition, and
 - (b) administering a therapeutically effective amount of a compound comprising a CTR1 specific inhibitor to the animal, wherein the compound administered to the animal treats the animal having, or at risk of having, the copper related disease, disorder and/or condition.
4. A method for treating, preventing and/or ameliorating damage to a cell, tissue or organ in an animal having, or at risk of having, a copper related disease, disorder and/or condition comprising

(a) selecting the animal having, or at risk of having, the copper related disease, disorder or condition, and

(b) administering a therapeutically effective amount of a compound comprising a CTR1 specific inhibitor to the animal,

wherein the compound administered to the animal treats, prevents and/or ameliorates damage to the cell, tissue and/or organ of the animal having, or at risk of having, the copper related disease, disorder and/or condition.

5. The method of any of claims 1, 3-4, wherein the copper related disease, disorder and/or condition is copper accumulation in cells, tissues and/or organs of the animal.

6. The method of any of claims 1, 3-5, wherein the copper related disease, disorder or condition is Wilson's Disease, Alzheimer's Disease, copper toxicosis, neurological diseases, amyloid related diseases, anemias, inflammatory diseases, cardiovascular diseases, fibrosis, cirrhosis and other copper related diseases, disorders and/or conditions or symptoms thereof.

7. A method for reducing copper accumulation in a cell, organ or tissue of an animal comprising administering a compound comprising a CTR1 specific inhibitor to the animal, thereby reducing the copper accumulation in the cell, organ or tissue of the animal.

8. The method of claim 7, wherein the organ is liver, brain. blood, kidney or cornea and the cell or tissue is from the liver, brain. blood, kidney or cornea.

9. The method of claim any preceeding claim, wherein CTR1 has a sequence as shown in any of SEQ ID NOs: 1-8.

10. The method of any preceding claim, wherein the CTR1 specific inhibitor is any of a nucleic acid, peptide, antibody, small molecule, and other agents capable of specifically inhibiting the expression of CTR1 mRNA and/or CTR1 protein.

11. The method of claim 10, wherein the nucleic acid is an antisense compound targeting CTR1.

12. The method of claim 10, wherein the antisense compound targeting CTR1 is an antisense oligonucleotide.

13. The method of claim 12, wherein the antisense oligonucleotide comprises a nucleobase sequence at least 80%, 85%, 90%, 95% or 100% complementary to any of the nucleobase sequences recited in SEQ ID NOs: 1-8.

14. The method of claim 12, wherein the antisense oligonucleotide consists of a single-stranded modified oligonucleotide.

15. The method of claim 12, wherein the antisense oligonucleotide consists of 12 to 30 linked nucleosides.

16. The method of claim 15, wherein the antisense oligonucleotide consists of 20 linked nucleosides.

17. The method of claim 12, wherein the antisense oligonucleotide comprises at least one modified internucleoside linkage, at least one modified sugar and/or at least one modified nucleobase.

18. The method of claim 17, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage, wherein the modified sugar is a bicyclic sugar or a 2'-O-methoxyethyl and wherein the modified nucleobase is a 5-methylcytosine.

19. The method of claim 14, wherein the modified oligonucleotide comprises:

- (a) a gap segment consisting of linked deoxynucleosides;
- (b) a 5' wing segment consisting of linked nucleosides;
- (c) a 3' wing segment consisting of 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.

20. A method for treating an animal having or at risk for a copper related disease comprising
- (a) selecting the animal having, or at risk of having, a copper related disease, and
 - (b) administering to the animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein the modified antisense oligonucleotide is complementary to a CTR1 nucleic acid as shown in any of SEQ ID NOs: 1-8, and wherein the compound administered to the animal treats the animal having, or at risk of having, the copper related disease.
21. The method of any preceding claim, wherein the animal is a human.
22. The method of any of claims 1, 3-4 or 20, wherein a marker of the copper related disease, disorder and/or condition is selected from one or more of copper accumulation in the liver, brain, blood, kidney or cornea of an animal and/or conditions or symptoms thereof.
23. The method of any preceding claim, wherein the compound is parenterally administered.
24. The method of claim 23, wherein the parenteral administration is any of subcutaneous or intravenous administration.
25. The method of any preceding claim, wherein the compound is administered once or more daily, once or more a week, once or more every two weeks or once or more a month.
26. The method of any preceding claim, further comprising a second agent.
27. The method of claim 26, wherein the second agent is administered concomitantly or sequentially with the compound.
28. The method of claim 26, wherein the second agent is a CTR1 inhibitor, metal chelator, copper absorption inhibitor, antioxidant, cholinesterase inhibitor, copper binding protein or a drug that induces production of the copper binding protein.

29. The method of claim 28, wherein the metal chelator is selected from any of dimercaprol, penicillamine, trientine and the copper absorption inhibitor is selected from any of tetrathiomolybdate and zinc salts.
30. The method of any preceding claim, wherein the compound is a salt form.
31. The method of any preceding claim, further comprising a pharmaceutically acceptable carrier or diluent.
32. The method of any preceding claim, wherein the compound targeting CTR1 reduces metal levels by at least 10%, 15%, 20%, 25% or 30%.
33. Use of a compound comprising a CTR1 specific inhibitor for treating a copper related disease, disorder and/or condition.
34. The use of claim 33, wherein the CTR1 specific inhibitor is any of a nucleic acid, peptide, antibody, small molecule, and other agents capable of specifically inhibiting the expression of CTR1 mRNA and/or CTR1 protein.
35. The use of claim 34, wherein the nucleic acid is an antisense compound.
36. The use of claim 35, wherein the antisense compound is a modified antisense oligonucleotide.
37. The use of claims 33-36, wherein CTR1 has a sequence as shown in any of SEQ ID NOs: 1-8.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/57358

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/11 ; C07H 21/02 (2013.01)

USPC - 514/44A; 536/23.1

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)

USPC: 514/44A; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/44A; 536/23.1 (text search)

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

Electronic data bases: PatBase, Google Patents, Google Scholar, GenCore sequence search (NT)

Search terms: CTR1 (synonyms: copper transporter 1, COPT1, hCTR1, SLC31A1), antisense, RNAi, administer, copper, cisplatin ototoxicity, industrial manganese elevation of copper levels

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHENG et al. Relative contribution of CTR1 and DMT1 in copper transport by the blood-CSF barrier: implication in manganese-induced neurotoxicity. Toxicol Appl Pharmacol 1 May 2012 Vol 260 No 3 Pages 285-293. Especially pg 2 para 1-2, pg 5 para 4, pg 15 fig 3C.	1-5, 20, 22, 33-36
X	MORE et al. Role of the copper transporter, CTR1, in platinum-induced ototoxicity. J. Neuroscience 14 July 2010 Vol 30 No 28 Pages 9500-9509. Especially abstract, pg 9505 fig 7; pg 9505 col 2 para 3- pg 9506 col1 para 1	7, 8
A	ALA et al. Wilson's disease. Lancet, 3 February 2007, Vol 369 No 9559 Pages 397-408. Entire article.	1-5, 7, 8

☐ Further documents are listed in the continuation of Box C.


* 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

26 November 2013 (26.11.2013)

Date of mailing of the international search report

20 DEC 2013

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
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Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/57358

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☒

in the international application as filed

☐

together with the international application in electronic form

☒

subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

GenCore ver 6.4.1 SEQ ID NOs: 1, 4, 5, 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/57358

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.: 6, 9-19, 21, 23-32, 37
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.