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(54) Title: GLUCOKINASE (GCK) IRNA COMPOSITIONS AND METHODS OF USE THEREOF

(57) Abstract: The invention relates to double stranded ribonucleic acid (dsRNA) compositions targeting a glucokinase (GCK) gene, as well as methods of inhibiting expression of a glucokinase (GCK) gene, and methods of treating subjects having a glycogen storage disease (GSD), e.g., type Ia GSD.



GLUCOKINASE (GCK) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

Related Applications

5 This application claims the benefit of priority of U.S. Provisional Patent Application No. 62/183,413, filed on June 23, 2015, U.S. Provisional Patent Application No. 62/200,207, filed on August 3, 2015, and U.S. Provisional Patent Application No. 62/260,876, filed on November 30, 2015. The entire contents of each of the foregoing applications are hereby incorporated herein by reference.

10 Sequence Listing

 The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 16, 2016, is named 121301-03720_SL.txt and is 523,685 bytes in size.

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Background of the Invention

 Hypoglycemia is a biochemical symptom indicating the presence of an underlying disease or disorder. Since glucose is the fundamental energy currency of the cell, diseases and disorders that affect its availability or use can cause hypoglycemia.

20 The body normally defends against hypoglycemia by decreasing insulin secretion and increasing glucagon, epinephrine, growth hormone, and cortisol secretion. These hormonal changes combine to increase hepatic glucose production, increase alternative fuel availability, and decrease glucose use. The increase in hepatic glucose production is initially caused by the breakdown of liver glycogen stores resulting from lower insulin
25 levels and increased glucagon levels. When glycogen stores become depleted and protein breakdown increases because of increased cortisol levels, hepatic gluconeogenesis replaces glycogenolysis as the primary source of glucose production. Decreased use of peripheral glucose occurs initially because of a fall in insulin levels and later because of increases in epinephrine, cortisol, and growth hormone levels.

30 All of these events increase lipolysis and plasma free fatty acid levels, which are then available as an alternative fuel source and act to competitively inhibit glucose use. Plasma free fatty acids also stimulate glucose production. Hypoglycemia occurs when one or more of these counterregulatory mechanisms fail because of, for example, the overuse of glucose (as in hyperinsulinism) or the underproduction of glucose (as in a
35 glycogen-storage diseases).

GSD is an inherited genetic disorder due to an absence or deficiency of one of the enzymes responsible for making or breaking down glycogen in the body. This enzyme deficiency causes either abnormal tissue concentrations of glycogen or incorrectly or abnormally formed glycogen and patients with glycogen storage diseases (GSD) may also have low blood glucose levels. GSD occurs in about one of 50,000 to 100,000 births. Some patients might die before diagnosis, while severe infantile forms and some milder forms might go unrecognized. Symptoms of GSD vary based on the enzyme that is missing and usually result from the buildup of glycogen or from the inability to produce glucose when needed. Because GSD occurs mainly in muscles and the liver, those areas show the most symptoms, such as, poor growth, muscle cramps, low blood sugar, enlarged liver, swollen belly, and abnormal blood chemistry.

Current treatments for glycogen storage diseases have been very limited and mainly focused on correcting hypoglycemia and other metabolic disturbances through dietary control, such as, by using a modified form of cornstarch, or a high-protein diet. Accordingly, there is a need in the art for alternative treatments for subjects having a glycogen storage disease (GSD), *e.g.*, type Ia GSD, which are independent of the underlying molecular defects.

Summary of the Invention

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a glucokinase (GCK) gene. The GCK gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The present invention also provides methods of using the iRNA compositions of the invention for inhibiting the expression of a GCK gene and/or for treating a subject who would benefit from inhibiting or reducing the expression of a GCK gene, *e.g.*, a subject suffering or prone to suffering from a glycogen storage disease (GSD), or one or more signs or symptoms of GSD, such as hypoglycemia, lactic acidosis, hyperuricemia, hyperlipidemia, hepatomegaly, kidney disease as a result of glycogen accumulation, hunger, jitteriness, lethargy, apnea, seizures, diaphoresis, confusion, headaches, dizziness, unusual mood or behavior changes, loss of consciousness, coma, muscle cramps, bleeding diathesis, short stature, osteoporosis, delayed puberty, gout, renal disease, systemic hypertension, pulmonary hypertension, hepatic adenomas, pancreatitis, anemia, vitamin D deficiency, polycystic ovaries, irregular menstrual cycles, menorrhagia, and/or eruptive xanthomata.

GCK in the liver of a subject is the rate limiting enzyme for both glucose uptake and glycogen synthesis. Furthermore, the liver is a major site for regulation of whole body glucose metabolism. Therefore, by inhibiting expression of a glucokinase gene, *e.g.*, a glucokinase gene in the liver of a subject, with a double stranded RNAi agent of

the invention, *e.g.*, an RNAi agent that contains and/or is coupled to a ligand, *e.g.*, GalNAc3, that directs the RNAi agent to a site of interest, *e.g.*, the liver, hepatic glucose uptake and storage will decrease, thus, inhibiting hypoglycemia, *e.g.*, fasting hypoglycemia, in subjects suffering or prone to suffering from a glycogen storage disease (GSD), *e.g.*, type Ia GSD, independent of the underlying cause. Furthermore, by
5 inhibiting expression of a glucokinase gene, *e.g.*, a glucokinase gene in the liver of a subject, with a double stranded RNAi agent of the invention, insulin resistance in the liver is selectively induced without altering insulin clearance in order to maintain safe glucose levels in subjects suffering or prone to suffering from a glycogen storage disease (GSD), *e.g.*, type Ia GSD. In addition, by inhibiting expression of a glucokinase gene,
10 *e.g.*, a glucokinase gene in the liver of a subject having a glycogen storage disease (GSD), *e.g.*, type Ia GSD, with a double stranded RNAi agent of the invention, hepatic glucose uptake and storage will decrease, thus, inhibiting hypoglycemia, *e.g.*, fasting hypoglycemia, inhibit hepatic glycogen storage to inhibit hepatomegaly, reduce lipid
15 abnormalities, lactic acidosis and/or hyperuricemia.

Accordingly, in one aspect, the present invention provides double stranded ribonucleic acids (RNAi) agents for inhibiting expression of a glucokinase (GCK) gene. The double stranded RNAi agents comprise a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no
20 more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:15.

In another aspect, the invention provides double stranded ribonucleic acids (RNAi) agents for inhibiting expression of glucokinase (GCK), wherein the double
25 stranded RNAi agents comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing no more than 3 nucleotides from any one of the antisense sequences listed in any one of Tables 2, 3, 6, and 7.

In certain embodiments, the double stranded RNAi agent comprises at least one
30 modified nucleotide. In one embodiment, the modified nucleotide comprises a 2'-O-methyl modified nucleotide. In another embodiment, the modified nucleotide comprises a 2'-fluoro modified nucleotide. In one embodiment, the modified nucleotide comprises a 3'-terminal deoxy-thymine (dT) nucleotide. In another embodiment, the modified nucleotide comprises a short sequence of deoxy-thymine (dT) nucleotides.

35 In certain embodiments, the dsRNA comprises no more than 4 (*i.e.*, 4, 3, 2, 1, or 0) unmodified nucleotides in the sense strand. In certain embodiments, the dsRNA comprises no more than 4 (*i.e.*, 4, 3, 2, 1, or 0) unmodified nucleotides in the antisense strand. In certain embodiments, the double stranded RNAi agent comprises no more

than 4 (*i.e.*, 4, 3, 2, 1, or 0) unmodified nucleotides in both the sense strand and the antisense strand. In certain embodiments, the double stranded RNAi agent comprises all modified nucleotides in the sense strand. In certain embodiments, the dsRNA comprises all modified nucleotides in the antisense strand. In certain embodiments, the double stranded RNAi agent comprises all modified nucleotides in both the sense strand and the antisense strand.

In one aspect, the present invention provides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of glucokinase (GCK), wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:15, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand is conjugated to a ligand attached at the 3'-terminus.

In certain embodiments, at least one of the modified nucleotides is selected from the group consisting of a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5'-methylphosphonate group, a nucleotide comprising a 5' phosphate or 5' phosphate mimic, a nucleotide comprising vinyl phosphate, a nucleotide comprising adenosine-glycol nucleic acid (GNA), a nucleotide comprising thymidine-glycol nucleic acid (GNA) S-Isomer, a nucleotide comprising 2-hydroxymethyl-tetrahydrofuran-5-phosphate, a nucleotide comprising 2'-deoxythymidine-3' phosphate, a nucleotide comprising 2'-deoxyguanosine-3'-phosphate, and a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group.

In certain embodiments, the modified nucleotides is/are independently selected from the group consisting of a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a 3'-terminal deoxy-thymine (dT) nucleotide, and a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group. In certain embodiments, the modified nucleotide is selected from the group

consisting of a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

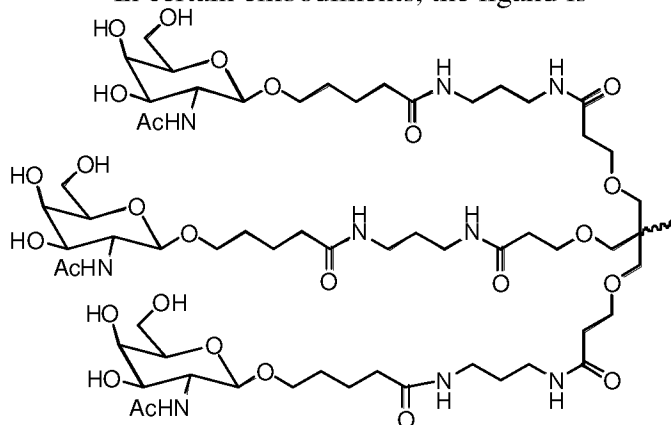
5 In certain embodiments, the double stranded RNAi agent comprises a region of complementarity at least 17 nucleotides in length. In certain embodiments, the double stranded RNAi agent comprises a region of complementarity 19-23 nucleotides in length. In certain embodiments, the double stranded RNAi agent comprises a region of complementarity 19-21 nucleotides in length. In certain embodiments, the double
10 stranded RNAi agent comprises a region of complementarity is 19 nucleotides in length. In certain embodiments, the double stranded RNAi agent comprises a region of complementarity is 21 nucleotides in length.

In certain embodiments, each strand of the double stranded RNAi agent is no more than 30 nucleotides in length. In certain embodiments, the double stranded RNAi
15 agent is at least 15 nucleotides in length.

In some embodiments, at least one strand of the dsRNA agent comprises a 3' overhang of at least 1 nucleotide, *e.g.*, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In some
20 embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In some embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide. In some embodiments, at least one strand comprises a 3' overhang of at least 2 nucleotides.

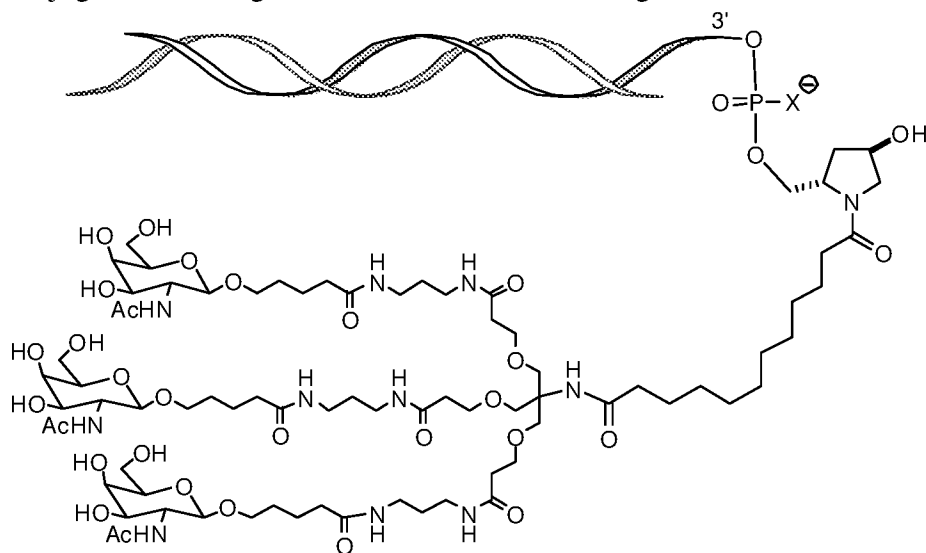
25 In certain embodiments, the double stranded RNAi agent further comprises a ligand. In certain embodiments, the ligand is conjugated to the 3' end of the sense strand of the double stranded RNAi agent. In certain embodiments, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

In certain embodiments, the ligand is



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In certain embodiments, the wherein the double stranded RNAi agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

5 In certain embodiments, the ligand is a cholesterol.

In certain embodiments, the ligand is one or more GalNAc derivatives attached through a monovalent, a bivalent, or a trivalent branched linker. The ligand may be conjugated to the 3' end of the sense strand of the polynucleotide agent, the 5' end of the sense strand of the polynucleotide agent, the 3' end of the antisense strand of the polynucleotide agent, the 5' end of the antisense strand of the polynucleotide agent.

In some embodiments, the polynucleotide agents of the invention comprise a plurality, *e.g.*, 2, 3, 4, 5, or 6, of GalNAc, each independently attached to a plurality of nucleotides of the polynucleotide agent through a plurality of monovalent linkers.

In one embodiment, the agents inhibit the expression of GCK and the sense strand and the antisense strand comprise nucleotide sequences selected from the group consisting of the nucleotide sequences of any one of the agents listed in any one of Tables 2, 3, 6, and 7.

The invention provides cells containing the double stranded RNAi agents provided herein.

20 In another aspect, the invention provides pharmaceutical composition for inhibiting expression of a glucokinase (GCK) gene comprising the double stranded RNAi agent provided herein. In certain embodiments, the pharmaceutical compositions further comprise a lipid formulation.

The invention also provides methods of inhibiting expression of a glucokinase (GCK) gene in a cell. The methods include contacting the cell with a double stranded RNAi agent described herein; and maintaining the cell produced for a time sufficient to obtain degradation of the mRNA transcript of a GCK gene, thereby inhibiting expression of the GCK gene in the cell.

In certain embodiments, the cell is within a subject. In certain embodiments, the subject is a human. In certain embodiments, the human subject suffers from a disease or disorder that would benefit from reduction in GCK expression, such as a glycogen storage disease (GSD), *e.g.*, type Ia GSD. In certain embodiments, the GCK expression is inhibited by at least about 5%, 10%, 15%, 20%, 25%, 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

The invention further provides methods of treating a subject having a disorder that would benefit from reduction in expression of a glucokinase (GCK) gene. The methods include administering to the subject a therapeutically effective amount of any of the double stranded RNAi agents provided herein, thereby treating the subject, such as a disease or disorder associated with a glycogen storage disease (GSD), *e.g.*, type Ia GSD.

In one aspect, the present invention provides methods of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction in expression of a glucokinase (GCK) gene. The methods include administering to the subject a prophylactically effective amount of a double stranded RNAi agent described herein, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in GCK expression.

In certain embodiments, administration of the double stranded RNAi agent to the subject causes an increase in one or more blood glucose and/or a decrease in GCK protein accumulation. In certain embodiments, administration of the double stranded RNAi agent to the subject, *e.g.*, a subject having a glycogen storage disease (GSD), *e.g.*, type Ia GSD, causes a decrease in one or more signs of a glycogen storage disease (GSD), *e.g.*, type Ia GSD, *e.g.*, hypoglycemia, lactic acidosis, hyperuricemia, hyperlipidemia, hepatomegaly, kidney disease as a result of glycogen accumulation, hunger, jitteriness, lethargy, apnea, seizures, diaphoresis, confusion, headaches, dizziness, unusual mood or behavior changes, loss of consciousness, coma, muscle cramps, bleeding diathesis, short stature, osteoporosis, delayed puberty, gout, renal disease, systemic hypertension, pulmonary hypertension, hepatic adenomas, pancreatitis, anemia, vitamin D deficiency, polycystic ovaries, irregular menstrual cycles, menorrhagia, and/or eruptive xanthomata.

The invention also provides methods of inhibiting the expression of a glucokinase (GCK) gene in a subject. The methods include method administering to the subject a therapeutically effective amount of any of the double stranded RNAi agents provided herein or a pharmaceutical composition comprising any of the double stranded RNAi agents provided herein, thereby inhibiting the expression of GCK in the subject.

In one aspect, the present invention provides methods of increasing the blood glucose levels, *e.g.*, fasting blood glucose levels, in a subject having a disease or disorder that would benefit from reduction in GCK expression. The methods include administering to the subject a therapeutically effective amount of any of the double stranded RNAi agents provided herein or a pharmaceutical composition comprising any of the double stranded RNAi agents provided herein, thereby increasing the blood glucose levels in the subject.

In another aspect, the present invention provides methods of decreasing plasma lactate levels in a subject having a disease or disorder that would benefit from reduction in GCK expression. The methods include administering to the subject a therapeutically effective amount of any of the double stranded RNAi agents provided herein or a pharmaceutical composition comprising any of the double stranded RNAi agents provided herein, thereby decreasing the blood lactate levels in the subject.

In yet another aspect, the present invention provides methods of decreasing plasma uric acid levels in a subject having a disease or disorder that would benefit from reduction in GCK expression. The methods include administering to the subject a therapeutically effective amount of any of the double stranded RNAi agents provided herein or a pharmaceutical composition comprising any of the double stranded RNAi agents provided herein, thereby decreasing the plasma uric acid levels in the subject.

In one aspect, the present invention provides methods of decreasing plasma triglyceride levels in a subject having a disease or disorder that would benefit from reduction in GCK expression. The methods include administering to the subject a therapeutically effective amount of any of the double stranded RNAi agents provided herein or a pharmaceutical composition comprising any of the double stranded RNAi agents provided herein, thereby decreasing plasma triglyceride levels in the subject.

In another aspect, the present invention provides methods of decreasing total plasma cholesterol levels in a subject having a disease or disorder that would benefit from reduction in GCK expression. The methods include administering to the subject a therapeutically effective amount of any of the double stranded RNAi agents provided herein or a pharmaceutical composition comprising any of the double stranded RNAi agents provided herein, thereby decreasing the total plasma cholesterol levels in said subject.

In one aspect, the present invention provides methods of decreasing hepatomegaly in a subject having a disease or disorder that would benefit from reduction in GCK expression. The methods include administering to the subject a therapeutically effective amount of any of the double stranded RNAi agents provided herein or a pharmaceutical composition comprising any of the double stranded RNAi agents provided herein, thereby decreasing hepatomegaly in the subject.

In certain embodiments, the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg. In certain embodiments, the dsRNA agent is administered about once per month, once every other two months, or once a quarter (*i.e.*, once every three months), for example, at a dose of about 0.1 mg/kg to about 5.0 mg/kg.

In certain embodiments, the double stranded RNA agent is administered to the subject once a week. In certain embodiments, the dsRNA agent is administered to the subject once a month. In certain embodiments, the dsRNA agent is administered once per quarter (*i.e.*, every three months).

In some embodiments, the methods of the invention further include administering an additional therapeutic to the subject. In one embodiment, the additional therapeutic is a sodium-glucose co-transporter 2 (SGLT2) inhibitor, *e.g.*, Dapagliflozin, Canagliflozin, Ipragliflozin (ASP-1941), Tofogliflozin, Empagliflozin, Sergliflozin etabonate, Remogliflozin etabonate (BHV091009), and Ertugliflozin (PF-04971729 / MK-8835).

In yet another aspect, the invention provides kits for performing the methods of the invention. In one aspect, the invention provides a kit for performing a method for inhibiting expression of a glucokinase (GCK) gene in a cell by contacting a cell with a double stranded RNAi agent in an amount effective to inhibit expression of the GCK in the cell. The kit comprises an RNAi agent and instructions for use and, optionally, means for administering the RNAi agent to a subject.

Detailed Description of the Invention

The present invention provides iRNA compositions, which affect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a glucokinase (GCK) gene. The GCK gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The present invention also provides methods of using the iRNA compositions of the invention for inhibiting the expression of a glucokinase (GCK) gene and/or for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a GCK gene, such as a glycogen storage disease (GSD), *e.g.*, type Ia GSD, and one or more of the signs or symptoms associated therewith.

The iRNAs of the invention include an RNA strand (the antisense strand) having a region which is about 30 nucleotides or less in length, *e.g.*, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23,

or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of a GCK gene.

In certain embodiments, the iRNAs of the invention include an RNA strand (the antisense strand) which can include longer lengths, for example up to 66 nucleotides, e.g., 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of a GCK gene. These iRNAs with the longer length antisense strands include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

The use of these iRNAs enables the targeted degradation of mRNAs of a GCK gene in mammals. Very low dosages of GCK iRNAs, in particular, can specifically and efficiently mediate RNA interference (RNAi), resulting in significant inhibition of expression of a GCK gene. Using cell-based assays, the present inventors have demonstrated that iRNAs targeting GCK can mediate RNAi, resulting in significant inhibition of expression of a GCK gene. Thus, methods and compositions including these iRNAs are useful for treating a subject who would benefit by a reduction in the levels and/or activity of a GCK protein, such as a subject having a glycogen storage disease (GSD), e.g., type Ia GSD.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a GCK gene, as well as compositions and methods for treating subjects having diseases and disorders that would benefit from inhibition and/or reduction of the expression of this gene.

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element, *e.g.*, a plurality of elements.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to". The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise.

The term "about" is used herein to mean within the typical ranges of tolerances in the art. For example, "about" can be understood as within about 2 standard deviations from the mean. In certain embodiments, about means +10%. In certain embodiments,

about means +5%. When about is present before a series of numbers or a range, it is understood that “about” can modify each of the numbers in the series or range.

The terms “GCK,” “glucokinase,” “hexokinase D,” and “hexokinase 4” refer to an enzyme that facilitates phosphorylation of glucose to glucose-6-phosphate having an amino acid sequence from any vertebrate or mammalian source, including, but not limited to, human, bovine, chicken, rodent, mouse, rat, porcine, ovine, primate, monkey, and guinea pig, unless specified otherwise. The term also refers to fragments and variants of native GCK that maintain at least one *in vivo* or *in vitro* activity of a native GCK. The term encompasses full-length unprocessed precursor forms of GCK as well as mature forms resulting from, *e.g.*, post-translational processing.

The sequence of a human GCK mRNA transcript (transcript variant 2) can be found at, for example, GenBank Accession No. GI: 15967158 (NM_033507; NCBI GeneID: 2645; SEQ ID NO:1). The sequence of another human GCK mRNA transcript (transcript variant 1) can be found at, for example, GenBank Accession No. GI: 167621407 (NM_000162); SEQ ID NO:2). The sequence of yet another human GCK mRNA transcript (transcript variant 3) can be found at, for example, GenBank Accession No. GI: 15967160 (NM_033508); SEQ ID NO:3). The predicted sequence of a rhesus GCK mRNA transcript (transcript variant X1) can be found at, for example, GenBank Accession No. GI: 544420246 (XM_005549685; SEQ ID NO:4). The predicted sequence of another rhesus GCK mRNA transcript (transcript variant X2) can be found at, for example, GenBank Accession No. GI: 544420248 (XM_005549686; SEQ ID NO:5). The predicted sequence of yet another rhesus GCK mRNA transcript (transcript variant X3) can be found at, for example, GenBank Accession No. GI: 544420250 (XM_005549687; SEQ ID NO:6). The predicted sequence of another rhesus GCK mRNA transcript (transcript variant X4) can be found at, for example, GenBank Accession No. GI: 544420252 (XM_005549688; SEQ ID NO:7). The sequence of a mouse GCK mRNA transcript (transcript variant 1) can be found at, for example, GenBank Accession No. GI: 565671706 (NM_010292; SEQ ID NO:8). The sequence of another mouse GCK mRNA transcript (transcript variant 2) can be found at, for example, GenBank Accession No. GI: 565671714 (NM_001287386; SEQ ID NO:9). The sequence of a rat GCK mRNA transcript (transcript variant 2) can be found at, for example, GenBank Accession No. GI: 399220372 (NM_012565; SEQ ID NO:10). The sequence of another rat GCK mRNA transcript (transcript variant 1) can be found at, for example, GenBank Accession No. GI: 399220370 (NM_001270849; SEQ ID NO:11). The sequence of yet another rat GCK mRNA transcript (transcript variant 3) can be found at, for example, GenBank Accession No. GI: 399220373 (NM_001270850; SEQ ID NO:12). Additional examples of GCK mRNA sequences are readily available using publicly available databases, *e.g.*, GenBank, UniProt, and OMIM.

As used herein, “target sequence” refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a GCK gene, including mRNA that is a product of RNA processing of a primary transcription product. In one embodiment, the target portion of the sequence will be at least long enough to
5 serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a GCK gene.

The target sequence may be from about 9-36 nucleotides in length, *e.g.*, about 15-30 nucleotides in length. For example, the target sequence can be from about 15-30 nucleotides, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-
10 19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of
15 the invention.

As used herein, the term “strand comprising a sequence” refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

“G,” “C,” “A,” “T,” and “U” each generally stand for a nucleotide that contains
20 guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that the term “ribonucleotide” or “nucleotide” can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an
25 oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another
30 example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

The terms “iRNA,” “RNAi agent,” “iRNA agent,” and “RNA interference agent”
35 as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The

iRNA modulates, *e.g.*, inhibits, the expression of GCK in a cell, *e.g.*, a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNAi that interacts with a target RNA sequence, *e.g.*, a GCK target mRNA sequence, to
5 direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into double stranded short interfering RNAs (siRNAs) comprising a sense strand and an antisense strand by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes these dsRNA into 19-23
10 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). These siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more
15 endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). Thus, in one aspect the invention relates to a single stranded RNA (ssRNA) (the antisense strand of an siRNA duplex) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.*, a GCK gene. Accordingly, the term "siRNA" is also used herein to
20 refer to an RNAi as described above.

In another embodiment, the RNAi agent may be a single-stranded RNA that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents bind to the RISC endonuclease, Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are generally 15-30 nucleotides and are chemically
25 modified. The design and testing of single-stranded RNAs are described in U.S. Patent No. 8,101,348 and in Lima *et al.*, (2012) *Cell* 150:883-894, the entire contents of each of which are hereby incorporated herein by reference. Any of the antisense nucleotide sequences described herein may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima *et al.*, (2012) *Cell* 150:883-
30 894.

In another embodiment, an "iRNA" for use in the compositions and methods of the invention is a double stranded RNA and is referred to herein as a "double stranded RNAi agent," "double stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA", refers to a complex of ribonucleic acid molecules,
35 having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, *i.e.*, a GCK gene. In some embodiments of the invention, a

double stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.*, an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

The majority of nucleotides of each strand of a dsRNA molecule may be
5 ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. In addition, as used in this specification, an “RNAi agent” may include ribonucleotides with chemical modifications; an RNAi agent may include substantial modifications at multiple nucleotides. As used herein, the term “modified nucleotide”
10 refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, and/or modified nucleobase. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.*, a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the invention include all types of modifications disclosed herein or
15 known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by “RNAi agent” for the purposes of this specification and claims.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 9 to 36 base pairs in length, *e.g.*, about 15-30 base pairs in length, for example, about 9, 10, 11, 12,
20 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-
25 22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands
30 are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a “hairpin loop.” A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at least 2, at least 3, at least 4, at least 5, at
35 least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23, or more unpaired nucleotides. In some embodiments, the hairpin loop can be 10 or fewer nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired

nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 nucleotides.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected.

5 Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand
10 of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs.

In one embodiment, an RNAi agent of the invention is a dsRNA, each strand of which comprises 19-23 nucleotides, that interacts with a target RNA sequence, *e.g.*, a GCK gene, without wishing to be bound by theory, long double stranded RNA
15 introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more
20 helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). In one embodiment, an RNAi agent of the invention is a dsRNA of 24-30 nucleotides that interacts with a target
25 RNA sequence, *e.g.*, a prolyl hydroxylase domain-containing gene, *i.e.*, a PHD1 target mRNA sequence, a PHD2 target mRNA sequence, or a PHD3 target mRNA sequence, to direct the cleavage of the target RNA.

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of an iRNA, *e.g.*, a dsRNA. For
30 example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or vice versa, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside
35 analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA.

In one embodiment of the dsRNA, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, e.g., 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, e.g., 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In one embodiment, the antisense strand of a dsRNA has a 1-10 nucleotide, e.g., a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end and/or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, e.g., a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end and/or the 5'-end. In certain embodiments, the overhang on the sense strand or the antisense strand, or both, can include extended lengths longer than 10 nucleotides, e.g., 1-30 nucleotides, 2-30 nucleotides, 10-30 nucleotides, or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the antisense strand of the duplex. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

The terms "blunt" or "blunt ended" as used herein in reference to a dsRNA mean that there are no unpaired nucleotides or nucleotide analogs at a given terminal end of a dsRNA, *i.e.*, no nucleotide overhang. One or both ends of a dsRNA can be blunt. Where both ends of a dsRNA are blunt, the dsRNA is said to be blunt ended. To be clear, a "blunt ended" dsRNA is a dsRNA that is blunt at both ends, *i.e.*, no nucleotide overhang at either end of the molecule. Most often such a molecule will be double stranded over its entire length.

The term "antisense strand" or "guide strand" refers to the strand of an iRNA, e.g., a dsRNA, which includes a region that is substantially complementary to a target sequence, e.g., a GCK mRNA. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, e.g., a GCK nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule.

Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, 3, or 2 nucleotides of the 5'- and/or 3'-terminus of the iRNA.

The term "sense strand" or "passenger strand" as used herein, refers to the strand of an iRNA that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

As used herein, the term "cleavage region" refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing (see, *e.g.*, "Molecular Cloning: A Laboratory Manual, Sambrook, *et al.* (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

Complementary sequences within an iRNA, *e.g.*, within a dsRNA as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3, or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.*, inhibition of gene expression via a RISC pathway. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such

overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as “fully complementary” for the purposes described herein.

“Complementary” sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogsteen base pairing.

The terms “complementary,” “fully complementary” and “substantially complementary” herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of an iRNA agent and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is “substantially complementary to at least part of” a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding GCK). For example, a polynucleotide is complementary to at least a part of a GCK mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding GCK.

Accordingly, in some embodiments, the antisense strand polynucleotides disclosed herein are fully complementary to the target GCK sequence. In other embodiments, the antisense strand polynucleotides disclosed herein are substantially complementary to the target GCK sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NO:1, or a fragment of SEQ ID NO:1, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target GCK sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 2, 3, 6, and 7, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2, 3, 6, and 7, such as at least 85%, 90%, 95% complementary, or 100% complementary.

In one embodiment, an RNAi agent of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target GCK sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NO:5, or a fragment of any one of SEQ ID NO:5, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

The term “inhibiting,” as used herein, is used interchangeably with “reducing,” “silencing,” “downregulating,” “suppressing” and other similar terms, and includes any level of inhibition.

The phrase “inhibiting expression of a GCK,” as used herein, includes inhibition of expression of any GCK gene (such as, *e.g.*, a mouse GCK gene, a rat GCK gene, a monkey GCK gene, or a human GCK gene) as well as variants or mutants of a GCK gene that encode a GCK protein.

“Inhibiting expression of a GCK gene” includes any level of inhibition of a GCK gene, *e.g.*, at least partial suppression of the expression of a GCK gene, such as an inhibition by at least about 20%. In certain embodiments, inhibition is by at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

The expression of a GCK gene may be assessed based on the level of any variable associated with GCK gene expression, *e.g.*, GCK mRNA level or GCK protein level. The expression of a GCK may also be assessed indirectly based on, *e.g.*, blood glucose levels, serum ketone body levels, and/or serum fatty acid levels. Inhibition may be assessed by a change in an absolute or relative level of one or more of these variables compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

In one embodiment, at least partial suppression of the expression of a GCK gene, is assessed by a reduction of the amount of GCK mRNA which can be isolated from or detected in a first cell or group of cells in which a GCK gene is transcribed and which has or have been treated such that the expression of a GCK gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or

group of cells but which has or have not been so treated (control cells). The degree of inhibition may be expressed in terms of:

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

The phrase “contacting a cell with an RNAi agent,” such as a dsRNA, as used
 5 herein, includes contacting a cell by any possible means. Contacting a cell with an
 RNAi agent includes contacting a cell *in vitro* with the iRNA or contacting a cell *in vivo*
 with the iRNA. The contacting may be done directly or indirectly. Thus, for example,
 the RNAi agent may be put into physical contact with the cell by the individual
 performing the method, or alternatively, the RNAi agent may be put into a situation that
 10 will permit or cause it to subsequently come into contact with the cell.

Contacting a cell *in vitro* may be done, for example, by incubating the cell with
 the RNAi agent. Contacting a cell *in vivo* may be done, for example, by injecting the
 RNAi agent into or near the tissue where the cell is located, or by injecting the RNAi
 agent into another area, *e.g.*, the bloodstream or the subcutaneous space, such that the
 15 agent will subsequently reach the tissue where the cell to be contacted is located. For
 example, the RNAi agent may contain and/or be coupled to a ligand, *e.g.*, GalNAc3, that
 directs the RNAi agent to a site of interest, *e.g.*, the liver. Combinations of *in vitro* and
in vivo methods of contacting are also possible. For example, a cell may also be
 contacted *in vitro* with an RNAi agent and subsequently transplanted into a subject.

20 In one embodiment, contacting a cell with an iRNA includes “introducing” or
 “delivering the iRNA into the cell” by facilitating or effecting uptake or absorption into
 the cell. Absorption or uptake of an iRNA can occur through unaided diffusive or active
 cellular processes, or by auxiliary agents or devices. Introducing an iRNA into a cell
 may be *in vitro* and/or *in vivo*. For example, for *in vivo* introduction, iRNA can be
 25 injected into a tissue site or administered systemically. *In vivo* delivery can also be done
 by a beta-glucan delivery system, such as those described in U.S. Patent Nos. 5,032,401
 and 5,607,677, and U.S. Publication No. 2005/0281781, the entire contents of which are
 hereby incorporated herein by reference. *In vitro* introduction into a cell includes
 methods known in the art such as electroporation and lipofection. Further approaches
 30 are described herein below and/or are known in the art.

The term “lipid nanoparticle” or “LNP” is a vesicle comprising a lipid layer
 encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, *e.g.*,
 an iRNA or a plasmid from which an iRNA is transcribed. LNPs are described in, for
 example, U.S. Patent Nos. 6,858,225, 6,815,432, 8,158,601, and 8,058,069, the entire
 35 contents of which are hereby incorporated herein by reference.

As used herein, a “subject” is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, a horse, and a whale), or a bird (*e.g.*, a duck or a goose). It is understood that the sequence of the GCK gene must be sufficiently complementary to the antisense strand of the iRNA agent for the agent to be used in the indicated species.

In an embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder or condition that would benefit from reduction in GCK expression; a human at risk for a disease, disorder or condition that would benefit from reduction in GCK expression; a human having a disease, disorder or condition that would benefit from reduction in GCK expression; and/or human being treated for a disease, disorder or condition that would benefit from reduction in GCK expression as described herein.

As used herein, the terms “treating” or “treatment” refer to a beneficial or desired result including, such as increasing blood glucose levels in a subject. The terms “treating” or “treatment” also include, but are not limited to, alleviation or amelioration of one or more symptoms of a glycogen storage disease (GSD), *e.g.*, type Ia GSD, or at least one sign or symptom associated with GSD, such as hypoglycemia, lactic acidosis, hyperuricemia, hyperlipidemia, hepatomegaly, kidney disease as a result of glycogen accumulation, hunger, jitteriness, lethargy, apnea, seizures, diaphoresis, confusion, headaches, dizziness, unusual mood or behavior changes, loss of consciousness, coma, muscle cramps, bleeding diathesis, short stature, osteoporosis, delayed puberty, gout, renal disease, systemic hypertension, pulmonary hypertension, hepatic adenomas, pancreatitis, anemia, vitamin D deficiency, polycystic ovaries, irregular menstrual cycles, menorrhagia, and eruptive xanthomata.

“Treatment” can also mean prolonging survival as compared to expected survival in the absence of treatment.

By “lower” in the context of a disease marker or symptom is meant a statistically significant decrease in such level. The decrease can be, for example, at least 10%, at least 20%, at least 30%, at least 40%, or more, down to a level accepted as within the range of normal for an individual without such disorder, or to below the level of detection of the assay. In certain embodiments, the decrease is down to a level accepted as within the range of normal for an individual without such disorder which can also be referred to as a normalization of a level. For example, lowering cholesterol to 180 mg/dl or lower would be considered to be within the range of normal for a subject. A subject having a cholesterol level of 230 mg/dl with a cholesterol level decreased to 210 mg/dl would have a cholesterol level that was decreased by 40% towards normal (230-

210/230-180 = 20/50 = 40% reduction). In certain embodiments, the reduction is the normalization of the level of a sign or symptom of a disease, a reduction in the difference between the subject level of a sign of the disease and the normal level of the sign for the disease (*e.g.*, to the upper level of normal when the value for the subject must be decreased to reach a normal value, and to the lower level of normal when the value for the subject must be increased to reach a normal level). In certain embodiments, the methods include a clinically relevant inhibition of expression of a GCK gene, *e.g.* as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of a GCK gene.

As used herein, “prevention” or “preventing,” when used in reference to a disease, disorder or condition thereof, that would benefit from a reduction in expression of a GCK gene, refers to a reduction in the likelihood that a subject will develop a symptom associated with such disease, disorder, or condition, *e.g.*, a glycogen storage disease (GSD), *e.g.*, type Ia GSD. The likelihood of developing type Ia GSD is reduced, for example, when an individual having one or more risk factors for type Ia GSD, *e.g.*, a genetic disorder, either fails to develop type Ia GSD, or signs or symptoms thereof, or develops type Ia GSD, or signs or symptoms thereof, with less severity relative to a population having the same risk factors and not receiving treatment as described herein. The failure to develop a disease, disorder or condition, or the reduction in the development of a symptom associated with such a disease, disorder or condition (*e.g.*, by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed symptoms delayed (*e.g.*, by days, weeks, months, or years) is considered effective prevention. Prevention can require administration of more than one dose of an agent described herein.

As used herein, the term “blood glucose level” refers to the level of glucose present in blood as determined by any routine method known in the art. It is understood that the glucose level in a subject sample is dependent on, for example, whether the subject has had a meal, whether the subject has fasted, the time of day and the level of activity of the subject. Therefore, the glucose level must be compared to an appropriate control to determine if the glucose level is, in fact, altered from a normal level or from a level obtained from the subject at an earlier time point, *e.g.*, prior to treatment. In general, blood glucose levels, *e.g.*, fasting blood glucose levels, in a subject that does not have a disease or disorder that would benefit from reduction in the levels of GCK as described herein is about 70 to about 120 mg/dL.

As used herein, the term “plasma lactate level” refers to the level of lactate present in blood as determined by any routine method known in the art. It is understood that the lactate level in a subject sample is dependent on, for example, whether the subject has fasted, the time of day and the level of activity of the subject. Therefore, the

lactate level must be compared to an appropriate control to determine if the lactate level is, in fact, altered from a normal level or from a level obtained from the subject at an earlier time point, *e.g.*, prior to treatment. In general, plasma lactate levels in a subject that does not have a disease or disorder that would benefit from reduction in the levels of GCK as described herein is about 0.5 to about 2.2 mmol/L.

As used herein, the term “plasma uric acid level” refers to the level of uric acid present in blood as determined by any routine method known in the art. It is understood that the lactate level in a subject sample is dependent on, for example, whether the subject has fasted, the time of day and the level of activity of the subject. Therefore, the uric acid level must be compared to an appropriate control to determine if the uric acid level is, in fact, altered from a normal level or from a level obtained from the subject at an earlier time point, *e.g.*, prior to treatment. In general, plasma uric acid levels in a subject that does not have a disease or disorder that would benefit from reduction in the levels of GCK as described herein is about 2.0 to about 5.0 mg/dL.

As used herein, the term “plasma triglyceride level” refers to the level of triglycerides present in blood as determined by any routine method known in the art. It is understood that the triglyceride level in a subject sample is dependent on, for example, whether the subject has fasted, the time of day and the level of activity of the subject. Therefore, the triglyceride level must be compared to an appropriate control to determine if the triglyceride level is, in fact, altered from a normal level or from a level obtained from the subject at an earlier time point, *e.g.*, prior to treatment. In general, plasma triglyceride levels in a subject that does not have a disease or disorder that would benefit from reduction in the levels of GCK as described herein is about 150 to about 200 mg/dL.

As used herein, the term “total cholesterol level” refers to the level of high density lipoprotein (HDL) plus low density lipoprotein (LDL) plus 20% of the triglyceride level as determined by any routine method known in the art. It is understood that the total cholesterol level in a subject sample is dependent on, for example, whether the subject has fasted, the time of day and the level of activity of the subject. Therefore, the total cholesterol level must be compared to an appropriate control to determine if the total cholesterol level is, in fact, altered from a normal level or from a level obtained from the subject at an earlier time point, *e.g.*, prior to treatment. In general, plasma cholesterol levels in a subject that does not have a disease or disorder that would benefit from reduction in the levels of GCK as described herein is about 100 to about 200 mg/dL.

As used herein, the term “hepatomegaly” refers to swelling of the liver beyond its normal size as determined by any routine method known in the art. It is understood that the size and weight of the liver in a subject that does not have a disease or disorder

that would benefit from reduction in the levels of GCK as described herein increases with age and body weight. Sex and body shape also influence the size of the liver, *e.g.*, by percussion, the mean liver size is about 7.5 centimeters in adult women and about 10.5 centimeters in adult men; it may be about 3 centimeters larger or smaller and still be normal.

As used herein, a “disease or disorder that would benefit from reduction in GCK expression” is a disease or disorder associated with or caused by a clinically relevant hypoglycemia. For example, this term includes any disorder, disease or condition resulting in one or more signs or symptoms of a glycogen storage disease (GSD), *e.g.*, type Ia GSD, including, but not limited to, hypoglycemia, lactic acidosis, hyperuricemia, hyperlipidemia, hepatomegaly, kidney disease as a result of glycogen accumulation, hunger, jitteriness, lethargy, apnea, seizures, diaphoresis, confusion, headaches, dizziness, unusual mood or behavior changes, loss of consciousness, coma, muscle cramps, bleeding diathesis, short stature, osteoporosis, delayed puberty, gout, renal disease, systemic hypertension, pulmonary hypertension, hepatic adenomas, pancreatitis, anemia, vitamin D deficiency, polycystic ovaries, irregular menstrual cycles, menorrhagia, and eruptive xanthomata.. In certain embodiments, a “disease or disorder that would benefit from reduction in GCK expression” meets the diagnostic requirements of a type Ia GSD.

“Glycogen storage disease” or “GSD” is an inherited genetic disorder due to an absence or deficiency of one of the enzymes responsible for making or breaking down glycogen in the body. This enzyme deficiency causes either abnormal tissue concentrations of glycogen or incorrectly or abnormally formed glycogen. There are about 11 known types of GSD, which are classified based on the missing or defective enzymes. For example, Type Ia GSD, the most common form of GSD, is caused by a genetic defect in the enzyme glucose-6-phosphatase. Hepatomegaly due to inappropriate glycogen accumulation and various metabolic disarrangements from inappropriate glucose-6-phosphate metabolism are predominant features of many of the various glycogen storage diseases, such as Types Ia, Ib, III, IV, VI and IX. Symptoms of GSD vary based on the enzyme that is missing and usually result from the buildup of glycogen or from the inability to produce glucose when needed. Glycogen storage disease is usually diagnosed in infancy or childhood, *e.g.*, at about age 3-4. Affected children typically present with hepatomegaly, lactic acidosis, hyperuricemia, hyperlipidemia, hypertriglyceridemia and/or hypoglycemic seizures. Further, affected children often have doll-like faces with fat cheeks, relatively thin extremities, short stature, and protuberant abdomen. Xanthoma and diarrhea may be present. Impaired platelet function can lead to a bleeding tendency with frequent epistaxis. The diagnostic criteria for GSD, *e.g.*, type Ia GSD, include, for example, fasting blood glucose

concentration lower than 60 mg/dL; plasma lactate higher than 2.5 mmol/L; plasma uric acid higher than 5.0 mg/dL; triglycerides higher than 250 mg/dL; total plasma cholesterol higher than 200 mg/dL; administration of glucagon or epinephrine (*i.e.*, a glucagon or epinephrine challenge test) causes little or no increase in blood glucose concentration, but both increase serum lactate concentrations significantly;

5 histopathologic liver findings which include distention of the liver cells by glycogen and fat; PAS positive and diastase sensitive glycogen that is uniformly distributed within the cytoplasm; normal or only modestly increased glycogen; and large and numerous lipid vacuoles; biallelic mutations in either G6PC (GSDIa) or SLC37A4 (GSDIb) (Veiga-da-

10 Cunha *et al* (1998) *Am J Hum Genet.* 63:976–83; Chou *et al* (2002) *Hum Mutat.* 29:921–30; Matern *et al* (2002) *Eur J Pediatr.* 161 Suppl 1:S10–9; Rake *et al* (2002) *Eur J Pediatr.* 161 Suppl 1:S20–34; and Ekstein *et al* (2004) *Am J Med Genet.* 129A:162–4); deficient, *e.g.*, (lower than 10% of normal) glucose-6-phosphatase (G6Pase) catalytic activity (the normal G6Pase enzyme activity level in liver is 3.50 ± 0.8 $\mu\text{mol/min/g}$ tissue,

15 although in rare individuals with milder clinical manifestations, the G6Pase enzyme activity can be higher, *e.g.*, >1.0 $\mu\text{mol/min/g}$ tissue and <2.0 $\mu\text{mol/min/g}$ tissue.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a glycogen storage disease (GSD), *e.g.*, type Ia GSD, is sufficient to effect treatment of the disease

20 (*e.g.*, by diminishing, ameliorating or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be

25 treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an iRNA that, when administered to a subject having a glycogen storage disease (GSD), *e.g.*, type Ia GSD, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the

30 course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the iRNA, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

35 A "therapeutically-effective amount" or "prophylactically effective amount" also includes an amount of an RNAi agent that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. iRNA employed in

the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of
5 sound medical judgment, suitable for use in contact with the tissues of human subjects and animal subjects without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid
10 filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject
15 being treated. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium
20 lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum
25 hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

30 The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs or localized regions. For example, samples may be
35 derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from the liver (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver, such as, *e.g.*, hepatocytes). In

some embodiments, a “sample derived from a subject” refers to blood drawn from the subject or plasma isolated therefrom, saliva, or urine, typically a 24 hour urine sample.

II. iRNAs of the Invention

5 Described herein are iRNAs which inhibit the expression of a GCK gene. In one embodiment, the iRNA agent includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a GCK gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human having a glycogen storage disease (GSD), *e.g.*, type Ia GSD. The dsRNA includes an antisense strand having a region of
10 complementarity which is complementary to at least a part of an mRNA formed in the expression of a GCK gene. The region of complementarity is about 30 nucleotides or less in length (*e.g.*, about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 nucleotides or less in length). Upon contact with a cell expressing the GCK gene, the iRNA inhibits the expression of the GCK gene (*e.g.*, a human, a primate, a non-primate, or a bird GCK
15 gene) by at least about 10% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting, or flowcytometric techniques. In preferred embodiments, inhibition of expression is determined by the qPCR method provided in the examples. For *in vitro* assessment of activity, percent inhibition is
20 determined using the methods provided herein at a single dose at, for example, a 10 nM duplex final concentration. For *in vivo* studies, the level after treatment can be compared to, for example, an appropriate historical control or a pooled population sample control to determine the level of reduction, *e.g.*, when a baseline value is not available for the subject.

25 A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the
30 expression of a GCK gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed
35 to being on separate oligonucleotides.

Generally, the duplex structure is between 15 and 30 base pairs in length, *e.g.*, between, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-

20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

Similarly, the region of complementarity to the target sequence is between 15 and 30 nucleotides in length, *e.g.*, between 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

In some embodiments, the dsRNA is between about 15 and about 23 nucleotides in length, or between about 25 and about 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well known in the art that dsRNAs longer than about 21-23 nucleotides can serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 9 to 36 base pairs, *e.g.*, about 10-36, 11-36, 12-36, 13-36, 14-36, 15-36, 9-35, 10-35, 11-35, 12-35, 13-35, 14-35, 15-35, 9-34, 10-34, 11-34, 12-34, 13-34, 14-34, 15-34, 9-33, 10-33, 11-33, 12-33, 13-33, 14-33, 15-33, 9-32, 10-32, 11-32, 12-32, 13-32, 14-32, 15-32, 9-31, 10-31, 11-31, 12-31, 13-32, 14-31, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In

another embodiment, an iRNA agent useful to target GCK expression is not generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.*, 1, 2, 3, or 4 nucleotides. dsRNAs having at least one
5 nucleotide overhang can have unexpectedly superior inhibitory properties relative to their blunt-ended counterparts. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end, or both ends of
10 either an antisense or sense strand of a dsRNA. In certain embodiments, longer, extended overhangs are possible.

A dsRNA can be synthesized by standard methods known in the art as further discussed below, *e.g.*, by use of an automated DNA synthesizer, such as are commercially available from, for example, BiosearchTM, Applied BiosystemsTM, Inc.

15 iRNA compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide strands
20 comprising unnatural or modified nucleotides can be easily prepared. Single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In certain aspects, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand sequence is
25 selected from the group of sequences provided in any of Tables 2, 3, 6, and 7 and the corresponding nucleotide sequence of the antisense strand of the sense strand is selected from the group of sequences provided in any of Tables 2, 3, 6, and 7. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated
30 in the expression of a GCK gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in any of Tables 2, 3, 6, and 7, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any of Tables 2, 3, 6, and 7. In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate
35 oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

It will be understood that, although the sequences in Tables 3 and 7 are described as modified and/or conjugated sequences, the RNA of the iRNA of the invention *e.g.*, a dsRNA of the invention, may comprise any one of the sequences set forth in any one of Tables 3 and 7 that is un-modified, un-conjugated, and/or modified and/or conjugated differently than described therein.

In another aspect, a double stranded ribonucleic acid (dsRNA) of the invention for inhibiting expression of GCK comprises, consists essentially of, or consists of a sense strand and an antisense strand, wherein the sense strand comprises the nucleotide sequence of a sense strand in any of Tables 2, 3, 6, and 7 and the antisense strand comprises the nucleotide sequence of the corresponding antisense strand in any of Tables 2, 3, 6, and 7.

The skilled person is well aware that dsRNAs having a duplex structure of between about 20 and 23 base pairs, *e.g.*, 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, (2001) *EMBO J.*, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided herein, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences provided herein, and differing in their ability to inhibit the expression of a GCK gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs described herein identify a site(s) in a GCK transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within this site(s). As used herein, an iRNA is said to target within a particular site of an RNA transcript if the iRNA promotes cleavage of the transcript anywhere within that particular site. Such an iRNA will generally include at least about 15 contiguous nucleotides from one of the sequences provided herein coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a GCK gene.

While a target sequence is generally about 15-30 nucleotides in length, there is wide variation in the suitability of particular sequences in this range for directing cleavage of any given target RNA. Various software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given

gene target, but an empirical approach can also be taken in which a “window” or “mask” of a given size (as a non-limiting example, 21 nucleotides) is literally or figuratively (including, *e.g.*, in silico) placed on the target RNA sequence to identify sequences in the size range that can serve as target sequences. By moving the sequence “window” progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified sequences (using assays as described herein or as known in the art) to identify those sequences that perform optimally can identify those RNA sequences that, when targeted with an iRNA agent, mediate the best inhibition of target gene expression. Thus, while the sequences identified herein represent effective target sequences, it is contemplated that further optimization of inhibition efficiency can be achieved by progressively “walking the window” one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

Further, it is contemplated that for any sequence identified herein, further optimization could be achieved by systematically either adding or removing nucleotides to generate longer or shorter sequences and testing those sequences generated by walking a window of the longer or shorter size up or down the target RNA from that point. Again, coupling this approach to generating new candidate targets with testing for effectiveness of iRNAs based on those target sequences in an inhibition assay as known in the art and/or as described herein can lead to further improvements in the efficiency of inhibition. Further still, such optimized sequences can be adjusted by, *e.g.*, the introduction of modified nucleotides as described herein or as known in the art, addition or changes in overhang, or other modifications as known in the art and/or discussed herein to further optimize the molecule (*e.g.*, increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with silencing pathway enzymes, increasing release from endosomes) as an expression inhibitor.

An iRNA agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, an iRNA as described herein contains no more than 3 mismatches. If the antisense strand of the iRNA contains mismatches to a target sequence, it is preferable that the area of mismatch is not located in the center of the region of complementarity. If the antisense strand of the iRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, for a 23 nucleotide iRNA agent the strand which is complementary to a region of a GCK gene, generally does not contain any mismatch within the central 13 nucleotides. The

methods described herein or methods known in the art can be used to determine whether an iRNA containing a mismatch to a target sequence is effective in inhibiting the expression of a GCK gene. Consideration of the efficacy of iRNAs with mismatches in inhibiting expression of a GCK gene is important, especially if the particular region of complementarity in a GCK gene is known to have polymorphic sequence variation within the population.

III. Modified iRNAs of the Invention

In one embodiment, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is unmodified, and does not comprise, *e.g.*, chemical modifications and/or conjugations known in the art and described herein. In another embodiment, the RNA of an iRNA of the invention, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the invention, substantially all of the nucleotides of an iRNA of the invention are modified. In other embodiments of the invention, all of the nucleotides of an iRNA of the invention are modified. iRNAs of the invention in which “substantially all of the nucleotides are modified” are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

The nucleic acids featured in the invention can be synthesized and/or modified by methods well established in the art, such as those described in “Current protocols in nucleic acid chemistry,” Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Modifications include, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (*e.g.*, at the 2'-position or 4'-position) or replacement of the sugar; and/or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

Modified RNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6,239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and US Pat RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S, and CH₂ component parts.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

In other embodiments, suitable RNA mimetics are contemplated for use in iRNAs, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the iRNAs of the invention are described in, for example, in Nielsen *et al.*, *Science*, 1991, 254, 1497-1500.

Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH₂--NH--CH₂--, --CH₂--N(CH₃)--O--CH₂--[known as a methylene (methylimino) or MMI backbone], --CH₂--O--N(CH₃)--CH₂--, --CH₂--N(CH₃)--N(CH₃)--CH₂-- and --N(CH₃)--CH₂--CH₂--[wherein the native phosphodiester backbone is represented as --O--P--O--CH₂--] of the above-referenced U.S. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506.

Modified RNAs can also contain one or more substituted sugar moieties. The iRNAs, *e.g.*, dsRNAs, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Exemplary suitable modifications include O[(CH₂)_nO]_mCH₃, O(CH₂)_nOCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, 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 the pharmacokinetic properties of an iRNA, or a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--CH₂CH₂OCH₃, also

known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e.*, a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH₂--O--CH₂--N(CH₂)₂. Further exemplary modifications include : 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

Other modifications include 2'-methoxy (2'-OCH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the RNA of an iRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. iRNAs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 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; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application. The entire contents of each of the foregoing are hereby incorporated herein by reference.

An iRNA can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 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 uracil and cytosine, 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, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in *Modified Nucleosides in Biochemistry, Biotechnology and Medicine*, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J. L., ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al.*, (1991) *Angewandte Chemie*,

International Edition, 30:613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include

5 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 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., dsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base

10 substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Patent Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066;

15 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

20 The RNA of an iRNA can also be modified to include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to

25 increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193).

In some embodiments, the iRNA of the invention comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is unlocked acyclic

30 nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'-C4' have been removed (*i.e.* the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (*i.e.* the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been

35 removed (see *Nuc. Acids Symp. Series*, 52, 133-134 (2008) and Fluiters *et al.*, *Mol. Biosyst.*, 2009, 10, 1039 hereby incorporated by reference).

Representative U.S. publications that teach the preparation of UNA include, but are not limited to, US Patent No. 8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

5 The RNA of an iRNA can also be modified to include one or more bicyclic sugar moieties. A “bicyclic sugar” is a furanosyl ring modified by the bridging of two atoms. A “bicyclic nucleoside” (“BNA”) is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of
10 the sugar ring. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH₂-O-2' bridge. This structure effectively “locks” the
15 ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the
20 polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention 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 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
25 “cEt”) and 4'-CH(CH₂OCH₃)—O-2' (and analogs thereof; see, *e.g.*, U.S. Pat. No. 7,399,845); 4'-C(CH₃)(CH₃)—O-2' (and analogs thereof; see *e.g.*, US Patent No. 8,278,283); 4'-CH₂—N(OCH₃)-2' (and analogs thereof; see *e.g.*, US Patent No. 8,278,425); 4'-CH₂—O—N(CH₃)-2' (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4'-CH₂—N(R)—O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting
30 group (see, *e.g.*, U.S. Pat. No. 7,427,672); 4'-CH₂—C(H)(CH₃)-2' (see, *e.g.*, Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂—C(=CH₂)-2' (and analogs thereof; see, *e.g.*, US Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

35 Additional representative U.S. Patents and US Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133; 7,084,125; 7,399,845; 7,427,672; 7,569,686;

7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

Any 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 WO 99/14226).

The RNA of an iRNA can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)-O-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

An iRNA of the invention may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2' and C4' carbons of ribose or the C3 and -C5' carbons of ribose. CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, US Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"-phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

Other modifications of the nucleotides of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, *e.g.*, a 5'-terminal phosphate or phosphate mimic on the antisense strand of an RNAi agent. Suitable phosphate mimics are disclosed in, for example US Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

IV. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the RNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the iRNA. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, (1989)

Proc. Natl. Acad. Sci. USA, 86: 6553-6556), cholic acid (Manoharan *et al.*, (1994) *Biorg. Med. Chem. Lett.*, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, (1992) *Ann. N.Y. Acad. Sci.*, 660:306-309; Manoharan *et al.*, (1993) *Biorg. Med. Chem. Lett.*, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, (1992) *Nucl. Acids Res.*, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, (1991) *EMBO J*, 10:1111-1118; Kabanov *et al.*, (1990) *FEBS Lett.*, 259:327-330; Svinarchuk *et al.*, (1993) *Biochimie*, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al.*, (1995) *Tetrahedron Lett.*, 36:3651-3654; Shea *et al.*, (1990) *Nucl. Acids Res.*, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, (1995) *Nucleosides & Nucleotides*, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, (1995) *Tetrahedron Lett.*, 36:3651-3654), a palmityl moiety (Mishra *et al.*, (1995) *Biochim. Biophys. Acta*, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, (1996) *J. Pharmacol. Exp. Ther.*, 277:923-937).

In one embodiment, a ligand alters the distribution, targeting or lifetime of an iRNA agent into which it is incorporated. In preferred embodiments a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. Preferred ligands will not take part in duplex pairing in a duplexed nucleic acid.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylglucosamine, N-acetylgalactosamine or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolid) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid or protein, *e.g.*, an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, mucin carbohydrate, multivalent lactose, monovalent or multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic. In certain embodiments, ligands include monovalent or multivalent galactose. In certain

embodiments, ligands include cholesterol.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine), and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, [MPEG]₂, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF- κ B.

The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, and/or intermediate filaments. The drug can be, for example, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins *etc.* Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin *etc.* Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

Ligand-conjugated oligonucleotides of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied BiosystemsTM (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In the ligand-conjugated oligonucleotides and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites

and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A. Lipid Conjugates

In one embodiment, the ligand or conjugate is a lipid or lipid-based molecule.

- 5 Such a lipid or lipid-based molecule preferably binds a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, neproxin or aspirin can be used. A lipid
10 or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, and/or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

- A lipid based ligand can be used to inhibit, *e.g.*, control the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA
15 more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

- In a preferred embodiment, the lipid based ligand binds HSA. Preferably, it binds HSA with a sufficient affinity such that the conjugate will be preferably
20 distributed to a non-kidney tissue. However, it is preferred that the affinity not be so strong that the HSA-ligand binding cannot be reversed.

- In another preferred embodiment, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be preferably distributed to the kidney. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid
25 based ligand.

- In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, *e.g.*, of the malignant or non-malignant type, *e.g.*, cancer cells. Exemplary vitamins include vitamin A, E, and K. Other
30 exemplary vitamins include are B vitamin, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

B. Cell Permeation Agents

- In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-
35 permeation agent. Preferably, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopodia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and

use of D-amino acids. The helical agent is preferably an alpha-helical agent, which preferably has a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined
5 three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

10 A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic
15 MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 25). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 26) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell
20 membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO: 27) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 28) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC)
25 combinatorial library (Lam *et al.*, Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or
30 direct conformational properties. Any of the structural modifications described below can be utilized.

An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.*, glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidomimetics may
35 include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Preferred conjugates of this ligand target PECAM-1 or VEGF.

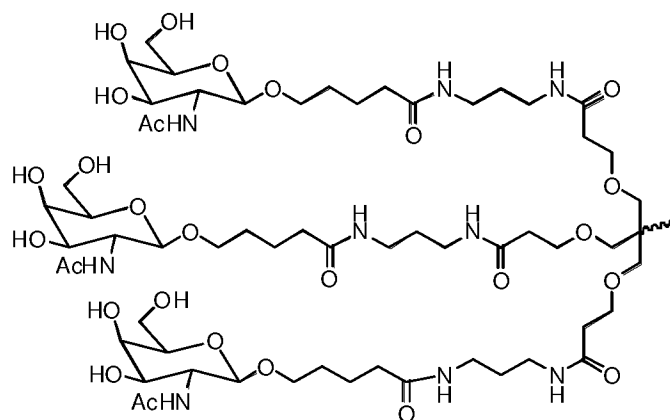
A “cell permeation peptide” is capable of permeating a cell, *e.g.*, a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, a α -helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β -defensin or bactenecin), or a peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni *et al.*, Nucl. Acids Res. 31:2717-2724, 2003).

C. Carbohydrate Conjugates

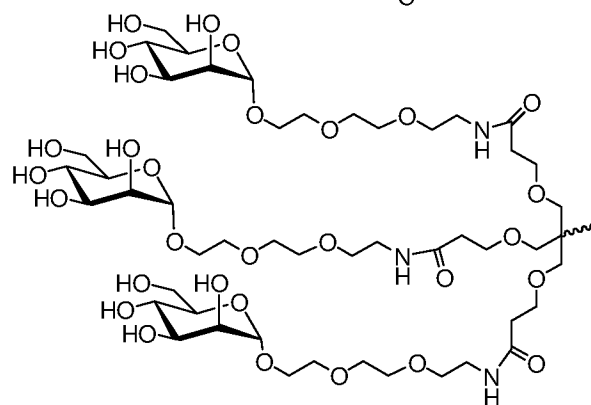
In some embodiments of the compositions and methods of the invention, an iRNA oligonucleotide further comprises a carbohydrate. The carbohydrate conjugated iRNA are advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, “carbohydrate” refers to a compound which is either a carbohydrate *per se* made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen, or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen, or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (*e.g.*, C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (*e.g.*, C5, C6, C7, or C8).

In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide.

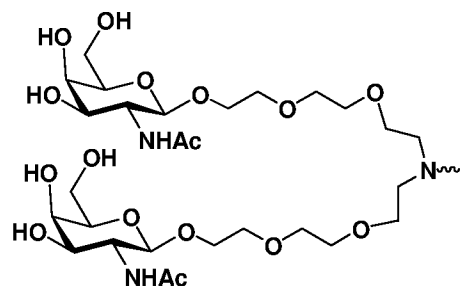
In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



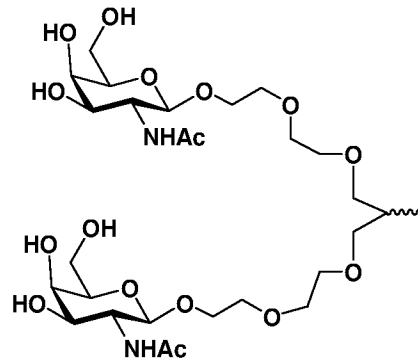
Formula II,



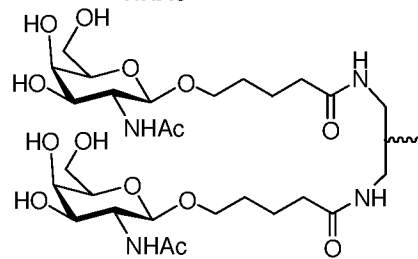
Formula III,



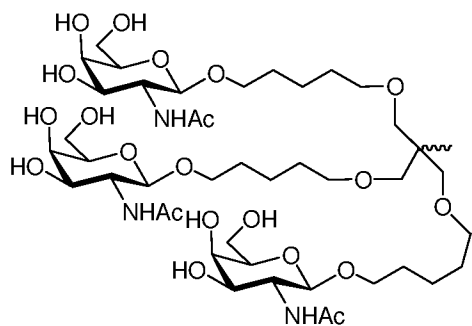
Formula IV,



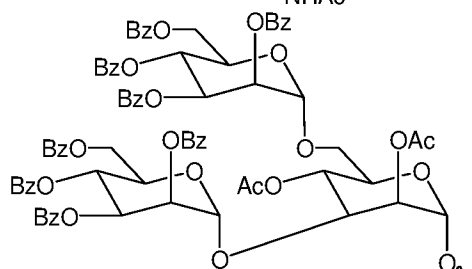
Formula V,



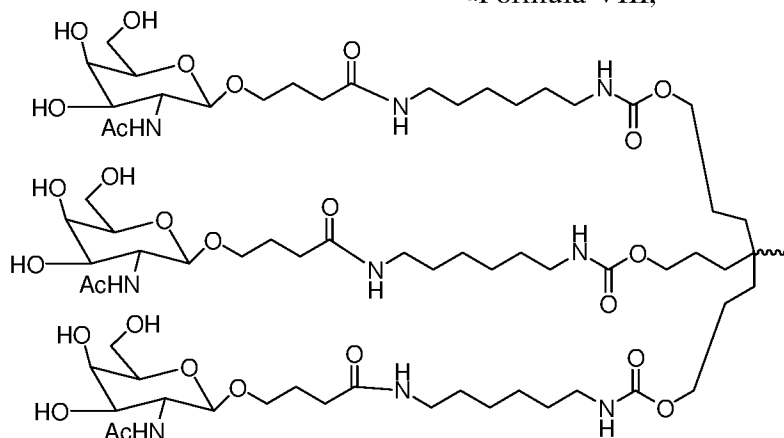
Formula VI,



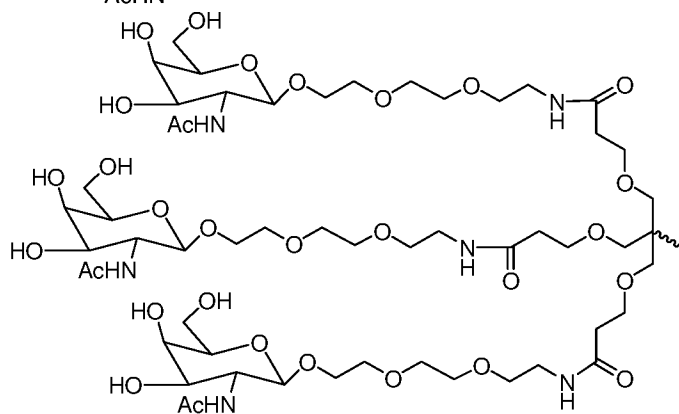
Formula VII,



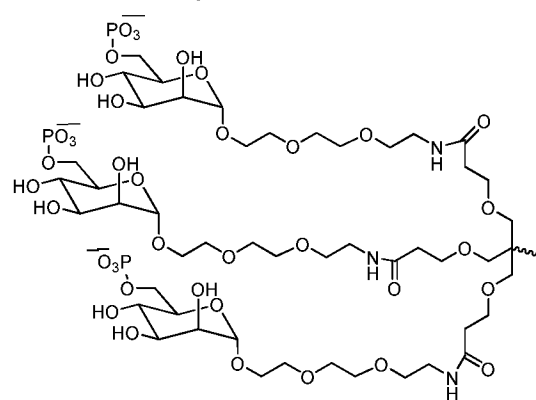
Formula VIII,



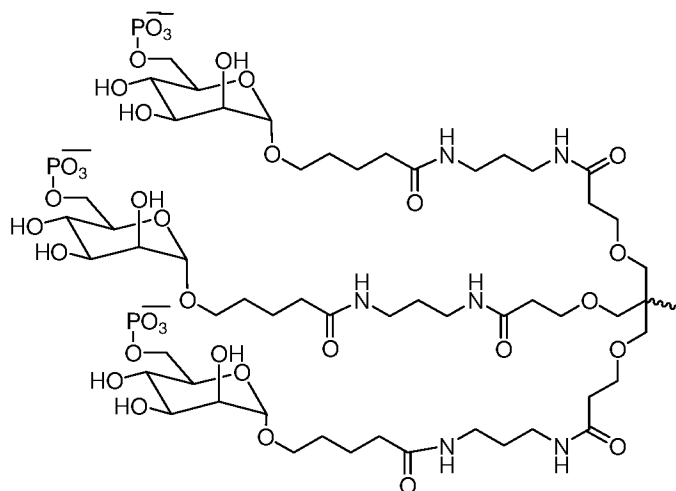
Formula IX,



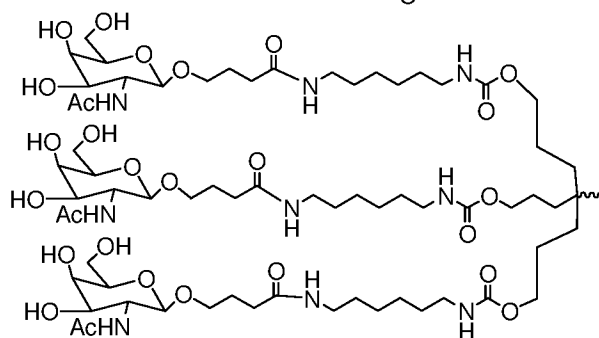
Formula X,



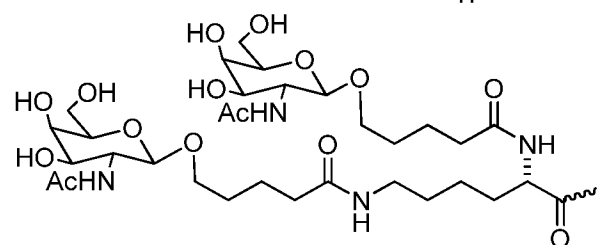
Formula XI,



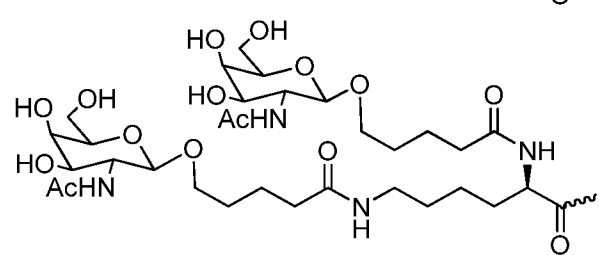
Formula XII,



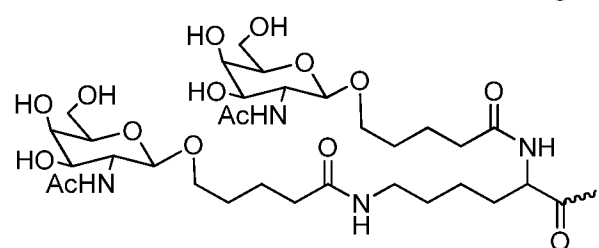
Formula XIII,



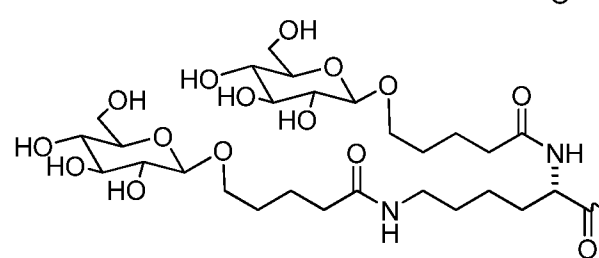
Formula XIV,



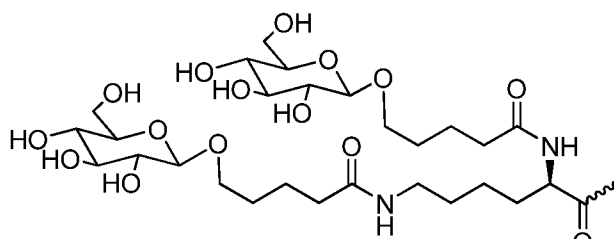
Formula XV,



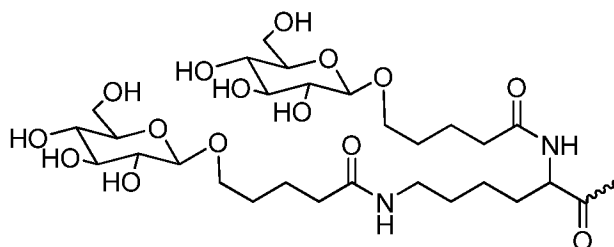
Formula XVI,



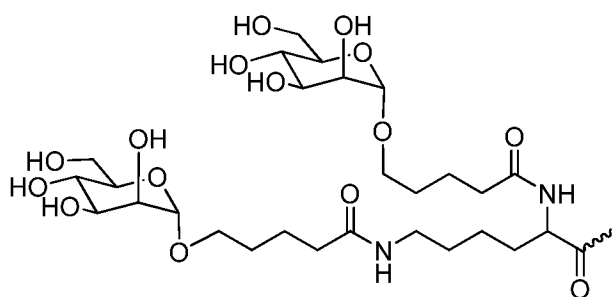
Formula XVII,



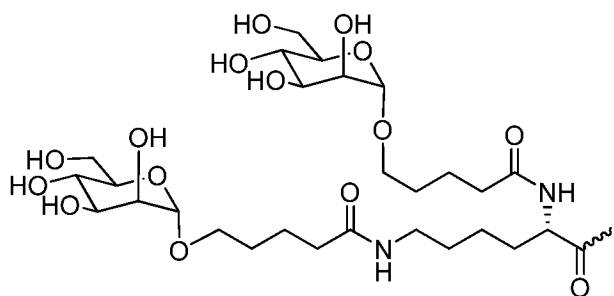
Formula XVIII,



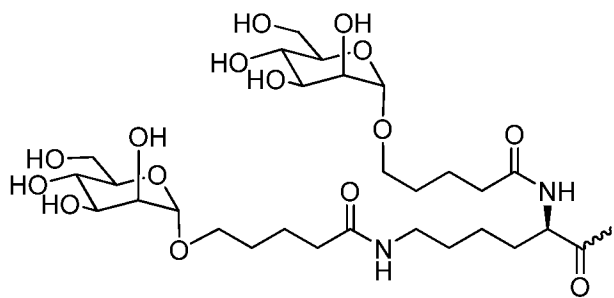
Formula XIX,



Formula XX,



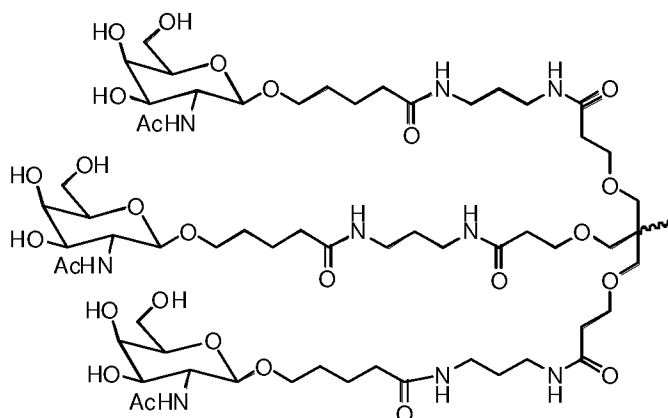
Formula XXI,



Formula XXII.

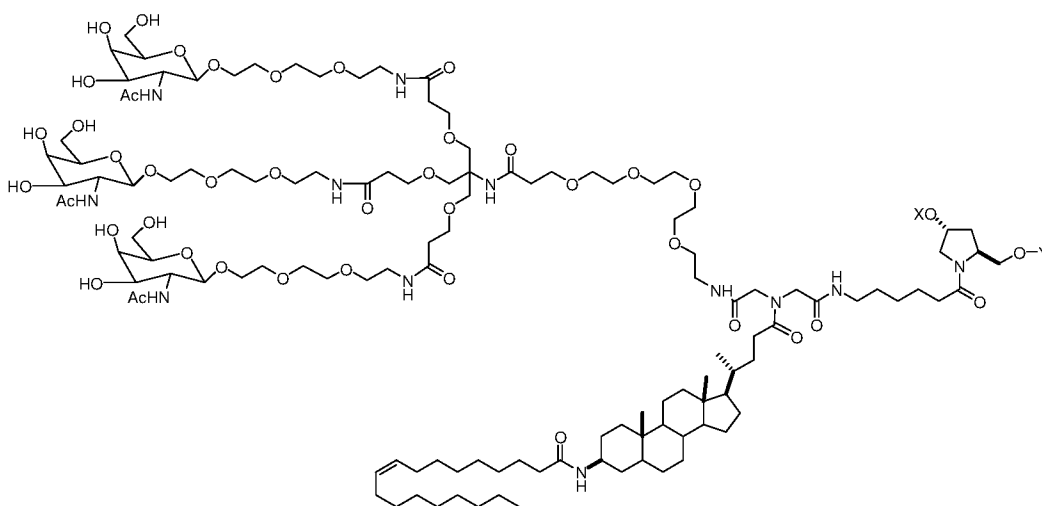
5

In one embodiment, the monosaccharide is an N-acetylgalactosamine, such as



Formula II.

Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



5 (Formula XXIII),

when one of X or Y is an oligonucleotide, the other is a hydrogen.

In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a trivalent linker.

In one embodiment, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In another embodiment, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each

unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator and/or a cell permeation peptide.

Additional carbohydrate conjugates (and linkers) suitable for use in the present invention include those described in PCT Publication Nos. WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.

10 D. Linkers

In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non cleavable.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.*, covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR₈, C(O), C(O)NH, SO, SO₂, SO₂NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclalkyl, heterocyclalkenyl, heterocyclalkynyl, aryl, heteroaryl, heterocycl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclalkyl, alkylheterocyclalkenyl, alkylheterocyclalkynyl, alkenylheterocyclalkyl, alkenylheterocyclalkenyl, alkenylheterocyclalkynyl, alkynylheterocyclalkyl, alkynylheterocyclalkenyl, alkynylheterocyclalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylheteroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO₂, N(R₈), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R₈ is hydrogen, acyl, aliphatic or substituted aliphatic. In one embodiment, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-17, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80

times, 90 times or more, or at least about 100 times faster in a target cell or under a first reference condition (which can, *e.g.*, be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, *e.g.*, be selected to mimic or represent conditions found in the blood or serum).

5 Cleavable linking groups are susceptible to cleavage agents, *e.g.*, pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, *e.g.*, oxidative or
10 reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, *e.g.*, those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

15 A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a cationic
20 lipid from the ligand inside the cell, or into the desired compartment of the cell.

 A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and
25 therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

 Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

30 In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a
35 second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to

make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

i. Redox cleavable linking groups

In one embodiment, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable “reductively cleavable linking group,” or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents known in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

ii. Phosphate-based cleavable linking groups

In another embodiment, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S-. Preferred embodiments are -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -S-P(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O-, -S-P(S)(H)-O-, -S-P(O)(H)-S-, -O-P(S)(H)-S-. A preferred embodiment is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

In another embodiment, a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (*e.g.*, about 6.0, 5.75, 5.5, 5.25, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula $-C=NN-$, $C(O)O-$, or $-OC(O)-$. A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

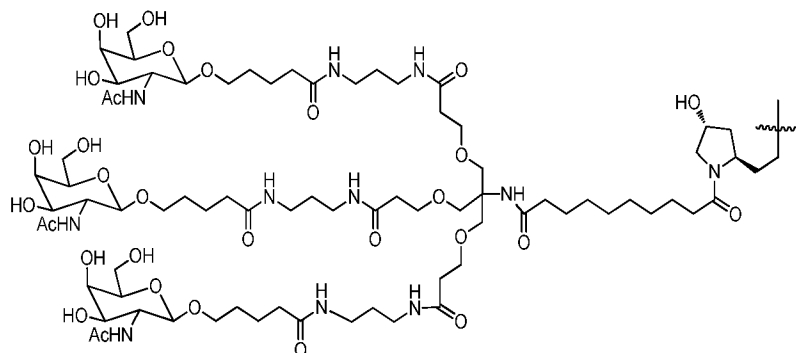
iv. Ester-based linking groups

In another embodiment, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene, and alkynylene groups. Ester cleavable linking groups have the general formula $-C(O)O-$, or $-OC(O)-$. These candidates can be evaluated using methods analogous to those described above.

v. Peptide-based cleaving groups

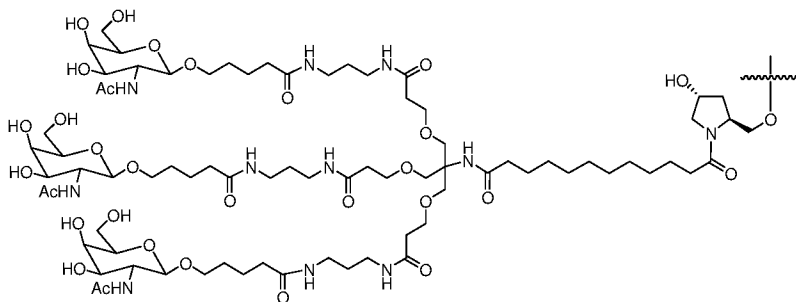
In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides, etc.) and polypeptides. Peptide-based cleavable groups do not include the amide group ($-C(O)NH-$). The amide group can be formed between any alkylene, alkenylene, or alkynylene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula $-NHCH(RA)C(O)NHCH(RB)C(O)-$, where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

In one embodiment, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,

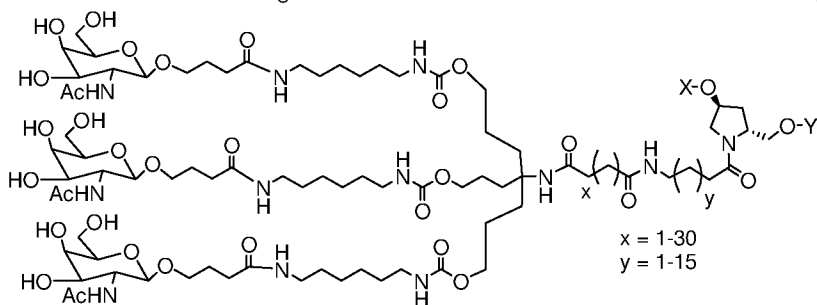


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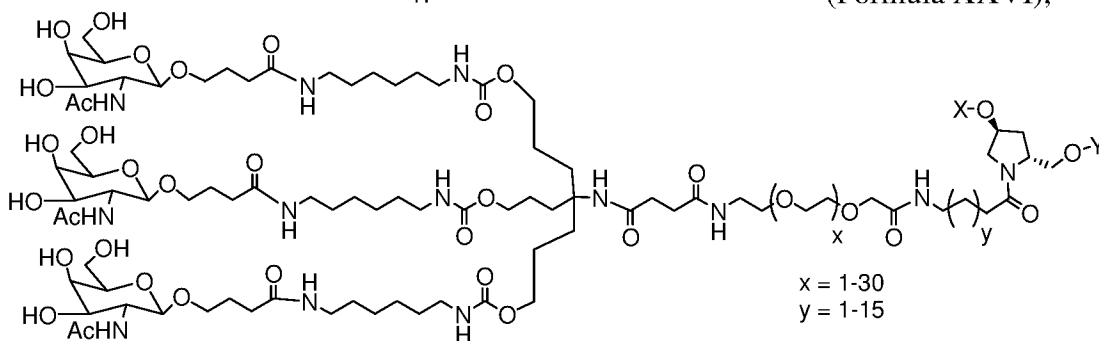
(Formula XXIV),



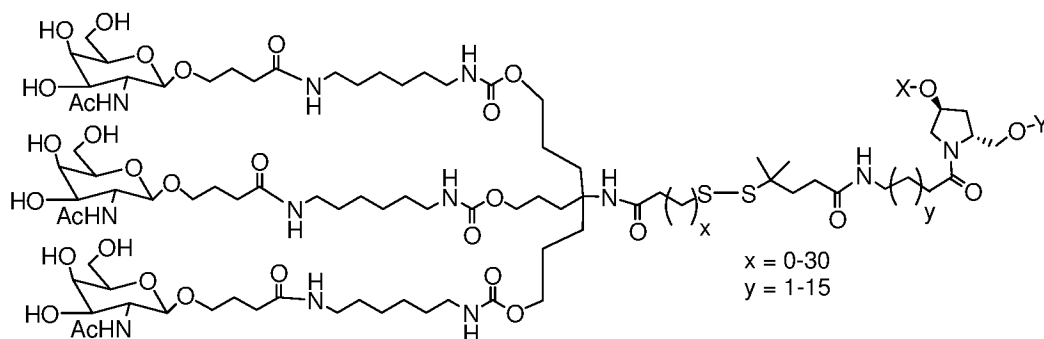
(Formula XXV),



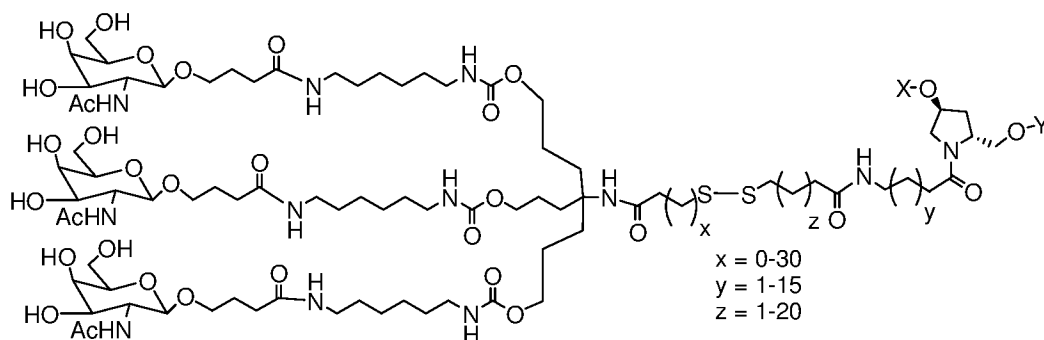
(Formula XXVI),



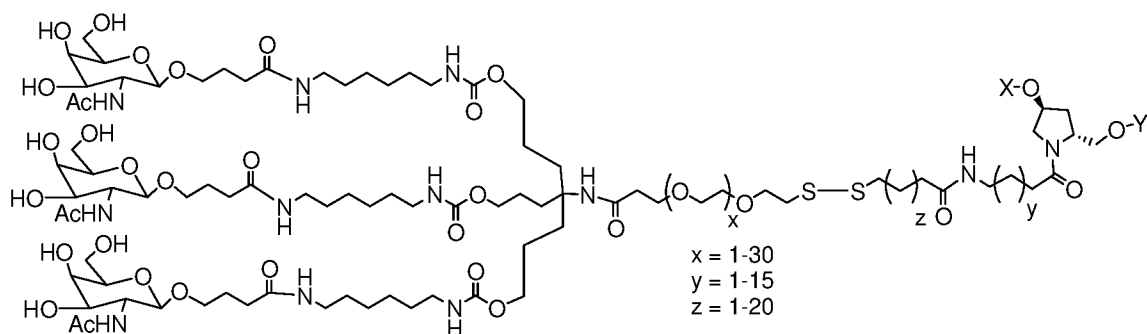
10 (Formula XXVII),



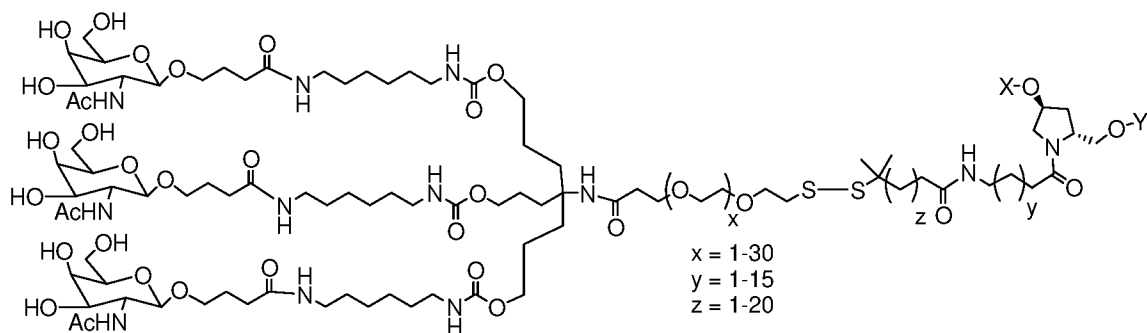
(Formula XXVIII),



5 (Formula XXIX),



(Formula XXX), and



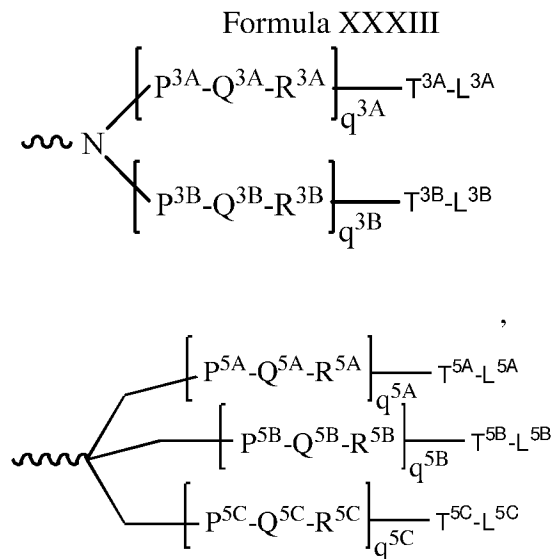
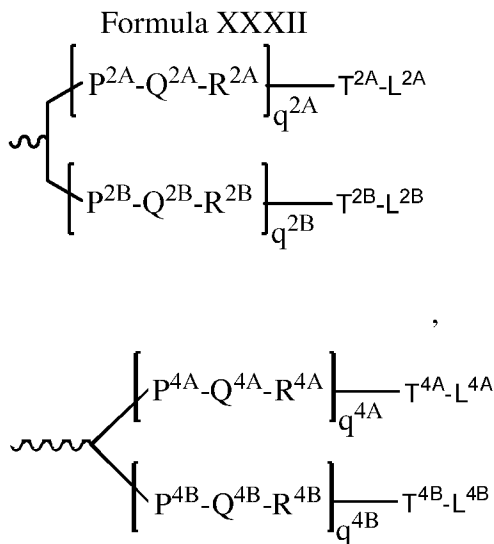
10

(Formula XXXI), when one of X or Y is an oligonucleotide, the other is a hydrogen.

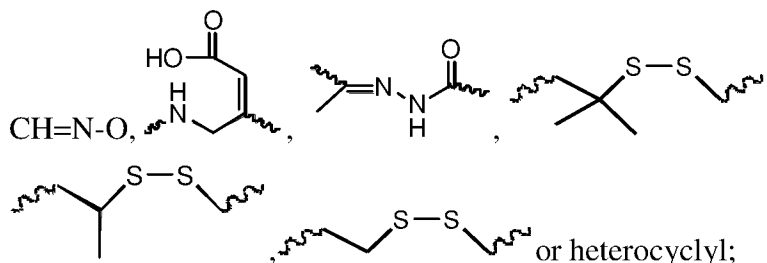
In certain embodiments of the compositions and methods of the invention, a ligand is one or more GalNAc (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XXXII) – (XXXV):

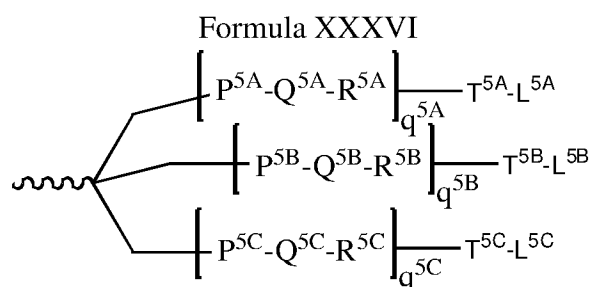
5



- 10 wherein:
 q^{2A} , q^{2B} , q^{3A} , q^{3B} , q^{4A} , q^{4B} , q^{5A} , q^{5B} and q^{5C} represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;
 P^{2A} , P^{2B} , P^{3A} , P^{3B} , P^{4A} , P^{4B} , P^{5A} , P^{5B} , P^{5C} , T^{2A} , T^{2B} , T^{3A} , T^{3B} , T^{4A} , T^{4B} , T^{5A} , T^{5B} , T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH₂,
 15 CH₂NH or CH₂O;
 Q^{2A} , Q^{2B} , Q^{3A} , Q^{3B} , Q^{4A} , Q^{4B} , Q^{5A} , Q^{5B} , Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO₂, N(R^N), C(R')=C(R''), C≡C or C(O);
 R^{2A} , R^{2B} , R^{3A} , R^{3B} , R^{4A} , R^{4B} , R^{5A} , R^{5B} , R^{5C} are each independently for each occurrence
 20 absent, NH, O, S, CH₂, C(O)O, C(O)NH, NHCH(R^a)C(O), -C(O)-CH(R^a)-NH-, CO,



L^{2A} , L^{2B} , L^{3A} , L^{3B} , L^{4A} , L^{4B} , L^{5A} , L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; and R^a is H or amino acid side chain. Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (XXXVI):



wherein L^{5A} , L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II, VII, XI, X, and XIII.

Representative U.S. patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an iRNA. The present invention also includes iRNA compounds that are chimeric compounds.

“Chimeric” iRNA compounds or “chimeras,” in the context of this invention, are iRNA compounds, preferably dsRNAs, which contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide in the case of a

dsRNA compound. These iRNAs typically contain at least one region wherein the RNA is modified so as to confer upon the iRNA increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the iRNA can serve as a substrate for enzymes capable of

5 cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of iRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter iRNAs when chimeric dsRNAs are used, compared to

10 phosphorothioate deoxy dsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to

15 enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. *et al.*, Biochem. Biophys. Res. Comm., 2007, 365(1):54-61; Letsinger *et al.*, Proc. Natl. Acad. Sci. USA, 1989, 86:6553), cholic acid (Manoharan *et al.*, Bioorg. Med. Chem. Lett.,

20 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al.*, Ann. N.Y. Acad. Sci., 1992, 660:306; Manoharan *et al.*, Bioorg. Med. Chem. Lett., 1993, 3:2765), a thiocholesterol (Oberhauser *et al.*, Nucl. Acids Res., 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, EMBO J., 1991, 10:111; Kabanov *et al.*, FEBS Lett., 1990, 259:327; Svinarchuk *et al.*, Biochimie, 1993, 75:49),

25 a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, Tetrahedron Lett., 1995, 36:3651; Shea *et al.*, Nucl. Acids Res., 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, Nucleosides & Nucleotides, 1995, 14:969), or adamantane acetic acid (Manoharan *et al.*, Tetrahedron Lett., 1995, 36:3651), a palmityl

30 moiety (Mishra *et al.*, Biochim. Biophys. Acta, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke *et al.*, J. Pharmacol. Exp. Ther., 1996, 277:923). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of an RNAs bearing an amino linker at one or more positions of the sequence.

35 The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

IV. Delivery of an iRNA of the Invention

The delivery of an iRNA of the invention to a cell *e.g.*, a cell within a subject, such as a human subject (*e.g.*, a subject in need thereof, such as a subject having a glycogen storage disease (GSD), *e.g.*, type Ia GSD) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an iRNA of the invention either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an iRNA, *e.g.*, a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the iRNA. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an iRNA of the invention (see *e.g.*, Akhtar S. and Julian RL., (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. The non-specific effects of an iRNA can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the iRNA molecule to be administered. Several studies have shown successful knockdown of gene products when an iRNA is administered locally. For example, intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, MJ. *et al.*, (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, SJ. *et al.* (2003) *Mol. Vis.* 9:210-216) were both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of a dsRNA in mice reduces tumor volume (Pille, J. *et al.* (2005) *Mol. Ther.* 11:267-274) and can prolong survival of tumor-bearing mice (Kim, WJ. *et al.*, (2006) *Mol. Ther.* 14:343-350; Li, S. *et al.*, (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G. *et al.*, (2004) *Nucleic Acids* 32:e49; Tan, PH. *et al.* (2005) *Gene Ther.* 12:59-66; Makimura, H. *et al.* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al.* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al.* (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya, Y., *et al.* (2005) *J. Neurophysiol.* 93:594-602) and to the lungs by intranasal administration (Howard, KA. *et al.*, (2006) *Mol. Ther.* 14:476-484; Zhang, X. *et al.*, (2004) *J. Biol. Chem.* 279:10677-

10684; Bitko, V. *et al.*, (2005) *Nat. Med.* 11:50-55). For administering an iRNA systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-nucleases in vivo. Modification of the

5 RNA or the pharmaceutical carrier can also permit targeting of the iRNA composition to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and

10 resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J. *et al.*, (2004) *Nature* 432:173-178). Conjugation of an iRNA to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, JO. *et al.*, (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the iRNA can be delivered using drug delivery systems such as

15 a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an iRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or

20 micelle (see *e.g.*, Kim SH. *et al.*, (2008) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al.* (2003) *J. Mol. Biol* 327:761-766; Verma, UN. *et al.*, (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS *et al.*, (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-

25 limiting examples of drug delivery systems useful for systemic delivery of iRNAs include DOTAP (Sorensen, DR., *et al.* (2003), *supra*; Verma, UN. *et al.*, (2003), *supra*), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS. *et al.*, (2006) *Nature* 441:111-114), cardiolipin (Chien, PY. *et al.*, (2005) *Cancer Gene Ther.* 12:321-328; Pal, A. *et al.*, (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME. *et al.*, (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA. *et al.*, (2007) *Biochem. Soc. Trans.* 35:61-67;

35 Yoo, H. *et al.*, (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be

found in U.S. Patent No. 7, 427, 605, which is herein incorporated by reference in its entirety.

A. Vector encoded iRNAs of the Invention

iRNA targeting the GCK gene can be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), 12:5-10; Skillern, A., *et al.*, International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, U.S. Pat. No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, (1995) *Proc. Natl. Acad. Sci. USA* 92:1292).

The individual strand or strands of an iRNA can be transcribed from a promoter on an expression vector. Where two separate strands are to be expressed to generate, for example, a dsRNA, two separate expression vectors can be co-introduced (*e.g.*, by transfection or infection) into a target cell. Alternatively each individual strand of a dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as inverted repeat polynucleotides joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

iRNA expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of an iRNA as described herein. Eukaryotic cell expression vectors are well known in the art and are available from a number of commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired nucleic acid segment. Delivery of iRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, *etc.*; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-

defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of an iRNA will generally require regulatory elements, *e.g.*, promoters, enhancers, *etc.*, to ensure the expression of the iRNA in target cells. Other aspects to consider for vectors and constructs are known in the art.

V. Pharmaceutical Compositions of the Invention

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for treating a disease or disorder associated with the expression or activity of a GCK gene, such as, a glycogen storage disease (GSD), *e.g.*, type Ia GSD, or one or more signs or symptoms of GSD, such as hypoglycemia, lactic acidosis, hyperuricemia, hyperlipidemia, hepatomegaly, kidney disease as a result of glycogen accumulation, hunger, jitteriness, lethargy, apnea, seizures, diaphoresis, confusion, headaches, dizziness, unusual mood or behavior changes, loss of consciousness, coma, muscle cramps, bleeding diathesis, short stature, osteoporosis, delayed puberty, gout, renal disease, systemic hypertension, pulmonary hypertension, hepatic adenomas, pancreatitis, anemia, vitamin D deficiency, polycystic ovaries, irregular menstrual cycles, menorrhagia, and eruptive xanthomata.

Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery, *e.g.*, by intravenous (IV) or for subcutaneous delivery. Another example is compositions that are formulated for direct delivery into the liver, *e.g.*, by infusion into the liver, such as by continuous pump infusion.

The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a GCK gene. In general, a suitable dose of an iRNA of the invention will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. Typically, a suitable dose of an iRNA of the invention will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, preferably about 0.3 mg/kg and about 3.0 mg/kg. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as every other day or once a year. In certain embodiments, the iRNA is administered about once per month to about once per quarter (*i.e.*, about once every three months).

After an initial treatment regimen, the treatments can be administered on a less frequent basis.

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and *in vivo* half-lives for the individual iRNAs encompassed by the invention can be made using conventional methodologies or on the basis of *in vivo* testing using an appropriate animal model, as described elsewhere herein.

Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as disorders of excess glucose that would benefit from reduction in the expression of GCK.

The 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 topical (*e.g.*, by a transdermal patch), pulmonary, *e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; subdermal, *e.g.*, via an implanted device; or intracranial, *e.g.*, by intraparenchymal, intrathecal or intraventricular, administration.

The iRNA can be delivered in a manner to target a particular tissue, such as the liver (*e.g.*, the hepatocytes of the liver).

Pharmaceutical compositions and formulations for topical administration can include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids, and powders. Conventional pharmaceutical carriers, aqueous, powder, or oily bases, thickeners and the like can be necessary or desirable. Coated condoms, gloves and the like can also be useful. Suitable topical formulations include those in which the iRNAs featured in the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents, and surfactants. Suitable lipids and liposomes include neutral (*e.g.*, dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline), negative (*e.g.*, dimyristoylphosphatidyl glycerol DMPG), and cationic (*e.g.*, dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). iRNAs featured in the invention can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, iRNAs

can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, monoolein, dilaurin, glyceryl 1-monocaprinate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C₁₋₂₀ alkyl ester (e.g., isopropylmyristate IPM), monoglyceride or diglyceride; or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in U.S. Patent No. 6,747,014, which is incorporated herein by reference.

A. iRNA Formulations Comprising Membranous Molecular Assemblies

10 An iRNA for use in the compositions and methods of the invention can be formulated for delivery in a membranous molecular assembly, e.g., a liposome or a micelle. As used herein, the term “liposome” refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, e.g., one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a
15 membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the iRNA composition. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the iRNA composition, although in some examples, it may. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane
20 is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the iRNA are delivered into the cell where the iRNA can specifically bind to a target RNA and can mediate RNAi. In some cases the liposomes are also specifically targeted, e.g., to direct
25 the iRNA to particular cell types.

A liposome containing a RNAi agent can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical
30 micelle concentration and may be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The RNAi agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the RNAi agent and condense around the RNAi agent to form a liposome. After condensation, the detergent is removed, e.g., by dialysis, to yield
35 a liposomal preparation of RNAi agent.

If necessary a carrier compound that assists in condensation can be added during the condensation reaction, *e.g.*, by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (*e.g.*, spermine or spermidine). pH can also adjusted to favor condensation.

5 Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as structural components of the delivery vehicle, are further described in, *e.g.*, WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Felgner, P. L. *et al.*, (1987) *Proc. Natl. Acad. Sci. USA* 8:7413-7417; U.S. Pat. No. 4,897,355; U.S. Pat. No. 5,171,678; 10 Bangham *et al.*, (1965) *M. Mol. Biol.* 23:238; Olson *et al.*, (1979) *Biochim. Biophys. Acta* 557:9; Szoka *et al.*, (1978) *Proc. Natl. Acad. Sci.* 75: 4194; Mayhew *et al.*, (1984) *Biochim. Biophys. Acta* 775:169; Kim *et al.*, (1983) *Biochim. Biophys. Acta* 728:339; and Fukunaga *et al.*, (1984) *Endocrinol.* 115:757. Commonly used techniques for 15 preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, *e.g.*, Mayer *et al.*, (1986) *Biochim. Biophys. Acta* 858:161. Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew *et al.*, (1984) *Biochim. Biophys. Acta* 775:169. These methods are readily adapted to packaging RNAi agent 20 preparations into liposomes.

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged nucleic acid molecules to form a stable complex. The positively charged nucleic acid/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH 25 within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang *et al.* (1987) *Biochem. Biophys. Res. Commun.*, 147:980-985).

Liposomes, which are pH-sensitive or negatively charged, entrap nucleic acids rather than complex with them. Since both the nucleic acid and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some nucleic 30 acid is entrapped within the aqueous interior of these liposomes. pH sensitive liposomes have been used to deliver nucleic acids encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou *et al.* (1992) *Journal of Controlled Release*, 19:269-274).

One major type of liposomal composition includes phospholipids other than 35 naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed

primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

5 Examples of other methods to introduce liposomes into cells *in vitro* and *in vivo* include U.S. Pat. No. 5,283,185; U.S. Pat. No. 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Felgner, (1994) *J. Biol. Chem.* 269:2550; Nabel, (1993) *Proc. Natl. Acad. Sci.* 90:11307; Nabel, (1992) *Human Gene Ther.* 3:649; Gershon, (1993) *Biochem.* 32:7143; and Strauss, (1992) *EMBO J.* 11:417.

10 Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver
15 cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporine A into different layers of the skin (Hu *et al.*, (1994) *S.T.P. Pharma. Sci.*, 4(6):466).

 Liposomes also include “sterically stabilized” liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when
20 incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG)
25 moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen *et al.*, (1987) *FEBS Letters*, 223:42; Wu *et al.*, (1993) *Cancer Research*,
30 53:3765).

 Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos *et al.* (*Ann. N.Y. Acad. Sci.*, (1987), 507:64) reported the ability of monosialoganglioside G_{M1}, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by
35 Gabizon *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, (1988), 85,:6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen *et al.*, disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb *et al.*) discloses liposomes comprising sphingomyelin.

Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim *et al.*).

In one embodiment, cationic liposomes are used. Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although
5 not able to fuse as efficiently with the plasma membrane, are taken up by macrophages in vivo and can be used to deliver RNAi agents to macrophages.

Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated RNAi agents
10 in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

A positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-
15 N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in delivery of RNAi agent (see, *e.g.*, Felgner, P. L. *et al.*, (1987) *Proc. Natl. Acad. Sci. USA* 8:7413-7417, and U.S. Pat. No. 4,897,355 for a description
20 of DOTMA and its use with DNA).

A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. Lipofectin™ (Bethesda Research Laboratories, Gaithersburg, Md.) is an
25 effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional
30 nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis(oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") (Boehringer Mannheim™, Indianapolis, Indiana) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

Other reported cationic lipid compounds include those that have been conjugated
35 to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoyleamide ("DOGS") (Transfectam™, Promega™,

Madison, Wisconsin) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPES") (see, e.g., U.S. Pat. No. 5,171,678).

Another cationic lipid conjugate includes derivatization of the lipid with cholesterol ("DC-Chol") which has been formulated into liposomes in combination with
 5 DOPE (See, Gao, X. and Huang, L., (1991) *Biochim. Biophys. Res. Commun.* 179:280). Lipopolylysine, made by conjugating polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. *et al.*, (1991) *Biochim. Biophys. Acta* 1065:8). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection
 10 than the DOTMA-containing compositions. Other commercially available cationic lipid products include DMRIE and DMRIE-HP (VicalTM, La Jolla, California) and LipofectamineTM (DOSPA) (Life TechnologyTM, Inc., Gaithersburg, Maryland). Other cationic lipids suitable for the delivery of oligonucleotides are described in WO 98/39359 and WO 96/37194.

15 Liposomal formulations are particularly suited for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer RNAi agent into the skin. In some implementations, liposomes are used for
 20 delivering RNAi agent to epidermal cells and also to enhance the penetration of RNAi agent into dermal tissues, e.g., into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, e.g., Weiner *et al.*, (1992) *Journal of Drug Targeting*, vol. 2,405-410 and du Plessis *et al.*, (1992) *Antiviral Research*, 18:259-265; Mannino, R. J. and Fould-
 25 Fogerite, S., (1998) *Biotechniques* 6:682-690; Itani, T. *et al.*, (1987) *Gene* 56:267-276; Nicolau, C. *et al.* (1987) *Meth. Enzymol.* 149:157-176; Straubinger, R. M. and Papahadjopoulos, D. (1983) *Meth. Enzymol.* 101:512-527; Wang, C. Y. and Huang, L., (1987) *Proc. Natl. Acad. Sci. USA* 84:7851-7855).

Non-ionic liposomal systems have also been examined to determine their utility
 30 in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome II (glyceryl distearate/ cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver a drug into the dermis of mouse skin. Such formulations with RNAi agent are useful for treating a
 35 dermatological disorder.

Liposomes that include iRNA can be made highly deformable. Such deformability can enable the liposomes to penetrate through pore that are smaller than the average radius of the liposome. For example, transfersomes are a type of

deformable liposomes. Transfersomes can be made by adding surface edge activators, usually surfactants, to a standard liposomal composition. Transfersomes that include RNAi agent can be delivered, for example, subcutaneously by infection in order to deliver RNAi agent to keratinocytes in the skin. In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. In addition, due to the lipid properties, these transfersomes can be self-optimizing (adaptive to the shape of pores, e.g., in the skin), self-repairing, and can frequently reach their targets without fragmenting, and often self-loading. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Other formulations amenable to the present invention are described in, for example, PCT Publication No. WO 2008/042973.

Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g., they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and

ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

- 5 If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates.
- 10 The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

- If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts
- 15 are the most used members of this class.

 If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines, and phosphatides.

- The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).
- 20

- The iRNA for use in the methods of the invention can also be provided as micellar formulations. "Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such
- 25 that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

- A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of the siRNA composition,
- 30 an alkali metal C₈ to C₂₂ alkyl sulphate, and a micelle forming compounds. Exemplary micelle forming compounds include lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl
- 35 glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof. The micelle forming compounds may be added at the same time or after addition of the

alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients but vigorous mixing in order to provide smaller size micelles.

In one method a first micellar composition is prepared which contains the siRNA composition and at least the alkali metal alkyl sulphate. The first micellar composition is then mixed with at least three micelle forming compounds to form a mixed micellar composition. In another method, the micellar composition is prepared by mixing the siRNA composition, the alkali metal alkyl sulphate and at least one of the micelle forming compounds, followed by addition of the remaining micelle forming compounds, with vigorous mixing.

Phenol and/or m-cresol may be added to the mixed micellar composition to stabilize the formulation and protect against bacterial growth. Alternatively, phenol and/or m-cresol may be added with the micelle forming ingredients. An isotonic agent such as glycerin may also be added after formation of the mixed micellar composition.

For delivery of the micellar formulation as a spray, the formulation can be put into an aerosol dispenser and the dispenser is charged with a propellant. The propellant, which is under pressure, is in liquid form in the dispenser. The ratios of the ingredients are adjusted so that the aqueous and propellant phases become one, *i.e.*, there is one phase. If there are two phases, it is necessary to shake the dispenser prior to dispensing a portion of the contents, *e.g.*, through a metered valve. The dispensed dose of pharmaceutical agent is propelled from the metered valve in a fine spray.

Propellants may include hydrogen-containing chlorofluorocarbons, hydrogen-containing fluorocarbons, dimethyl ether, and diethyl ether. In certain embodiments, HFA 134a (1,1,1,2 tetrafluoroethane) may be used.

The specific concentrations of the essential ingredients can be determined by relatively straightforward experimentation. For absorption through the oral cavities, it is often desirable to increase, *e.g.*, at least double or triple, the dosage for through injection or administration through the gastrointestinal tract.

B. Lipid particles

iRNAs, *e.g.*, dsRNAs of in the invention may be fully encapsulated in a lipid formulation, *e.g.*, a LNP, or other nucleic acid-lipid particle.

As used herein, the term "LNP" refers to a stable nucleic acid-lipid particle. LNPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate). LNPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (*e.g.*, sites physically separated from the administration site). LNPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. The particles of the present invention typically have a mean diameter of

about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid-lipid particles of the present invention are resistant in aqueous solution to

5 degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.*, U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; U.S. Publication No. 2010/0324120 and PCT Publication No. WO 96/40964.

In one embodiment, the lipid to drug ratio (mass/mass ratio) (*e.g.*, lipid to dsRNA ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to
10 about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. Ranges intermediate to the above recited ranges are also contemplated to be part of the invention.

The cationic lipid can be, for example, N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleoyloxypropylamine (DODMA), 1,2-DiLinoleoyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleoxy-3-(dimethylamino)acetoxyp propane (DLin-DAC), 1,2-Dilinoleoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleoyloxy-3-dimethylaminopropane (DLin-2-DMA), 1,2-Dilinoleoxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt
25 (DLin-TAP.Cl), 1,2-Dilinoleoxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleoxy-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof,
30 (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyldidodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid can
35 comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

In another embodiment, the compound 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane can be used to prepare lipid-siRNA nanoparticles. Synthesis of 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane is described in United States provisional patent application number 61/107,998 filed on October 23, 2008, which is
 5 herein incorporated by reference.

In one embodiment, the lipid-siRNA particle includes 40% 2, 2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0 ± 20 nm and a 0.027 siRNA/Lipid Ratio.

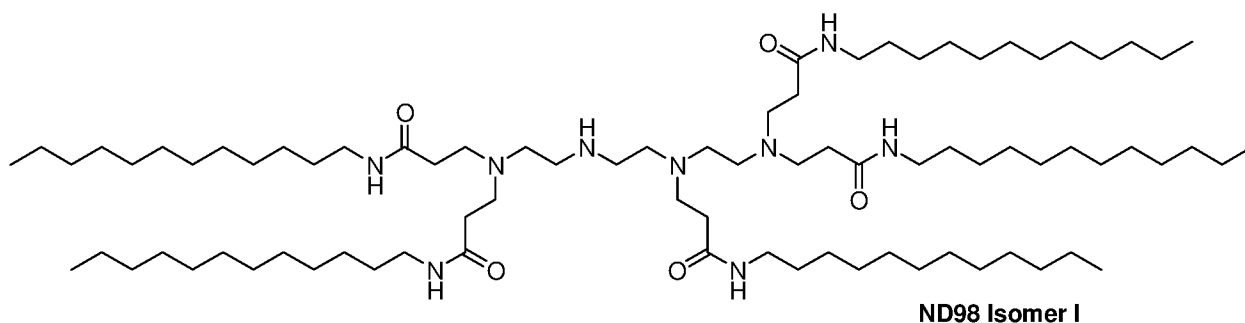
10 The ionizable/non-cationic lipid can be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyl dioleoylphosphatidylcholine (POPC), palmitoyl dioleoylphosphatidylethanolamine (POPE), dioleoyl-
 15 phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE),
 20 cholesterol; or a mixture thereof. The non-cationic lipid can be from about 5 mol % to about 90 mol %, about 10 mol %, or about 58 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol
 25 (DAG), a PEG-dialkylxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl (C_{12}), a PEG-dimyristyloxypropyl (C_{14}), a PEG-dipalmitoyloxypropyl (C_{16}), or a PEG-distearoyloxypropyl (C_{18}). The conjugated lipid that prevents aggregation of particles can be from 0 mol % to about 20 mol % or about 2 mol % of the total lipid
 30 present in the particle.

In some embodiments, the nucleic acid-lipid particle further includes cholesterol at, e.g., about 10 mol % to about 60 mol % or about 48 mol % of the total lipid present in the particle.

In one embodiment, the lipidoid ND98·4HCl (MW 1487) (see US20090023673, filed 3/26/2008, which is incorporated herein by reference), Cholesterol (Sigma-AldrichTM), and PEG-Ceramide C16 (AvantiTM Polar Lipids) can be used to prepare lipid-dsRNA nanoparticles (*i.e.*, LNP01 particles). Stock solutions of each in ethanol can be prepared as follows: ND98, 133 mg/ml; cholesterol, 25 mg/ml, PEG-Ceramide
 35

C16, 100 mg/ml. The ND98, cholesterol, and PEG-Ceramide C16 stock solutions can then be combined in a, *e.g.*, 42:48:10 molar ratio. The combined lipid solution can be mixed with aqueous dsRNA (*e.g.*, in sodium acetate pH 5) such that the final ethanol concentration is about 35-45% and the final sodium acetate concentration is about 100-300 mM. Lipid-dsRNA nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (*e.g.*, 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion step can be omitted. Ethanol removal and simultaneous buffer exchange can be accomplished by, for example, dialysis or tangential flow filtration. Buffer can be exchanged with, for example, phosphate buffered saline (PBS) at about pH 7, *e.g.*, about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, or about pH 7.4.



Formula 1

LNP01 formulations are described, *e.g.*, in International Application Publication No. WO 2008/042973, which is hereby incorporated by reference.

Additional exemplary lipid-dsRNA formulations are described in the table below.

	Ionizable/Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio
SNALP-1	1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1
2-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA ~ 7:1

	Ionizable/Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1)	Tech G1/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:siRNA: 11:1

	Ionizable/Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio
LNP15	MC3	MC3/DSPC/Chol/PEG-DSG/GalNAc-PEG-DSG 50/10/35/4.5/0.5 Lipid:siRNA: 11:1
LNP16	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP17	MC3	MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP18	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 12:1
LNP19	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:siRNA: 8:1
LNP20	MC3	MC3/DSPC/Chol/PEG-DPG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP21	C12-200	C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP22	XTC	XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

PEG-DMG: PEG-didimyristoyl glycerol (C14-PEG, or PEG-C14) (PEG with avg
5 mol wt of 2000)

PEG-DSG: PEG-distyryl glycerol (C18-PEG, or PEG-C18) (PEG with avg mol wt of 2000)

PEG-cDMA: PEG-carbamoyl-1,2-dimyristyloxypropylamine (PEG with avg mol wt of 2000) SNALP (1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA))

comprising formulations are described in International Publication No.

WO2009/127060, filed April 15, 2009, which is hereby incorporated by reference.

XTC comprising formulations are described in, for example, PCT Publication No. WO 2010/088537, the entire contents of which are hereby incorporated herein by reference.

MC3 comprising formulations are described, *e.g.*, in U.S. Publication No. 2010/0324120, filed June 10, 2010, the entire contents of which are hereby incorporated by reference.

ALNY-100 comprising formulations are described in, for example, PCT Publication No. WO 2010/054406, the entire contents of which are hereby incorporated herein by reference.

C12-200 comprising formulations are described in, for example, PCT Publication No. WO 2010/129709, the entire contents of which are hereby incorporated herein by reference.

Compositions and formulations for oral administration include 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 some embodiments, oral formulations are those in which dsRNAs featured in the invention are administered in conjunction with one or more penetration enhancer surfactants and chelators. Suitable surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucolic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride or a diglyceride; or a pharmaceutically acceptable salt thereof (*e.g.*, sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the invention can be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes,

polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, 5 polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (*e.g.*, p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcyanoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, 10 poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Patent 6,887,906, US Publn. No. 20030027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

Compositions and formulations for parenteral, intraparenchymal (into the brain), 15 intrathecal, intraventricular, or intrahepatic 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.

Pharmaceutical compositions of the present invention include, but are not limited 20 to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids, and self-emulsifying semisolids. Particularly preferred are formulations that target the liver when treating hepatic disorders such as hepatic carcinoma.

25 The pharmaceutical formulations of the present invention, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by 30 uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid 35 syrups, soft gels, suppositories, and enemas. The compositions of the present invention can also be formulated as suspensions in aqueous, non-aqueous, or mixed media. Aqueous suspensions can further contain substances which increase the viscosity of the

suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension can also contain stabilizers.

C. Additional Formulations

i. Emulsions

5 The compositions of the present invention can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, 10 in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi 15 *et al.*, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting 20 composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution in either aqueous phase, oily phase or itself as a separate phase. 25 Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do 30 not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the 35 dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion can be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of

stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories:

synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin, and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments, and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman,

Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols, and phosphatides that can readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used can be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (see *e.g.*, Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of ease of formulation, as well as efficacy from an absorption and bioavailability standpoint (see *e.g.*, Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins, and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

ii. Microemulsions

In one embodiment of the present invention, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, BrijTM 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750),

decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, or 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions can, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase can typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase can include, but is not limited to, materials such as Captex™ 300, Captex™ 355, Capmul™ MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils, and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (see *e.g.*, U.S. Patent Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides *et al.*, *Pharmaceutical Research*, 1994, 11, 1385-1390; Ritschel, *Meth. Find. Exp. Clin. Pharmacol.*, 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (see *e.g.*, U.S. Patent Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides *et al.*, *Pharmaceutical Research*, 1994, 11, 1385; Ho *et al.*, *J. Pharm. Sci.*, 1996, 85, 138-143). Often microemulsions can form spontaneously when their components are brought together at ambient temperature. This can be particularly advantageous when formulating thermolabile drugs, peptides or iRNAs. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of iRNAs and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of iRNAs and nucleic acids.

Microemulsions of the present invention can also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the iRNAs and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention can be classified as belonging to one of five

broad categories--surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Each of these classes has been discussed above.

iii. Microparticles

5 an RNAi agent of the invention may be incorporated into a particle, *e.g.*, a microparticle. Microparticles can be produced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

iv. Penetration Enhancers

10 In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly iRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the
15 membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers can be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating
20 non-surfactants (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92). Such compounds are well known in the art.

v. Carriers

Certain compositions of the present invention also incorporate carrier compounds
25 in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (*i.e.*, does not possess biological activity per se) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The
30 coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney, or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate dsRNA in hepatic tissue can
35 be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'-isothiocyano-stilbene-2,2'-disulfonic acid (Miyao *et al.*, DsRNA Res. Dev., 1995, 5, 115-121; Takakura *et al.*, DsRNA & Nucl. Acid Drug Dev., 1996, 6, 177-183.

vi. *Excipients*

In contrast to a carrier compound, a “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 is 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 suitable for non-parenteral administration which do not deleteriously react with nucleic acids 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.

Formulations for topical administration of nucleic acids can include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions can also contain buffers, diluents, and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone, and the like.

vii. *Other Components*

The compositions of the present invention can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of

the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents, and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if
5 desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings, and/or aromatic substances; and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

10 Aqueous suspensions can contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol, and/or dextran. The suspension can also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA compounds and (b) one or more agents which function by a non-RNAi mechanism and which are useful in treating a GCK-associated disorder.
15 Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio
20 LD₅₀/ED₅₀. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage can vary within this range
25 depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*,
30 achieving a decreased concentration of the polypeptide) that includes the IC₅₀ (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

35 In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by GCK expression. In any event, the administering physician can adjust the amount and timing of iRNA administration on the

basis of results observed using standard measures of efficacy known in the art or described herein.

VI. Methods of the Invention

5 The present invention also provides methods of using an iRNA of the invention and/or a composition containing an iRNA of the invention to reduce and/or inhibit glucokinase (GCK) expression in a cell. The methods include contacting the cell with a dsRNA of the invention and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of a GCK gene, thereby inhibiting expression of the
10 GCK gene in the cell. Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of GCK may be determined by determining the mRNA expression level of GCK using methods routine to one of ordinary skill in the art, *e.g.*, northern blotting, qRT-PCR; by determining the protein level of GCK using methods routine to one of ordinary skill in the art, such as
15 western blotting, immunological techniques. A reduction in the expression of GCK may also be assessed indirectly by measuring a decrease in biological activity of GCK.

In the methods of the invention the cell may be contacted *in vitro* or *in vivo*, *i.e.*, the cell may be within a subject.

A cell suitable for treatment using the methods of the invention may be any cell
20 that expresses a GCK gene. A cell suitable for use in the methods of the invention may be a mammalian cell, *e.g.*, a primate cell (such as a human cell or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), a non-primate cell (such as a cow cell, a pig cell, a camel cell, a llama cell, a horse cell, a goat cell, a rabbit cell, a sheep cell, a hamster, a guinea pig cell, a cat cell, a dog cell, a rat cell, a mouse cell, a lion cell, a tiger
25 cell, a bear cell, or a buffalo cell), a bird cell (*e.g.*, a duck cell or a goose cell), or a whale cell. In one embodiment, the cell is a human cell, *e.g.*, a human liver cell.

GCK expression is inhibited in the cell by at least about 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, 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, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or about 100%. In preferred embodiments, GCK expression is inhibited by at least 20%.

The *in vivo* methods of the invention may include administering to a subject a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is
35 complementary to at least a part of an RNA transcript of the GCK gene of the mammal to be treated. When the organism to be treated is a mammal such as a human, the composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*,

intraventricular, intraparenchymal, and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection.

In some embodiments, the administration is *via* a depot injection. A depot injection may release the iRNA in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, *e.g.*, a desired inhibition of GCK, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

In some embodiments, the administration is *via* a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the iRNA to the liver.

The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

In one aspect, the present invention also provides methods for inhibiting the expression of a GCK gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets a GCK gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the GCK gene, thereby inhibiting expression of the GCK gene in the cell. Reduction in gene expression can be assessed by any methods known in the art and by methods, *e.g.* qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known in the art and by methods, *e.g.* ELISA, described herein. In one embodiment, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in GCK gene and/or protein expression.

The present invention further provides methods of treatment of a subject in need thereof. The treatment methods of the invention include administering an iRNA of the invention to a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of GCK expression, in a therapeutically effective amount of an iRNA targeting a GCK gene or a pharmaceutical composition comprising an iRNA targeting a GCK gene.

In addition, the present invention provides methods of increasing the blood glucose levels, *e.g.*, fasting blood glucose levels, in a subject having a disease or disorder that would benefit from a reduction in GCK expression. The methods include administering an iRNA of the invention to the subject in a therapeutically effective amount of an iRNA targeting a GCK gene or a pharmaceutical composition comprising an iRNA targeting a GCK gene.

Further, the present invention provides methods of decreasing plasma lactate levels in a subject having a disease or disorder that would benefit from a reduction in GCK expression, *e.g.*, a GSD, *e.g.*, type Ia GSD. The methods include administering an iRNA of the invention to the subject in a therapeutically effective amount of an iRNA targeting a GCK gene or a pharmaceutical composition comprising an iRNA targeting a GCK gene.

The present invention also provides methods of decreasing plasma uric acid levels in a subject having a disease or disorder that would benefit from a reduction in GCK expression, *e.g.*, a GSD, *e.g.*, type Ia GSD. The methods include administering an iRNA of the invention to the subject in a therapeutically effective amount of an iRNA targeting a GCK gene or a pharmaceutical composition comprising an iRNA targeting a GCK gene.

The present invention provides methods of decreasing plasma triglyceride levels in a subject having a disease or disorder that would benefit from a reduction in GCK expression, *e.g.*, a GSD, *e.g.*, type Ia GSD. The methods include administering an iRNA of the invention to the subject in a therapeutically effective amount of an iRNA targeting a GCK gene or a pharmaceutical composition comprising an iRNA targeting a GCK gene.

The present invention further provides methods of decreasing total plasma cholesterol levels in a subject having a disease or disorder that would benefit from a reduction in GCK expression, *e.g.*, a GSD, *e.g.*, type Ia GSD. The methods include administering an iRNA of the invention to the subject in a therapeutically effective amount of an iRNA targeting a GCK gene or a pharmaceutical composition comprising an iRNA targeting a GCK gene.

The present invention also provides methods of decreasing hepatomegaly in a subject having a disease or disorder that would benefit from a reduction in GCK expression, *e.g.*, a GSD, *e.g.*, type Ia GSD. The methods include administering an iRNA of the invention to the subject in a therapeutically effective amount of an iRNA targeting a GCK gene or a pharmaceutical composition comprising an iRNA targeting a GCK gene.

An iRNA of the invention may be administered as a “free iRNA.” A free iRNA is administered in the absence of a pharmaceutical composition. The naked iRNA may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the iRNA can be adjusted such that it is suitable for administering to a subject.

Alternatively, an iRNA of the invention may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

Subjects that would benefit from a reduction and/or inhibition of GCK gene expression include those having a glycogen storage disease (GSD), *e.g.*, type Ia GSD. In one embodiment, subjects that would benefit from a reduction and/or inhibition of GCK gene expression are those having one or more signs or symptoms associated with a glycogen storage disease (GSD), *e.g.*, type Ia GSD, including, but not limited to, hypoglycemia, lactic acidosis, hyperuricemia, hyperlipidemia, hepatomegaly, kidney disease as a result of glycogen accumulation, hunger, jitteriness, lethargy, apnea, seizures, diaphoresis, confusion, headaches, dizziness, unusual mood or behavior changes, loss of consciousness, coma, muscle cramps, bleeding diathesis, short stature, osteoporosis, delayed puberty, gout, renal disease, systemic hypertension, pulmonary hypertension, hepatic adenomas, pancreatitis, anemia, vitamin D deficiency, polycystic ovaries, irregular menstrual cycles, menorrhagia, and eruptive xanthomata.

Treatment of a subject that would benefit from a reduction and/or inhibition of GCK gene expression and normalization of blood glucose levels includes therapeutic treatment (*e.g.*, of a subject suffering from a glycogen storage disease (GSD)) and prophylactic treatment (*e.g.*, of a subject that does not meet the diagnostic criteria of a glycogen storage disease (GSD), or a subject who may be at risk of developing a glycogen storage disease (GSD)).

The invention further provides methods for the use of an iRNA or a pharmaceutical composition thereof, *e.g.*, for treating a subject that would benefit from reduction and/or inhibition of GCK expression, *e.g.*, a subject having a glycogen storage disease (GSD), *e.g.*, type Ia GSD, in combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders. For example, in certain embodiments, an iRNA targeting GCK is administered to a subject having a GSD in combination with, *e.g.*, antihypertensive agents, such as Diazoxide, somatostatin analogues, such as Octreotide, calcium channel blockers, such as, Nifedipine, Thiazide diuretics, such as chlorothiazide (*e.g.*, in combination with Diazoxide), intravenous infusion of glucagon, parenterally

administered dextrose; and/or partial pancreatectomy. In some embodiments, an iRNA targeting GCK is administered to a subject having type Ia GSD in combination with, *e.g.*, a sodium-glucose co-transporter 2 (SGLT2) inhibitor, *e.g.*, Dapagliflozin, Canagliflozin, Ipragliflozin (ASP-1941), Tofogliflozin, Empagliflozin, Sergliflozin etabonate, Remogliflozin etabonate (BHV091009), and Ertugliflozin (PF-04971729 / MK-8835).

The iRNA and additional therapeutic agents may be administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

In one embodiment, the method includes administering a composition featured herein such that expression of the target GCK gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 18, 24 hours, 28, 32, or about 36 hours. In one embodiment, expression of the target GCK gene is decreased for an extended duration, *e.g.*, at least about two, three, four days or more, *e.g.*, about one week, two weeks, three weeks, or four weeks or longer.

Preferably, the iRNAs useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target GCK gene. Compositions and methods for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

Administration of the dsRNA according to the methods of the invention may result in a reduction of the severity, signs, symptoms, and/or markers of such diseases or disorders in a patient with a glycogen storage disease (GSD), *e.g.*, type Ia GSD. By “reduction” in this context is meant a statistically significant decrease in such level. The reduction can be, for example, at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker, or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of a glycogen storage disease (GSD) may be assessed, for example, by periodic monitoring of, *e.g.*, blood glucose levels. Comparisons of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection

with the administration of an iRNA targeting GCK or pharmaceutical composition thereof, "effective against" a glycogen storage disease (GSD) indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as a improvement of symptoms, a
 5 cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating a glycogen storage disease (GSD) and the related causes and effects.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to
 10 develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment. Efficacy for a given iRNA drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an
 15 experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

Alternatively, the efficacy can be measured by a reduction in the severity of disease as determined by one skilled in the art of diagnosis based on a clinically accepted disease severity grading scale such as those provided above. Any positive
 20 change resulting in *e.g.*, lessening of severity of disease measured using the appropriate scale, represents adequate treatment using an iRNA or iRNA formulation as described herein.

Subjects can be administered a therapeutic amount of dsRNA, such as about 0.01 mg/kg to about 200 mg/kg.

25 The iRNA can be administered by intravenous infusion over a period of time, on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. Administration of the iRNA can reduce GCK levels, *e.g.*, in a cell, tissue, blood, urine, or other compartment of the patient by at least about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36,
 30 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, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% or more.

Before administration of a full dose of the iRNA, patients can be administered a smaller dose, such as a 5% infusion reaction, and monitored for adverse effects, such as
 35 an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (*e.g.*, TNF-alpha or INF-alpha) levels.

Alternatively, the iRNA can be administered subcutaneously, *i.e.*, by subcutaneous injection. One or more injections may be used to deliver the desired daily dose of iRNA to a subject. The injections may be repeated over a period of time. The administration may be repeated on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as every other day or to once a year. In certain embodiments, the iRNA is administered about once per month to about once per quarter (*i.e.*, about once every three months).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the iRNAs and methods featured in the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

Example 1. iRNA Design, Synthesis, Selection, and *In Vitro* Evaluation

This Example describes methods for the design, synthesis, selection, and *in vitro* evaluation of GCK iRNA agents.

Source of reagents

Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

Bioinformatics

A set of siRNAs targeting the human GCK, “glucokinase (hexokinase 4)”, (human: NCBI refseqID NM_033507; NCBI GeneID: 2645), as well as toxicology-species GCK orthologs (cynomolgus monkey: XM_005549685; mouse: NM_010292; rat, NM_012565) were designed using custom R and Python scripts. The human NM_033507 REFSEQ mRNA, version 1, has a length of 2442 bases. The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every

potential 19mer iRNA from position 1 through position 2442 (the coding region and 3' UTR) was determined with a linear model derived the direct measure of mRNA knockdown from more than 20,000 distinct iRNA designs targeting a large number of vertebrate genes. Subsets of the GCK iRNAs were designed with perfect or near-perfect matches between human, cynomolgus and rhesus monkey. A further subset was designed with perfect or near-perfect matches to mouse and rat GCK orthologs. For each strand of the iRNA, a custom Python script was used in a brute force search to measure the number and positions of mismatches between the iRNA and all potential alignments in the target species transcriptome. Extra weight was given to mismatches in the seed region, defined here as positions 2-9 of the antisense oligonucleotide, as well the cleavage site of the iRNA, defined here as positions 10-11 of the antisense oligonucleotide. The relative weight of the mismatches was 2.8; 1.2: 1 for seed mismatches, cleavage site, and other positions up through antisense position 19. Mismatches in the first position were ignored. A specificity score was calculated for each strand by assuming the value of each weighted mismatch. Preference was given to iRNAs whose antisense score in human and cynomolgus monkey was ≥ 2.0 and predicted efficacy was $\geq 50\%$ knockdown of the GCK transcript.

Synthesis of GCK sequences

Synthesis of GCK Single Strands and Duplexes

GCK siRNA sequences were synthesized at 1 μmol scale on Mermade 192 synthesizer (BioAutomation) using the solid support mediated phosphoramidite chemistry. The solid support was controlled pore glass (500 $^{\circ}\text{A}$) loaded with custom GalNAc ligand or universal solid support (AM biochemical). Ancillary synthesis reagents, 2'-F and 2'-O-Methyl RNA and deoxy phosphoramidites were obtained from Thermo-Fisher (Milwaukee, WI) and Hongene (China). 2'F, 2'-O-Methyl, RNA, DNA and other modified nucleosides were introduced in the sequences using the corresponding phosphoramidites. Synthesis of 3' GalNAc conjugated single strands was performed on a GalNAc modified CPG support. Custom CPG universal solid support was used for the synthesis of antisense single strands. Coupling time for all phosphoramidites (100 mM in acetonitrile) was 5 min employing 5-Ethylthio-1H-tetrazole (ETT) as activator (0.6 M in acetonitrile). Phosphorothioate linkages were generated using a 50 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1, 2, 4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA) in anhydrous acetonitrile/pyridine (1:1 v/v). Oxidation time was 3 minutes. All sequences were synthesized with final removal of the DMT group ("DMT off").

Upon completion of the solid phase synthesis, single strands were cleaved from the solid support and deprotected in sealed 96 deep well plates using 200 μ L Aqueous Methylamine reagent at 60 °C for 20 minutes. For sequences containing 2' ribo residues (2'-OH) that are protected with tert-butyl dimethyl silyl (TBDMS) group, a second step
5 deprotection was performed using TEA.3HF (triethylamine trihydro fluoride) reagent. To the methylamine deprotection solution, 200 μ L of dimethyl sulfoxide (DMSO) and 300 μ L TEA.3HF reagent was added and the solution was incubated for additional 20min at 60°C. At the end of cleavage and deprotection step, the synthesis plate was allowed to come to room temperature and was precipitated by addition of 1mL of acetontile:
10 ethanol mixture (9:1). The plates were cooled at -80 °C for 2 hrs and the supernatant decanted carefully with the aid of a multi-channel pipette. The oligonucleotide pellet was re-suspended in 20mM NaOAc buffer and were desalted using a 5 mL HiTrap size exclusion column (GE Healthcare) on an AKTA Purifier System equipped with an A905 autosampler and a Frac 950 fraction collector. Desalted samples were collected in 96
15 well plates. Samples from each sequence were analyzed by LC-MS to confirm the identity, UV (260 nm) for quantification and a selected set of samples by IEX chromatography to determine purity.

Annealing of GCK single strands was performed on a Tecan liquid handling robot. Equimolar mixture of sense and antisense single strands were combined and
20 annealed in 96 well plates. After combining the complementary single strands, the 96 well plate was sealed tightly and heated in an oven at 100 °C for 10 minutes and allowed to come slowly to room temperature over a period 2-3 hours. The concentration of each duplex was normalized to 10uM in 1X PBS and then submitted for in vitro screening assays.

25

Cell culture and transfections for single dose and dose response studies

Primary mouse hepatocytes (PMH) (GIBCO) or Primary *Cynomolgus* monkey hepatocytes (PCH) (Celsis) were transfected by adding 4.9 μ L of Opti-MEM plus 0.1 μ L of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 μ L of
30 siRNA duplexes per well into a 384-well plate and incubated at room temperature for 15 minutes. Forty μ L of William's E Medium (Life Tech) containing about 5×10^3 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10nM and 0.1nM final duplex concentration.

35

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen, part #: 610-12)

Total RNA was isolated using an automated protocol on a BioTek-EL406 platform using DYNABEADS (Invitrogen, cat#61012). Briefly, 50µl of Lysis/Binding Buffer and 25µl of lysis buffer containing 3µl of magnetic beads were added to the plate with cells. Plates were incubated on an electromagnetic shaker for 10 minutes at room temperature and then magnetic beads were captured and the supernatant was removed. Bead-bound RNA was then washed 2 times with 150µl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150µl Elution Buffer, re-captured and supernatant removed.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813)

Ten µl of a master mix containing 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl 10x Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H₂O per reaction was added to the RNA isolated as described above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2h 37°C. Plates were then incubated at 80°C for 8 minutes.

Real time PCR

Two µl of cDNA was added to a master mix containing 0.5µl GAPDH TaqMan Probe (Applied Biosystems Cat #4326317E), or 0.5µl of Custom made Cyno GAPDH Taqman Probe, 0.5µl GCK mouse probe (Mm00439129 m1) or 0.5µl cyno probe (Mf02827184 m1) and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plate (Roche cat # 04887301001). Real time PCR is done in an Roche Lightcycler Real Time PCR system (Roche) using the $\Delta\Delta C_t$ (RQ) assay. Each duplex was tested in two independent transfections.

To calculate relative fold change, real time data are analyzed using the $\Delta\Delta C_t$ method and normalized to assays performed with cells transfected with a non-targeting control siRNA, AD-1955

The sense and antisense sequences of AD-1955 are:

SENSE: cuuAcGcuGAGuAcuucGAdTsdT (SEQ ID NO: 29)

ANTISENSE: UCGAAGuACUcAGCGuAAGdTsdT (SEQ ID NO: 30).

Table 1: Abbreviations of nucleotide monomers used in nucleic acid sequence representation.

It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation	Nucleotide(s)
A	Adenosine-3'-phosphate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine -3'-phosphorothioate
Us	uridine -3'-phosphorothioate
N	any nucleotide (G, A, C, T or U)
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'- phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'- phosphorothioate
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'- phosphorothioate
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage

Abbreviation	Nucleotide(s)
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl]-4-hydroxyprolinol Hyp-(GalNAc-alkyl) ₃
dT	2'-deoxythymidine-3'-phosphate
dC	2'-deoxycytidine-3'-phosphate

A detailed list of the unmodified GCK sense and antisense strand sequences is shown in Table 2 and a detailed list of the modified GCK sense and antisense strand sequences is shown in Table 3.

5 Table 4 shows the results of a single dose screen in primary mouse hepatocytes transfected with the indicated modified siRNAs. Data are expressed as percent of message remaining relative to cells treated with a non-targeting control siRNA.

10 Table 5 shows the results of a single dose screen in primary *Cynomologous* hepatocytes transfected with the indicated modified siRNAs. Data are expressed as percent of message remaining relative to cells treated with a non-targeting control siRNA.

Table 2. GCK Unmodified Sequences

Duplex ID	Sense ID	Sense Sequence (5'-3')	SEQ ID NO:	Position in NM_033507.1	Antisense ID	Antisense Sequence (5'-3')	SEQ ID NO:	Position in NM_033507.1	cross-sp
AD-69366	A-139669	GAGGACCUUGAAGAGGUG AUA	31	_253-273_s	A-139670	UAUACACCUUUCUACAGGUCCU CCU	120	_251-273_as	hemr
AD-69368	A-139673	GGACCUUGAAGAGGUGAU GAA	32	_255-275_s	A-139674	UUCAUACACCUUUCUACAGGUC CUC	121	_253-275_as	hemr
AD-69411	A-139758	GGACCUUGAAGAGGUGAU A	33	_257-277_s	A-139759	UAUACACCUUUCUACAGGUCC	122	_255-277_as	hemr
AD-69413	A-139762	ACCUGAAGAGGUGAUGA A	34	_259-279_s	A-139763	UUAUACACCUUUCUACAGGU	123	_257-279_as	hemr
AD-69367	A-139671	GGUGAUGAGACGGAUGCA GAA	35	_267-287_s	A-139672	UUCUGCAUCCGUCUCAUCAC CUU	124	_265-287_as	hemr
AD-69369	A-139675	UGAUGAGACGGAUGCAGA AGA	36	_269-289_s	A-139676	UCUUCUGCAUCCGUCUCAUC ACC	125	_267-289_as	hemr
AD-69412	A-139760	UGAUGAGACGGAUGCAGA A	37	_271-291_s	A-139761	UUCUGCAUCCGUCUCAUCA	126	_269-291_as	hemr
AD-69414	A-139764	AUGAGACGGAUGCAGAAG A	38	_273-293_s	A-139765	UCUUCUGCAUCCGUCUCAU	127	_271-293_as	hemr
AD-69371	A-139679	ACGGAUGCAGAAGGAGAU GGA	39	_276-296_s	A-139680	UCCAUCUCCUUCUGCAUCCG UCU	128	_274-296_as	hemr
AD-69416	A-139768	GGAUGCAGAAGGAGAUAG A	40	_280-300_s	A-139769	UCCAUCUCCUUCUGCAUCC	129	_278-300_as	hemr
AD-69370	A-139677	ACCCAUGAAGAGGCCAGU GUA	41	_316-336_s	A-139678	UACACUGGCCUUCUUCAGGG UCU	130	_314-336_as	hemr
AD-69415	A-139766	CCAUGAAGAGGCCAGUGU A	42	_320-340_s	A-139767	UACACUGGCCUUCUUCAGG	131	_318-340_as	hemr
AD-69372	A-139681	CAGUGUGAAGAUUGCUGCC CAA	43	_330-350_s	A-139682	UUGGGCAGCAUCUUCACACU GGC	132	_328-350_as	hc
AD-69417	A-139770	GUGUGAAGAUUGCUGCCCA A	44	_334-354_s	A-139771	UUGGGCAGCAUCUUCACAC	133	_332-354_as	hc
AD-69373	A-139683	GUGAAGGUGGGAGAAGGU GAA	45	_436-456_s	A-139684	UUCACCUUUCUCCACCUUCA CCA	134	_434-456_as	hc
AD-69418	A-139772	GAAAGGUGGGAGAAGGUGA	46	_440-460_s	A-139773	UUCACCUUUCUCCACCUUC	135	_438-460_as	hc

AD-69374	A-139685	A	GAGCGUGAAGACCAACA CCA	47	_468-488_s	A-139686	UGGUGUUUGGUCUUCACGC UCCA	136	_466-488_as	hc
AD-69419	A-139774	A	GCGUGAAGACCAAAACACC	48	_472-492_s	A-139775	UGGUGUUUGGUCUUCACGC	137	_470-492_as	hc
AD-69375	A-139687	A	GAAGACCAAAACACCAAGAU GUA	49	_474-494_s	A-139688	UACAUCUGGUGUUUGGUCU UCAC	138	_472-494_as	hcmr
AD-69420	A-139776	A	AGACCAAAACACCAAGUGU	50	_478-498_s	A-139777	UACAUCUGGUGUUUGGUCU	139	_476-498_as	hcmr
AD-69376	A-139689	A	GACUCCUGGACAAAGCAU CAA	51	_565-585_s	A-139690	UUGAUGCUUUGUCCAGGAAG UCGG	140	_563-585_as	hcmr
AD-69377	A-139691	A	CUUCCUGGACAAAGCAUCA GAU	52	_567-587_s	A-139692	AUCUGAUGCUUUGUCCAGGA AGUC	141	_565-587_as	hcmr
AD-69421	A-139778	A	CUUCCUGGACAAAGCAUCA	53	_569-589_s	A-139779	UUGAUGCUUUGUCCAGGAAG	142	_567-589_as	hcmr
AD-69422	A-139780	U	UCCUGGACAAAGCAUCAGA	54	_571-591_s	A-139781	AUCUGAUGCUUUGUCCAGGA	143	_569-591_as	hcmr
AD-69378	A-139693	A	CCUGGACAAAGCAUCAGAU GAA	55	_570-590_s	A-139694	UUCAUCUGAUGCUUUGUCCAG GAA	144	_568-590_as	hcmr
AD-69379	A-139695	A	CUGGACAAAGCAUCAGAU AAA	56	_571-591_s	A-139696	UUUCAUCUGAUGCUUUGUCCA GGA	145	_569-591_as	hcmr
AD-69423	A-139782	A	UGGACAAAGCAUCAGAU A	57	_574-594_s	A-139783	UUCAUCUGAUGCUUUGUCCA	146	_572-594_as	hcmr
AD-69424	A-139784	A	GGACAAAGCAUCAGAU A	58	_575-595_s	A-139785	UUUCAUCUGAUGCUUUGUCC	147	_573-595_as	hcmr
AD-69381	A-139699	A	AGGCACGAAAGACAUCGAU AAA	59	_634-654_s	A-139700	UUUAUCGAUGUCUUCGUGCC UCA	148	_632-654_as	hc
AD-69426	A-139788	A	GCACGAAGACAUCGAU A	60	_638-658_s	A-139789	UUUAUCGAUGUCUUCGUGC	149	_636-658_as	hc
AD-69382	A-139701	A	ACAUCGAUAAGGGCAUCC UUA	61	_644-664_s	A-139702	UAGGGAUGCCCCUUAUCGAU GUCU	150	_642-664_as	hc
AD-69427	A-139790	A	AUCGAUAAGGGCAUCCU A	62	_648-668_s	A-139791	UAGGGAUGCCCCUUAUCGAU	151	_646-668_as	hc
AD-69383	A-139703	A	CUGGACCAAGGGCUUCAA GGA	63	_669-689_s	A-139704	UCCUUGAAGCCCCUUGGUCCA GUU	152	_667-689_as	hcmr
AD-69428	A-139792	A	GGACCAAGGGCUUCAAGG	64	_673-693_s	A-139793	UCCUUGAAGCCCCUUGGUCC	153	_671-693_as	hcmr

AD-69384	A-139705	A	GGGCUUCAAGGCCUCAGG AGA	65			A-139706	UCUCCUGAGGCCUUUAAGCC CUU	154			676-698_as	hc
AD-69429	A-139794	A	GCUUCAAGGCCUCAGGAG	66			A-139795	UCUCCUGAGGCCUUUAAGC	155			680-702_as	hc
AD-69385	A-139707	AUA	CAGGAGCAGAAAGGGAACA	67			A-139708	UAUUGUUCCCUUUCUGCUCCU GAG	156			690-712_as	hc
AD-69386	A-139709	GUA	GGAGCAGAAAGGGAACAAU	68			A-139710	UACAUUGUCCCUUUCUGCUC CUG	157			692-714_as	hc
AD-69430	A-139796	A	GGAGCAGAAAGGGAACAAU	69			A-139797	UAUUGUUCCCUUUCUGCUCC	158			694-716_as	hc
AD-69387	A-139711	UCA	GAGCAGAAAGGGAACAAUG	70			A-139712	UGACAUUGUCCCUUUCUGCU CCU	159			693-715_as	hc
AD-69431	A-139798	A	AGCAGAAAGGGAACAAUGU	71			A-139799	UACAUUGUCCCUUUCUGCU	160			696-718_as	hc
AD-69432	A-139800	A	GCAGAAAGGGAACAAUGUC	72			A-139801	UGACAUUGUCCCUUUCUGC	161			697-719_as	hc
AD-69388	A-139713	GUA	GACUUUGAAAUGGAUGUG	73			A-139714	UACCACAUCCAUUUUCAAAGU CCC	162			749-771_as	hcmr
AD-69389	A-139715	GGA	CUUUGAAAUGGAUGUGGU	74			A-139716	UCCACCACAUCCAUUUUCAA GUC	163			751-773_as	hcmr
AD-69433	A-139802	A	CUUUGAAAUGGAUGUGGU	75			A-139803	UACCACAUCCAUUUUCAAAG	164			753-775_as	hcmr
AD-69434	A-139804	A	UUGAAAUGGAUGUGGUGG	76			A-139805	UCCACCACAUCCAUUUUCAA	165			755-777_as	hcmr
AD-69391	A-139719	AAU	UGAAAUGGAUGUGGUGGC	77			A-139720	AUUGCCACCACAUCCAUUUUC AAA	166			754-776_as	hcmr
AD-69436	A-139808	U	AAUUGGAUGUGGUGGCAA	78			A-139809	AUUGCCACCACAUCCAUUU	167			758-780_as	hcmr
AD-69392	A-139721	GUA	AUGGAUGUGGUGGCAAUG	79			A-139722	UACCAUUGCCACCACAUCCA UUU	168			758-780_as	hcmr
AD-69402	A-139721	GUA	AUGGAUGUGGUGGCAAUG	80			A-139741	UACCAUUGCCACCACAUCCA UCU	169			758-780_as	mr
AD-69393	A-139723	GAA	GGAUGUGGUGGCAAUGGU	81			A-139724	UUCACCAUUGCCACCACAUCA CAU	170			760-782_as	hcmr
AD-69437	A-139810	GGAUGUGGUGGCAAUGGU	82				A-139811	UACCAUUGCCACCACAUCCA	171			762-784_as	hcmr

AD-69438	A-139812	A	AUGUGGUGGCAAUUGGUGA	83			A-139813	UUACCAUUGCCACCACAU	172			_764-786_as	hcmr
AD-69395	A-139727	A	GCAAUGGUGAAUGACACG GUA	84			A-139728	UACCGUGUCAUUCACCAUUG CCA	173			_770-792_as	hc
AD-69440	A-139816	A	AAUGGUGAAUGACACGGU	85			A-139817	UACCGUGUCAUUCACCAU	174			_774-796_as	hc
AD-69396	A-139729	A	CAGUGCGAGGUCGGCAUG AUA	86			A-139730	UAUCAUGCCGACCUCCGCACU GAU	175			_824-846_as	hcmr
AD-69441	A-139818	A	GUGCGAGGUCGGCAUGAU	87			A-139819	UAUCAUGCCGACCUCCGCAC	176			_828-850_as	hcmr
AD-69397	A-139731	A	ACAUGGAGGAGAUGCAGA AUA	88			A-139732	UAUUCUGCAUCUCCUCCAU UAG	177			_870-892_as	hc
AD-69442	A-139820	A	AUGGAGGAGAUGCAGAAU	89			A-139821	UAUUCUGCAUCUCCUCCAU	178			_874-896_as	hc
AD-69394	A-139725	A	GAUGCAGAAUGUGGAGCU GGU	90			A-139726	ACCAGCUCCACAUUCUGCAU CUC	179			_880-902_as	hcmr
AD-69439	A-139814	U	UGCAGAAUGUGGAGCUGG	91			A-139815	ACCAGCUCCACAUUCUGCA	180			_884-906_as	hcmr
AD-69398	A-139733	A	GUGGACGAGAGCUCUGCA AAA	92			A-139734	UUUUGCAGAGCUCUCGUCCA CCA	181			_998- 1020_as	hc
AD-69443	A-139822	A	GGACGAGAGCUCUGCAAA	93			A-139823	UUUUGCAGAGCUCUCGUCC	182			_1002- 1024_as	hc
AD-69380	A-139697	A	AAGUACAUGGGCGAGCUG GUA	94			A-139698	UACCAGCUCGCCCAUGUACU UGC	183			_1055- 1077_as	hcmr
AD-69425	A-139786	A	GUACAUGGGCGAGCUGGU	95			A-139787	UACCAGCUCGCCCAUGUAC	184			_1059- 1081_as	hcmr
AD-69403	A-139742	A	AGGUCUGUGGACGAAAAAC CUA	96			A-139743	UAGGUUUUCGUCCACGAGCC UGA	185			_1091- 1113_as	hc
AD-69447	A-139830	A	GCUCGUGGACGAAAAACCU	97			A-139831	UAGGUUUUCGUCCACGAGC	186			_1095- 1117_as	hc
AD-69404	A-139744	A	CGUGGACGAAAAACCUUCU	98			A-139745	AAGAGCAGGUUUUCGUCCAC GAG	187			_1096- 1118_as	hc
AD-69405	A-139746	A	GUGGACGAAAAACCUUCUC UUA	99			A-139747	UAGAGCAGGUUUUCGUCC ACGA	188			_1097- 1119_as	hc
AD-69448	A-139832	A	UGGACGAAAAACCUUCUCU	100			A-139833	AAGAGCAGGUUUUCGUCCAC	189			_1100- _1100-	hc

AD-69449	A-139834	U	GGACGAAACCCUGCUCU	101			A-139835	UAAGAGCAGGUUUUCGUCC	190	1122_as	
AD-69406	A-139748	CGCAAGCAGAUCAAC	AUA	102			A-139749	UAUGUUUAGAUUCUGCUUG	191	1101-1123_as	hc
AD-69450	A-139836	CAAGCAGAUCAACA	CAU	103			A-139837	UAUGUUUAGAUUCUGCUUG	192	1202-1224_as	hc
AD-69390	A-139717	AGCUGCGAGAUACCCUUC	AUA	104			A-139718	UAUGAAAGGUGAUUCUGGCAG	193	1206-1228_as	hc
AD-69435	A-139806	CUGCGAGAUACCCUUC	AUA	105			A-139807	UAUGAAAGGUGAUUCUGGCAG	194	1466-1488_as	hcmr
AD-69408	A-139752	CCAGUCCUGGCCAUUUUC	UUA	106			A-139753	UAAGAAAAUUGGCCAGGACU	195	1470-1492_as	hcmr
AD-69452	A-139840	AGUCCUGGCCAUUUUCU	AUA	107			A-139841	UAAGAAAAUUGGCCAGGACU	196	2047-2069_as	hc
AD-69409	A-139754	CACUGAGUGGCUUGUGAU	UCU	108			A-139755	AGAAUCACAAGCCACUCAGU	197	2051-2073_as	hc
AD-69453	A-139842	CUGAGUGGCUUGUGAUUC	UUA	109			A-139843	AGAAUCACAAGCCACUCAG	198	2168-2190_as	hc
AD-69410	A-139756	AAUGUUAAAAGUUUUAAA	CAU	110			A-139757	AUGUUUAAAACUUUUAAACA	199	2172-2194_as	hc
AD-69454	A-139844	UGUUAAAAGUUUUAAAACA	UUA	111			A-139845	AUGUUUAAAACUUUUAAACA	200	2411-2433_as	hc
AD-69444	A-139824	AGCAGAAAGGGAACAACA	AUA	112			A-139825	UAUGUUUUUUUUUUUUUUUU	201	2415-2437_as	hc
AD-69399	A-139735	GGAGCAGAAAGGGAACAACA	AUA	113			A-139736	UAUGUUUUUUUUUUUUUUUU	202	696-718_as	mr
AD-69445	A-139826	UCUCCGAGAUUCUAUCAA	AUA	114			A-139827	UUUGAUAGCAUUCUGGAGA	203	692-714_as	mr
AD-69400	A-139737	CUUCUCCGAGAUUCUAUC	AAA	115			A-139738	UUUGAUAGCAUUCUGGAGA	204	723-745_as	mr
AD-69446	A-139828	AGAUUGGAUGUGGUGGCAA	UAU	116			A-139829	AUUGCCACCACAUCUUCU	205	719-741_as	mr
AD-69401	A-139739	UGAGAUUGGAUGUGGUGGC	AAU	117			A-139740	AUUGCCACCACAUCUUCU	206	758-780_as	mr
AD-69451	A-139838	CUGCGAAAUACCCUUCU	CAU	118			A-139839	AAUGAAAGGUGAUUUUCGCAG	207	754-776_as	mr
										1470-	mr

[illegible]

Table 3. GCK Modified Sequences

Duplex ID	Sense ID	Sense sequence (5'-3')	SEQ ID NO:	Antisense ID	Antisense sequence (5'-3')	SEQ ID NO:	mRNA sequence	SEQ ID NO:
AD-69366	A-139669	gsaggacCfuGfAfAfgaaggauaalL96	209	A-139670	usAfsucaCfcUfuFcuucAfgGfuccucscsu	298	AGGAGGACCCUGAAGAA GGUGAUA	387
AD-69368	A-139673	gsgsaccuGfaAfGfAfaggugauaalL96	210	A-139674	usUfseauCfaCfCfucuuUfcAf ggucscsuc	299	GAGGACCUGAAGAAAGG UGAUGAA	388
AD-69411	A-139758	GGACCCUGAAGAAGGUGAUAdTdT	211	A-139759	UAUCAACCUUCUUCAGG UCCdTdT	300	GGACCUGAAGAAGGUG AUA	389
AD-69413	A-139762	ACCUGAAGAAGGUGAU GAAdTdT	212	A-139763	UUCAUCACCUUCUUA GGUdTdT	301	ACCUGAAGAAGGUGAU GAA	390
AD-69367	A-139671	gsgsugauGfaGfAfCfggangcaaalL96	213	A-139672	usUfscugCfaUfcfegucUfcAf uacacsusu	302	AAGGUGAUGAGACGGA UGCAGAA	391
AD-69369	A-139675	usgsaugaGfaCfGfGfaugcagaalL96	214	A-139676	usCfsnuuCfGfCfaFuecgUfcU fcaucascsc	303	GGUGAUGAGACGGAUG CAGAAAGA	392
AD-69412	A-139760	UGAUGAGACGGAUGCA GAAdTdT	215	A-139761	UUCUGCAUCCGUCUCA UCAdTdT	304	UGAUGAGACGGAUGCA GAA	393
AD-69414	A-139764	AUGAGACGGAUGCAGAGAdTdT	216	A-139765	UCUUCUGCAUCCGUCU CAUdTdT	305	AUGAGACGGAUGCAGA AGA	394
AD-69371	A-139679	ascsggauGfcAfGfAfaggagau ggaL96	217	A-139680	usCfscauCfuCfCfucuuGfcAf uccguscscu	306	AGACGGAUGCAGAAAGG AGAUGGA	395
AD-69416	A-139768	GGAUUGCAGAAAGGAGAU GGAdTdT	218	A-139769	UCCAUCUCCUUCUGCA UCCdTdT	307	GGAUGCAGAAAGGAGAU GGA	396
AD-69370	A-139677	ascsccauGfaAfGfAfggccaguguaL96	219	A-139678	usAfscaCfGfCfcauUfcAf uggguscscu	308	AGACCCAUGAAGAGAGC CAGUGUA	397
AD-69415	A-139766	CCAUGAAGAGGCCAGU GUAdTdT	220	A-139767	UACACUGGCCUUCUUA UGGdTdT	309	CCAUGAAGAGGCCAGU GUA	398
AD-69372	A-139681	csasguguGfaAfGfAfugcugcc	221	A-139682	usUfsgggCfaGfCfcauUfcA	310	GCCAGUGAAGAAGUGC	399

AD-69417	A-139770	caal96	GUGUGAAGAUGCUGCC CAAdTdT	222	A-139771	fcagsgsc	UGCCCAA	311	GUGUGAAGAUGCUGCC CAA	400
AD-69373	A-139683	gsusgaagGfuGfGfGfagaaggu gaal96	223	A-139684	usUfscacCfuUfCfuuccAfcCf uucacsca	312	UGUGAAGGUGGGAGA AGUGAA	401		
AD-69418	A-139772	GAAGGUGGGAGAAAGGU GAAdTdT	224	A-139773	UUCACCUUCUCCCAACC UUCdTdT	313	GAAAGGUGGGAGAAAGGU GAA	402		
AD-69374	A-139685	gsasgcguGfaAfGfAfccaaaca ccal96	225	A-139686	usGfsgugUfuUfGfgucUfcA fcgucscsa	314	UGGAGCGUGAAGACCA AACACCA	403		
AD-69419	A-139774	GCGUGAAGACCAACA CCAdTdT	226	A-139775	UGGUGUUUGGUCUUA CGCdTdT	315	GCGUGAAGACCAACA CCA	404		
AD-69375	A-139687	gsasagacCfaAfAfCfaccagau gual96	227	A-139688	usAfscanCfuGfGfgucUfcG fucucscsc	316	GUGAAGACCAAAACACC AGAUGUA	405		
AD-69420	A-139776	AGACCAAAACACCAGAU GUAdTdT	228	A-139777	UACAUCUGGUGUUUGG UCUdTdT	317	AGACCAAAACACCAGAU GUA	406		
AD-69376	A-139689	gsascuucCfuGfGfAfcagcau caal96	229	A-139690	usUfsgauGfcUfUfguccAfcG faagucsgsg	318	CCGACUCCUGGACAA GCAUCAA	407		
AD-69377	A-139691	csusuccuGfgAfCfAfagaucan gaul96	230	A-139692	asUfscugAfUfGfCfuuguCfcA fggaagsusc	319	GACUCCUGGACAAAGC AUCAGAU	408		
AD-69421	A-139778	CUUCCUGGACAAAGCAU CAAdTdT	231	A-139779	UUGAUGCUUGUCCAGG AAGdTdT	320	CUUCCUGGACAAAGCAU CAA	409		
AD-69422	A-139780	UCCUGGACAAAGCAUCA GAUdTdT	232	A-139781	AUCUGAUGCUUGUCCA GGAdTdT	321	UCCUGGACAAAGCAUCA GAU	410		
AD-69378	A-139693	cscsuggaCfaAfGfCfaucagau gaal96	233	A-139694	usUfscanCfuGfAfugcuUfgU fccaggsasa	322	UCCUGGACAAAGCAUC AGAUGAA	411		
AD-69379	A-139695	csusggacAfaGfCfAfucagau aal96	234	A-139696	usUfsucaUfcUfGfaugeUfuG fuccaggsa	323	UCCUGGACAAAGCAUCA GAUGAAA	412		
AD-69423	A-139782	UGGACAAAGCAUCAGAU GAAdTdT	235	A-139783	UUAUCUGAUGCUUGU CCAdTdT	324	UGGACAAAGCAUCAGAU GAA	413		
AD-69424	A-139784	GGACAAAGCAUCAGAU AAAdTdT	236	A-139785	UUUAUCUGAUGCUUG UCCdTdT	325	GGACAAAGCAUCAGAU AAA	414		
AD-69381	A-139699	asgsgeacGfaAfGfAfeaucgau aal96	237	A-139700	usUfsuanCfGfAfUfgucUfcG fugccscsa	326	UGAGGCACGAAGACAU CGAUAAA	415		
AD-69426	A-139788	GCACGAAAGACAUCCGAU AAAdTdT	238	A-139789	UUUAUCGAUGUCUUCG UGCdTdT	327	GCACGAAAGACAUCCGAU AAA	416		

AD-69382	A-139701	ascsauegAfuAfAfGfggeaucc uuuL96	239	A-139702	usAfsaggAfuGfCfccuuAfuC fgauguscsu	328	AGACAUCGAUAAGGGC AUCCUUA	417
AD-69427	A-139790	AUCGAUUAAGGGCAUCC UUAdTdT	240	A-139791	UAAGGAUGCCCUUAUC GAUdTdT	329	AUCGAUAAGGGCAUCC UUA	418
AD-69383	A-139703	csusggacCfaAfGfGfgcucaa ggaL96	241	A-139704	usCfscuuGfaAfGfccuUfgG fuccagsusu	330	AACUGGACCAAGGGCU UCAAGGA	419
AD-69428	A-139792	GGACCAAGGGCUUCA GGAdTdT	242	A-139793	UCCUUGAAGGCCCUUGG UCCdTdT	331	GGACCAAGGGCUUCA GGA	420
AD-69384	A-139705	gsgsgcuuCfaAfGfGfcccagg agaL96	243	A-139706	usCfsuccUfgAfGfgecuUfgA fagecccsusu	332	AAGGCUUCAAGGCCU CAGGAGA	421
AD-69429	A-139794	GCUUCAAGGCCUCAGG AGAdTdT	244	A-139795	UCUCCUGAGGCCCUUGA AGCdTdT	333	GCUUCAAGGCCUCAGG AGA	422
AD-69385	A-139707	csasggagCfaGfAfAfgggaaca auaL96	245	A-139708	usAfsuugUfuCfCfcccUfgC fuccugsasg	334	CUCAGGAGCAGAAAGGG AACAAUA	423
AD-69386	A-139709	gsgsagcaGfaAfGfGfgaacaau gual96	246	A-139710	usAfscauUfgUfuUfcccUfcU fgeuccsug	335	CAGGAGCAGAAAGGGAA CAAUGUA	424
AD-69430	A-139796	GGAGCAGAAGGGGAACA AUAdTdT	247	A-139797	UAUUGUUCUUUCUUGC UCCdTdT	336	GGAGCAGAAGGGGAACA AUA	425
AD-69387	A-139711	gsasggagAfaGfGfGfgaacaug ucaL96	248	A-139712	usGfsacaUfuGfUfucccUfuCf ugcuescsu	337	AGGAGCAGAAAGGGAAC AAUGUCA	426
AD-69431	A-139798	AGCAGAAAGGGAACAAU GUAdTdT	249	A-139799	UACAUUGUUCUUUCUUCU GCUDTdT	338	AGCAGAAAGGGAACAAU GUA	427
AD-69432	A-139800	GCAGAAAGGGGAACAAUG UCAdTdT	250	A-139801	UGACAUUGUUUCCCUUC UGCdTdT	339	GCAGAAAGGGAACAAUG UCA	428
AD-69388	A-139713	gsascuuuGfaAfAfUfggaugug gual96	251	A-139714	usAfsecaCfaUfCfcauuUfcAf aagucscsc	340	GGGACUUUGAAAUUGGA UGUGGUA	429
AD-69389	A-139715	csusuugaAfaUfGfGfauguggu ggaL96	252	A-139716	usCfseacCfaCfAfuccaUfuUf caaagsusc	341	GACUUUGAAAUUGGAUG UGGUGGA	430
AD-69433	A-139802	CUUUGAAAUUGGAUGUG GUAdTdT	253	A-139803	UACCACAUCCAUUUCA AAGdTdT	342	CUUUGAAAUUGGAUGUG GUA	431
AD-69434	A-139804	UUGAAAUGGAUGUGGU GGAdTdT	254	A-139805	UCCACCACAUCCAUUU CAAdTdT	343	UUGAAAUGGAUGUGGU GGA	432
AD-69391	A-139719	usgsaaauGfgAfUfGfugguggc aaul96	255	A-139720	asUfsugeCfaCfCfacauCfcAf uuucasasa	344	UUUGAAAUGGAUGUGG UGGCAAU	433
AD-69436	A-139808	AAAUUGGAUGUGGUGGC AAUdTdT	256	A-139809	AUUGCCACCACAUCCA UUUdTdT	345	AAAUUGGAUGUGGUGGC AAU	434

AD-69392	A-139721	asusggauGfuGfGfUfggeaang gual96	257	A-139722	usAfsceaUfuGfCfcaaccAfeAf uceaususu	346	AAUGGAUGUGGUGGC AAUGGUA	435
AD-69402	A-139721	asusggauGfuGfGfUfggeaang gual96	258	A-139741	usAfsceaUfuGfCfcaaccAfeAf uceaususu	347	AGAUGGAUGUGGUGGC AAUGGUA	436
AD-69393	A-139723	gsausauGfuGfGfUfggeaang gual96	259	A-139724	usUfscaCfaUfuUfggeaCfeAf cauccsasu	348	AUGGAUGUGGUGGCAA UGGUGAA	437
AD-69437	A-139810	GGAUGUGGUGGCAAUG GUAdTdT	260	A-139811	UACCAUUGCCACCACA UCCdTdT	349	GGAUGUGGUGGCAAUG GUA	438
AD-69438	A-139812	AUGUGUGGCAAUGGU GAAdTdT	261	A-139813	UUCACCAUUGCCACCACA CAUdTdT	350	AUGUGGUGGCAAUGGU GAA	439
AD-69395	A-139727	gsausauGfuGfGfUfggeaang gual96	262	A-139728	usAfscaUfuGfCfcaaccAfeAf aungcsasu	351	UGGCAAUGGUGAAUGA CACGGUA	440
AD-69440	A-139816	AAUGGUGAAUGACACG GUAdTdT	263	A-139817	UACCGUGUCAUUCACC AUdTdT	352	AAUGGUGAAUGACACG GUA	441
AD-69396	A-139729	csausuGfuGfGfUfggeaang aual96	264	A-139730	usAfscaUfuGfCfcaaccAfeAf cagcsasu	353	AUCAGUGCGAGGUGCG CAUGAUA	442
AD-69441	A-139818	GUGCGAGGUGCGCAUG AUAdTdT	265	A-139819	UACAUGCCGACCUCG CACdTdT	354	GUGCGAGGUGCGCAUG AUA	443
AD-69397	A-139731	asausuGfuGfGfUfggeaang aual96	266	A-139732	usAfsuucUfuGfCfcaaccAfeAf fcaugsasu	355	CUACAUGGAGGAGAGUG CAGAAUA	444
AD-69442	A-139820	AUGGAGGAGAUAGCAGA AUAdTdT	267	A-139821	UAUUCUGCAUUCUCCUC CAUdTdT	356	AUGGAGGAGAUAGCAGA AUA	445
AD-69394	A-139725	gsausuGfuGfGfUfggeaang gual96	268	A-139726	asCfscaUfuGfCfcaaccAfeAf gcaucsasu	357	GAGAUGCAGAAUGUGG AGCUGGU	446
AD-69439	A-139814	UGCAGAAUGUGGAGCU GGUdTdT	269	A-139815	ACCAGCUCCACAUCU GCAdTdT	358	UGCAGAAUGUGGAGCU GGU	447
AD-69398	A-139733	gsausuGfuGfGfUfggeaang aual96	270	A-139734	usUfsuucUfuGfCfcaaccAfeAf fcaucsasu	359	UGGUGGAGGAGAGCUC UGCAAAA	448
AD-69443	A-139822	GGACGAGAGCUCUGCA AAAdTdT	271	A-139823	UUUUGCAGAGCUCUCCG UCCdTdT	360	GGACGAGAGCUCUGCA AAA	449
AD-69380	A-139697	asausuGfuGfGfUfggeaang gual96	272	A-139698	usAfscaUfuGfCfcaaccAfeAf uauucsasu	361	GCAAGUACAUGGGCGA GCUGGUA	450
AD-69425	A-139786	GUACAUGGGCGAGCUG GUAdTdT	273	A-139787	UACCAUGCUCGCCCAUG UACdTdT	362	GUACAUGGGCGAGCUG GUA	451
AD-69403	A-139742	asgsuucGfuGfGfUfggeaang cual96	274	A-139743	usAfsuucUfuGfCfcaaccAfeAf fagccsasu	363	UCAGGCUCGUGGACGA AAACCUA	452

AD-69447	A-139830	GCUCGUGGACGAAAC CUAdTdT	275	A-139831	UAGGUUUUCGUCCACG AGCdTdT	364	GCUCGUGGACGAAAC CUA	453
AD-69404	A-139744	csgsuggaCfGfAfAfafacugcu cuuL96	276	A-139745	asAfsagCfGfGfuuuuCfGfU fccacgsag	365	CUCGUGGACGAAACCC UGCUCUU	454
AD-69405	A-139746	gsusggacGfAfAfAfafceugcuc uuuL96	277	A-139747	usAfsagaGfAfGfuuuuUfcG fuaccsgsa	366	UCGUGGACGAAACCCU GCUCUUA	455
AD-69448	A-139832	UGGACGAAACCCUGCU CUUdTdT	278	A-139833	AAGAGCAGGUUUUUCGU CCAdTdT	367	UGGACGAAACCCUGCU CUU	456
AD-69449	A-139834	GGACGAAACCCUGCUC UUAdTdT	279	A-139835	UAAGAGCAGGUUUUUCG UCCdTdT	368	GGACGAAACCCUGCUC UUA	457
AD-69406	A-139748	csgscaagCfGfAfUfcuacaaca uuL96	280	A-139749	usAfsuguUfGfUfAfgaucUfGfC fuucgsgsgu	369	ACCGCAAGCAGAUCUA CAACAUA	458
AD-69450	A-139836	CAAGCAGAUUCUACAAC AUAdTdT	281	A-139837	UAUGUUUGUAGAUCUCG UUGdTdT	370	CAAGCAGAUUCUACAAC AUA	459
AD-69390	A-139717	asgscugcGfGfAfUfcacuuuc auuL96	282	A-139718	usAfsugaAfgGfUfegaucUfcG fcagcugsgg	371	CCAGCUGCGAGAUCAC CUUCAUA	460
AD-69435	A-139806	CUGCGAGAUCAACCUUC AUAdTdT	283	A-139807	UAUGAAAGGUGAUCUCG CAGdTdT	372	CUGCGAGAUCAACCUUC AUA	461
AD-69408	A-139752	cscsagucCfuGfGfCfcauuuuc uuuL96	284	A-139753	usAfsagaAfaAfUfegccAfgG facugsgsgu	373	ACCCAGUCCUGGCCAU UUUCUUA	462
AD-69452	A-139840	AGUCCUGGCCAUUUUUC UUAdTdT	285	A-139841	UAAGAAAUUGGCCAGG ACUdTdT	374	AGUCCUGGCCAUUUUUC UUA	463
AD-69409	A-139754	csascugaGfuGfGfCfhuugau ucuL96	286	A-139755	asGfsaauCfaCfAfagccAfeUf caguggsasu	375	AUCACUGAGUGGCCUUG UGAUUCU	464
AD-69453	A-139842	CUGAGUGGCUUUGUGAU UCUdTdT	287	A-139843	AGAAUUCACAAGCCACU CAGdTdT	376	CUGAGUGGCUUUGUGAU UCU	465
AD-69410	A-139756	asasuguuAfaAfAfGfuuuuuuu cauL96	288	A-139757	asUfsguuUfaAfAfacuUfuA facaususuu	377	AAAUGUUUAAAAGUUU UAAACAU	466
AD-69454	A-139844	UGUUAAAAGUUUUUAAA CAUdTdT	289	A-139845	AUGUUUAAAACUUUUA ACAdTdT	378	UGUUAAAAGUUUUUAAA CAU	467
AD-69444	A-139824	AGCAGAAAGGGAACAAC AUAdTdT	290	A-139825	UAUGUUUGUCCCCUUCU GCUDdTdT	379	AGCAGAAAGGGAACAAC AUA	468
AD-69399	A-139735	gsgsagaGfAfGfGfgaacaac auuL96	291	A-139736	usAfsuguUfGfUfUfcccUfU fgeucsgsg	380	CCGAGCAGAAAGGGAA CAACAUA	469
AD-69445	A-139826	UCUCCGAGAUUCUAUC AAAdTdT	292	A-139827	UUUGAUAGCAUCUCGG AGAdTdT	381	UCUCCGAGAUUCUAUC AAA	470

AD-69400	A-139737	csusueucCfGfAfGfAfugeuauc aalL96	293	A-139738	usUfsugaUfaGfCfaueuCfGg fagaagsusc	382	GACUUCUCCCGAGAUGC UAUCAA	471
AD-69446	A-139828	AGAUGGAUGUGGUGGC AAUdTdT	294	A-139829	AUUGCCACCCACAUCCA UCUdTdT	383	AGAUGGAUGUGGUGGC AAU	472
AD-69401	A-139739	usgsagauGfGfAfUfGfuggg caauL96	295	A-139740	asUfsugcCfaCfCfaueuCfAf ucucasasa	384	UUUGAGAUGGAUGUGG UGGCAAU	473
AD-69451	A-139838	CUGCGAAAUCAACCUUC AAUdTdT	296	A-139839	AAUGAAGGUGAUUUCG CAGdTdT	385	CUGCGAAAUCAACCUUC AAU	474
AD-69407	A-139750	asascugcGfaAfAfUfcaccuuc auuL96	297	A-139751	asAfsugaAfgGfUfgauuUfcG fcaguusgsg	386	CCAAUCUGCGAAAUCAAC CUUCAUU	475

Table 4. GCK Single Dose Screen in Primary Mouse Hepatocytes

	Primary Mouse Hepatocytes			
Duplex Name	10nM Avg	10nM SD	0.1nM Avg	0.1nM SD
AD-69366	82.2	54.1	70.2	12.8
AD-69368	47.8	5.3	91.7	3.6
AD-69411	17.6	4.8	27.9	2.8
AD-69413	15.9	5.1	43.7	4.8
AD-69367	58.2	11.0	92.0	10.5
AD-69369	60.3	10.6	88.0	6.5
AD-69412	24.1	5.3	71.7	15.6
AD-69414	52.5	7.7	82.7	7.2
AD-69371	77.5	9.7	89.1	12.3
AD-69416	11.8	2.4	25.1	4.0
AD-69370	99.5	10.1	96.9	5.1
AD-69415	90.3	9.3	91.3	15.2
AD-69372	27.9	2.2	49.8	10.2
AD-69417	29.9	2.1	63.6	12.6
AD-69373	96.7	17.1	104.7	48.8
AD-69418	86.2	34.0	62.0	9.9
AD-69374	33.7	4.8	71.7	7.8
AD-69419	17.1	3.2	44.8	4.4
AD-69375	72.0	8.3	104.5	5.3
AD-69420	20.3	2.0	46.6	7.2
AD-69376	22.1	2.3	59.0	9.7
AD-69377	23.0	2.0	59.2	6.6
AD-69421	16.1	2.1	29.9	3.5
AD-69422	15.2	1.2	38.9	2.4
AD-69378	24.8	2.1	70.1	4.2
AD-69379	11.2	0.9	18.0	3.2
AD-69423	11.7	1.3	41.1	3.6
AD-69424	9.5	1.6	13.9	3.5
AD-69381	73.6	13.3	99.9	36.4
AD-69426	84.5	6.3	76.5	6.1
AD-69382	91.9	5.6	91.6	3.3
AD-69427	97.6	2.5	88.8	4.5

AD-69383	95.9	3.2	100.5	9.3
AD-69428	32.7	4.3	66.1	5.0
AD-69384	88.3	9.8	97.9	8.6
AD-69429	90.7	10.6	95.9	5.7
AD-69385	29.2	4.2	45.1	4.4
AD-69386	92.5	9.0	97.8	9.8
AD-69430	21.2	1.4	31.8	2.1
AD-69387	69.7	9.0	77.8	7.1
AD-69432	69.5	3.2	79.6	13.6
AD-69388	66.9	11.3	84.7	11.9
AD-69389	76.3	18.3	86.9	6.0
AD-69433	70.0	2.6	76.4	5.0
AD-69434	97.6	6.6	71.6	9.5
AD-69391	21.7	5.1	59.8	5.6
AD-69436	19.9	1.9	54.3	14.6
AD-69392	39.2	4.8	81.5	12.7
AD-69402	32.2	5.1	62.9	12.4
AD-69393	34.9	5.4	78.5	13.0
AD-69437	14.0	2.5	27.3	4.6
AD-69438	11.7	0.7	28.4	2.5
AD-69395	28.2	6.8	55.4	12.2
AD-69440	19.4	2.8	34.7	7.3
AD-69396	29.7	3.0	57.2	9.8
AD-69441	23.8	4.1	44.6	7.4
AD-69397	39.5	11.8	67.9	11.6
AD-69442	29.3	2.4	40.1	8.4
AD-69394	39.4	4.2	79.5	3.2
AD-69439	37.6	3.2	65.9	3.0
AD-69398	92.2	9.6	111.3	16.2
AD-69443	115.1	14.5	97.4	5.1
AD-69380	23.6	3.2	65.0	11.2
AD-69425	11.9	1.2	29.4	6.5
AD-69403	64.2	13.0	77.0	13.9
AD-69447	87.4	7.9	84.3	11.7
AD-69404	67.2	10.6	83.9	16.6

AD-69405	69.0	10.9	86.1	23.6
AD-69448	107.8	6.4	120.4	36.9
AD-69449	129.6	37.4	120.2	6.4
AD-69406	63.8	4.7	91.7	10.8
AD-69450	84.5	12.4	109.5	5.3
AD-69390	16.9	4.2	38.9	11.6
AD-69435	9.0	1.5	20.9	3.5
AD-69408	67.3	4.7	83.6	16.3
AD-69452	92.6	10.4	111.8	5.1
AD-69409	74.8	4.8	79.7	12.3
AD-69453	87.2	1.4	93.7	3.0
AD-69410	71.2	4.9	90.2	9.4
AD-69454	90.0	10.9	86.0	7.8
AD-69399	24.4	2.5	45.8	6.1
AD-69445	16.0	0.6	44.4	4.4
AD-69400	12.2	1.4	34.8	2.3
AD-69446	14.9	1.3	30.4	2.9
AD-69401	15.4	1.6	29.4	4.9
AD-69451	6.9	1.3	19.2	3.6
AD-69407	4.1	0.4	11.8	2.8
AD-1955	101.283	16.1184		

Table 5. GCK Single Dose Screen in Primary Cynomologous Hepatocytes

	Primary Cyno Hepatocytes			
duplexName	10nM Avg	10nM SD	0.1nM Avg	0.1nM SD
AD-69366	29.8	14.3	37.4	9.3
AD-69368	12.2	4.6	28.1	4.9
AD-69411	14.2	4.5	22.6	10.8
AD-69413	12.1	3.2	30.0	14.0
AD-69367	14.9	2.6	39.9	7.1
AD-69369	36.6	14.2	54.2	9.5
AD-69412	19.3	6.5	31.2	4.6
AD-69414	26.4	11.9	63.6	8.7
AD-69371	50.2	20.5	91.3	17.3

AD-69416	24.5	8.3	21.8	3.6
AD-69370	43.4	10.3	69.4	11.6
AD-69415	15.4	6.2	34.2	13.2
AD-69372	19.8	7.9	23.7	8.7
AD-69417	23.8	10.5	31.4	16.1
AD-69373	87.7	22.1	66.8	10.6
AD-69418	20.4	4.9	25.7	6.4
AD-69374	24.7	15.0	41.8	6.2
AD-69419	21.9	6.7	32.8	12.9
AD-69375	20.6	5.5	49.5	16.0
AD-69420	15.3	2.9	41.8	21.6
AD-69376	17.7	5.3	22.4	12.1
AD-69377	21.6	5.6	26.4	6.2
AD-69421	22.1	4.7	22.1	8.9
AD-69422	22.7	5.7	38.2	11.8
AD-69378	14.7	6.6	31.9	26.2
AD-69379	10.5	2.7	14.4	2.9
AD-69423	20.3	8.2	42.2	10.8
AD-69424	20.0	5.1	17.5	10.3
AD-69381	46.6	12.8	56.9	20.6
AD-69426	49.3	8.4	76.7	29.0
AD-69382	74.0	22.7	54.6	14.2
AD-69427	33.9	5.9	66.3	13.8
AD-69383	63.4	18.6	81.4	22.7
AD-69428	39.4	16.0	69.7	10.0
AD-69384	58.1	17.4	89.1	22.8
AD-69429	30.9	7.3	47.9	17.9
AD-69385	27.3	9.2	37.0	7.7
AD-69386	41.4	16.6	61.3	18.6
AD-69430	24.2	8.8	29.8	13.7
AD-69387	28.2	3.0	30.9	11.1
AD-69432	23.5	7.8	24.4	5.3
AD-69388	63.7	11.0	70.8	7.3
AD-69389	59.7	13.8	77.8	15.6
AD-69433	37.0	3.3	62.2	31.0

AD-69434	76.3	27.9	94.1	8.0
AD-69391	22.0	11.2	25.2	10.0
AD-69436	24.5	4.8	41.3	2.7
AD-69392	23.3	10.1	52.9	11.4
AD-69402	21.9	7.6	59.1	29.2
AD-69393	22.9	2.3	37.3	2.9
AD-69437	21.8	6.6	30.4	7.7
AD-69438	21.5	5.9	32.4	9.6
AD-69395	17.4	5.7	32.3	4.6
AD-69440	25.7	15.0	35.1	13.5
AD-69396	28.8	9.3	16.6	5.1
AD-69441	26.3	8.4	39.2	8.9
AD-69397	62.4	33.3	48.6	26.1
AD-69442	41.0	12.3	50.4	12.5
AD-69394	38.0	12.9	65.7	13.1
AD-69439	30.2	9.0	51.6	10.8
AD-69398	21.1	3.8	23.1	6.5
AD-69443	20.0	7.6	29.3	4.2
AD-69380	43.8	15.3	64.7	26.0
AD-69425	35.5	9.8	38.5	8.5
AD-69403	34.5	12.0	35.1	7.0
AD-69447	23.5	7.4	55.0	21.7
AD-69404	41.2	23.9	30.0	5.5
AD-69405	20.2	7.7	35.0	14.4
AD-69448	53.1	16.4	42.3	6.3
AD-69449	37.8	14.7	42.6	4.0
AD-69406	21.6	13.7	23.4	7.1
AD-69450	19.6	4.5	23.9	5.1
AD-69390	18.8	8.0	21.0	4.2
AD-69435	20.0	17.3	23.9	9.7
AD-69408	40.0	17.9	34.1	18.5
AD-69452	28.9	9.6	45.5	13.6
AD-69409	40.0	10.8	52.7	31.5
AD-69453	39.2	6.5	57.6	20.9
AD-69410	62.0	8.7	78.6	10.5

AD-69454	86.5	6.9	102.4	27.8
AD-69399	88.8	17.5	81.6	21.4
AD-69445	68.9	19.5	118.3	26.5
AD-69400	32.7	11.9	31.1	10.1
AD-69446	23.5	10.8	38.3	4.1
AD-69401	28.4	18.3	24.7	5.7
AD-69451	79.6	23.5	102.9	41.6
AD-69407	16.8	7.7	29.2	12.6
AD-1955	102.912	29.2078		

Example 2. iRNA Design, Synthesis, Selection, and *In Vitro* Evaluation

This Example describes methods for the design, synthesis, selection, and *in vitro* evaluation of additional GCK iRNA agents.

5

Bioinformatics

A set of siRNAs targeting human glucokinase (GCK) (human NCBI refseqID: NM_033507; NCBI GeneID: 2645) were designed using custom R and Python scripts. The human GCK REFSEQ mRNA has a length of 2442 bases. The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 10 through position 2442 was determined with a linear model derived the direct measure of mRNA knockdown from more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. The custom Python script built the set of siRNAs by systematically selecting an siRNA every 11 bases along the target mRNA nucleotide sequence starting at position 10. At each of the positions, the neighboring siRNA (one position to the 5' end of the mRNA, one position to the 3' end of the mRNA) was swapped into the design set if the predicted efficacy was better than the efficacy at the exact every-eleventh siRNA. Low complexity siRNAs, *e.g.*, those with Shannon Entropy measures below 1.35, were excluded from the set.

20

Cell culture and transfections

Primary Cyno Hepatocytes (PCH) cells were transfected by adding 4.9µl of Opti-MEM plus 0.1µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of siRNA duplexes per well into a 384-well plate and incubated at room temperature for 15 minutes. Forty µl of EMEM containing about 5×10^3 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 20nM final duplex concentration.

25

Total RNA isolation using DYNABEADS mRNA Isolation Kit

RNA was isolated using an automated protocol on a BioTek-EL406 platform using DYNABEADS (Invitrogen, cat#61012). Briefly, 50µl of Lysis/Binding Buffer and 25µl of lysis buffer containing 3µl of magnetic beads were added to the plate with cells.

- 5 Plates were incubated on an electromagnetic shaker for 10 minutes at room temperature and then magnetic beads were captured and the supernatant was removed. Bead-bound RNA was then washed two times with 150µl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150µl Elution Buffer, re-captured, and the supernatant was removed.

10

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813)

Ten µl of a master mix containing 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl 10x Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H₂O per reaction was added to RNA isolated as described above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2 hours at 37°C.

15

Real time PCR

- 20 Two µl of cDNA were added to a master mix containing 0.5µl of Custom Cyno GAPDH TaqMan Probe, 0.5µl cyno GCK probe (Mf02827184 m1) and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was performed in a LightCycler480 Real Time PCR system (Roche) using the $\Delta\Delta C_t$ (RQ) assay. Each duplex was tested in four independent
- 25 transfections.

To calculate relative fold change, real time data were analyzed using the $\Delta\Delta C_t$ method and normalized to assays performed with cells transfected with 20nM AD-1955, or mock transfected cells.

- 30 A detailed list of the unmodified GCK sense and antisense strand sequences is shown in Table 6 and a detailed list of the modified GCK sense and antisense strand sequences is shown in Table 7.

- Table 8 shows the results of a single dose screen in primary *Cynomolgus* hepatocytes (PCH) transfected with the indicated modified siRNAs. Data are expressed as percent of message remaining relative to cells treated with a non-targeting control
- 35 siRNA, AD-1955.

Table 6: GCK Unmodified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Antisense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO:	Position in NM_033507.1
AD-71009	A-142377	CUGCCAGCCUCAGGCAGCU	476	A-142378	AGCUGCCUGAGGCUGGCAG	684	24-42
AD-71010	A-142379	UCAGGCAGCUCUCCAUCCA	477	A-142380	UGGAUGGAGAGCUGCCUGA	685	33-51
AD-71011	A-142381	CCAUCCAAAGCAGCCGUUGA	478	A-142382	UCAACGGCUGCUUGGAUGG	686	45-63
AD-71012	A-142383	AGCCGUUGCUGCCACAGGA	479	A-142384	UCCUGUGGCAGCAACGGCU	687	55-73
AD-71013	A-142385	ACAGCGGGGCCUACGCUA	480	A-142386	UAGCGUAAGGCCCGCCUGU	688	68-86
AD-71014	A-142387	UUACGCUCCAAGGCUACAA	481	A-142388	UUGUAGCCUUGGAGCGUAA	689	79-97
AD-71015	A-142389	AAGGCUACAGCAUGUGCUA	482	A-142390	UAGCACAUUGCUGUAGCCUU	690	88-106
AD-71016	A-142391	UGUGCUAGGCCUCAGCAGA	483	A-142392	UCUGCUGAGGCCUAGCACAA	691	100-118
AD-71017	A-142393	UCAGCAGGCAGGAGCAUCU	484	A-142394	AGAUGCUCUCCUGCCUGUGA	692	111-129
AD-71018	A-142395	AGCAUCUCUGCCUCCCAA	485	A-142396	UUUUGGAGGCAGAGAUCCU	693	123-141
AD-71019	A-142397	CCUCCCAAAGCAUCUACCU	486	A-142398	AGGUAGAUGCUCUUUGGGAGG	694	133-151
AD-71020	A-142401	UAGCCCCUCGGAGAGAUCA	487	A-142402	UCAUCUCUCCGAGGGGCUA	695	154-172
AD-71021	A-142403	AGAGAUUGCGAUGGAUGUA	488	A-142404	UACAUCCAUCGCCAUCUCU	696	165-183
AD-71022	A-142405	UGGAUGUCACAAAGGAGCCA	489	A-142406	UGGCUCCUUGUGACAUCCA	697	176-194
AD-71023	A-142407	AGGAGCCAGGCCAGACAA	490	A-142408	UUGUCUGGGCCUGGCUCUU	698	187-205
AD-71024	A-142411	ACUCUGGUAGAGCAGAUCA	491	A-142412	UGAUCUGCUCUACCAAGAU	699	211-229
AD-71025	A-142413	AGCAGAUCCUUGGCAGAGUU	492	A-142414	AACUCUGCCAGGAUCUCUGU	700	221-239
AD-71026	A-142415	CAGAGUCCAGCUGCAGGA	493	A-142416	UCCUGCAGCUGGAACUCUG	701	233-251
AD-71027	A-142417	AGCUGCAGGAGGAGGACCU	494	A-142418	AGGUCCUCCUCCUGCAGCU	702	242-260
AD-71028	A-142419	AGGACCUGAAAGAGGUGAU	495	A-142420	AUCACCCUUCUUCAGGUCCU	703	254-272
AD-71029	A-142421	AAGGUAGUAGACGGAUGA	496	A-142422	UCAUCCGUCUCAUCACCUU	704	265-283
AD-71030	A-142423	CGGAUGCAGAAAGGAGAUCA	497	A-142424	UCAUCUCCUUCUGCAUCCG	705	277-295

AD-71031	A-142425	AAGGAGAUGGACCGCGGCA	498	A-142426	UGCCGCGGUCCAUCUCCUU	706	286-304
AD-71032	A-142427	CGCGGCCUGAGGCGUGAGA	499	A-142428	UCUCCAGCCUACAGGCCGCG	707	298-316
AD-71033	A-142429	CUGGAGACCAUGAAGAGA	500	A-142430	UCUCUUAUUGGUCUCCAG	708	310-328
AD-71034	A-142431	CAUGAAGAGGCCAGUGUGA	501	A-142432	UCACACUGGCCUUCUUAUG	709	319-337
AD-71035	A-142433	CAGUGUAGAAGUCUGCCA	502	A-142434	UGGCAGCAUCUUCACACUG	710	330-348
AD-71036	A-142435	UGCUGCCACCUACGUGCA	503	A-142436	UGCACGUAGGUGGGCAGCA	711	341-359
AD-71037	A-142437	UACGUGCGCUCCACCCCAA	504	A-142438	UUGGGGUGGAGCGCACGUA	712	352-370
AD-71038	A-142439	ACCCAGAAGGCUACAGAAA	505	A-142440	UUUCUGAGCCUUCUGGGU	713	364-382
AD-71039	A-142441	UCAGAAUCGGGGACUUA	506	A-142442	UGAAGUCCCGACUUCUGA	714	376-394
AD-71040	A-142443	GGGACUUCUUCUCCUUGA	507	A-142444	UCAGGGAGAGGAAGUCCCC	715	385-403
AD-71041	A-142445	UCCUGGACCUUGGGUGGCA	508	A-142446	UGCCACCCAGGUCCAGGGA	716	397-415
AD-71042	A-142447	UGGGUGGCACUAAUUA	509	A-142448	UUGAAGUUAUGUGCCACCCA	717	407-425
AD-71043	A-142449	ACUUCAGGGUGAUGCUGGU	510	A-142450	ACCAGCAUCACCCUGAAGU	718	419-437
AD-71044	A-142453	AGGUGGGAGAGGUGAGGA	511	A-142454	UCCUCACCUUUCUCCACCU	719	440-458
AD-71045	A-142457	CAGUGGAGCGUGAAGACCA	512	A-142458	UGGUCUUCACGCUCCACUG	720	463-481
AD-71046	A-142461	CCAGAUUACUCCAUCCCA	513	A-142462	UGGGAUGGAGUACAUCUGG	721	486-504
AD-71047	A-142467	ACCGGCACUGCUGAGAU	514	A-142468	UCAUCUCAGCAGUGCCGGU	722	517-535
AD-71048	A-142469	AGAUUCUUCGACUACAU	515	A-142470	AUGUAGUCGAAGAGCAUCU	723	530-548
AD-71049	A-142471	UCGACUACAUUCUCUGAGUA	516	A-142472	UACUCAGAGAUUAUCGCA	724	539-557
AD-71050	A-142473	UCUGAGUGCAUCUCCGACU	517	A-142474	AGUCCGGAGAUACACUCAGA	725	550-568
AD-71051	A-142475	UCCGACUUCUUGGACAAAG	518	A-142476	UCUUGUCCAGGAAGUCGGA	726	562-580
AD-71052	A-142477	GACAAAGCAUCAGAUAAAC	519	A-142478	GUUUCAUCUGAUGCUUUGUC	727	574-592
AD-71053	A-142479	AGAUAAACACAAAGAGCU	520	A-142480	AGCUUCUUGUGUUUUAUCU	728	584-602
AD-71054	A-142481	AGAAUCUGCCCCUUGGGCUU	521	A-142482	AAGCCCAGGGCAGCUUCU	729	596-614
AD-71055	A-142483	CCUGGGCUUACCUUCUCA	522	A-142484	UGAGAAAGGUGAAGCCCAGG	730	606-624
AD-71056	A-142485	ACCUUCUCCUUUCCUUGA	523	A-142486	UCACAGGAAGGAGAAAGGU	731	616-634
AD-71057	A-142487	CUGAGGGCACGAAGACAU	524	A-142488	AUGUCUUCGUGCCUCACAG	732	629-647

AD-71058	A-142489	GAAGACAUCGAUAAGGGCA	525	A-142490	UGCCCUUAUCGAUGUCUUC	733	640-658
AD-71059	A-142491	GAUAGGGCAUCCUUCUCA	526	A-142492	UGAGAAGGAUGCCCUUAUC	734	649-667
AD-71060	A-142493	UUCUCAACUGGACCAAGGA	527	A-142494	UCCUUGGUCCAGUUGAGAA	735	662-680
AD-71061	A-142495	ACCAAGGGCUUCAAGGCCU	528	A-142496	AGCCUUGAAGCCCUUGGU	736	673-691
AD-71062	A-142497	CAAGGCCUCAGGAGCAGAA	529	A-142498	UUCUGCUCCUGAGGCCUUG	737	684-702
AD-71063	A-142499	AGGAGCAGAAAGGGAACAAU	530	A-142500	AUUGUCCCUUCUGCUCCU	738	693-711
AD-71064	A-142501	AACAAUGUCUGGGGCUUA	531	A-142502	UAAAGCCCCACGACAUUGUU	739	706-724
AD-71065	A-142503	UGGGCUUCUCGAGACGA	532	A-142504	UCGUCUCGCAGAAAGCCCA	740	716-734
AD-71066	A-142505	CGAGACGCUAUCAAACGGA	533	A-142506	UCCGUUUGAUAGCGUCUCG	741	727-745
AD-71067	A-142507	AAACGGAGAGGGGACUUA	534	A-142508	UAAAGUCCCCUCUCCGUUU	742	739-757
AD-71068	A-142509	GGGACUUUGAAAUUGGAUGU	535	A-142510	ACAUCCAUUUCAAAGUCCC	743	749-767
AD-71069	A-142513	GCAUUGGUGAAUGACACGA	536	A-142514	UCGUGUCAUUCACCAUUGC	744	772-790
AD-71070	A-142515	AAUGACACGGUGGCCACGA	537	A-142516	UCGUGGCCACCGUGUCAUU	745	781-799
AD-71071	A-142517	GCCACGAUGAUUCCUGCU	538	A-142518	AGCAGGAGAUCAUCGUGGC	746	793-811
AD-71072	A-142519	UCCUGCUACUACGAAGACA	539	A-142520	UGUCUUCGUAGUAGCAGGA	747	805-823
AD-71073	A-142523	AGUGCGAGGUCGGCAUGAU	540	A-142524	AUCAUGCCGACCUCGCACU	748	827-845
AD-71074	A-142525	GGCAUGAUCGUGGGCACGA	541	A-142526	UCGUGCCCCACGAUCAUGCC	749	838-856
AD-71075	A-142527	GUGGGCACGGGCGUGCAAUA	542	A-142528	UAUUGCAGCCCGUGCCAC	750	847-865
AD-71076	A-142529	UGCAAUGCCUGCUACAUGA	543	A-142530	UCAUGUAGCAGGCAUUGCA	751	859-877
AD-71077	A-142531	UACAUGGAGGAGAUGCAGA	544	A-142532	UCUGCAUCUCCUCCAUUGA	752	871-889
AD-71078	A-142533	AGAUGCAGAAUGUGGAGCU	545	A-142534	AGCUCCACAUUCUGCAUCU	753	881-899
AD-71079	A-142535	UGUGGAGCUGUGGAGGGA	546	A-142536	UCCUCCACCAGCUCCACA	754	891-909
AD-71080	A-142537	UGGAGGGGACGAGGGCCA	547	A-142538	UGGCCCUUGUCCCCCUCCA	755	902-920
AD-71081	A-142539	GAGGGCCGAUGUGCGUUA	548	A-142540	UGAGGCACAUUGCGGCCUUC	756	913-931
AD-71082	A-142541	UGCGUCAAUACCGAGUGGA	549	A-142542	UCCACUCGGUUAUUGACGCA	757	925-943
AD-71083	A-142543	CGAGUGGGGCGCCUUCGGA	550	A-142544	UCCGAAGGCGCCCCACUCG	758	936-954
AD-71084	A-142545	GCCUUCGGGGACUCCGGCA	551	A-142546	UGCCGGAGUCCCCCGAAGGC	759	946-964

AD-71085	A-142547	UCCGGCGAGCUGGACGAGU	552	A-142548	ACUCGUCCAGCUCGCCGGA	760	958-976
AD-71086	A-142549	GACGAGUUCUGCUGGAGU	553	A-142550	ACUCCAGCAGGAACUCGUC	761	970-988
AD-71087	A-142551	UGCUGGAGUAUGACCGCCU	554	A-142552	AGGCGGUCAUACUCCAGCA	762	980-998
AD-71088	A-142553	GACCGCCUGGUGGACGAGA	555	A-142554	UCUCGUCCACCCAGGCGGUC	763	991-1009
AD-71089	A-142555	GGACGAGAGCUCUGCAAAC	556	A-142556	GUUUGCAGAGCUCUCGUCC	764	1002-1020
AD-71090	A-142557	UCUGCAAACCCCGGUCAGA	557	A-142558	UCUGACCGGGGUUUGCAGA	765	1012-1030
AD-71091	A-142559	GGUCAGCAGCUGUAUGAGA	558	A-142560	UCUCAUACAGCUCUGACC	766	1024-1042
AD-71092	A-142561	UAUGAGAAAGCUCAUAGGUA	559	A-142562	UACCUAUGAGCUUCUCAUA	767	1036-1054
AD-71093	A-142563	UCAUAGGUGGCAAGUACAU	560	A-142564	AUGUACUUGCCACCUAUGA	768	1046-1064
AD-71094	A-142565	AAGUACAUGGCGGAGCUGA	561	A-142566	UCAGCUCGCCCCAUGUACUU	769	1057-1075
AD-71095	A-142567	GCGAGCUGGUGCGGCUUGU	562	A-142568	ACAAGCCGCACACGUCGC	770	1067-1085
AD-71096	A-142569	GGCUUGUGCUGCUCAGGCU	563	A-142570	AGCCUGAGCAGCACAAAGCC	771	1079-1097
AD-71097	A-142571	UCAGGCUCUGGACGAAAA	564	A-142572	UUUUCGUCCACGAGCCUGA	772	1091-1109
AD-69448	A-139832	UGGACGAAAAACUCUCUUU	565	A-139833	AAGAGCAGGUUUUCGUCCA	773	1100-1118
AD-71098	A-142573	UGCUCUCCACGGGGAGGA	566	A-142574	UCCUCCCCGUGGAAGAGCA	774	1112-1130
AD-71099	A-142575	GGGAGGCCUCCGAGCAGCU	567	A-142576	AGCUGCUCGGAGGCCUCCC	775	1124-1142
AD-71100	A-142577	CGAGCAGCUGCGCACACGA	568	A-142578	UCGUGUGCGCAGCUGCUCG	776	1134-1152
AD-71101	A-142581	AGCCUUCGAGACGCGCUUA	569	A-142582	UAAGCGCGUUCGAAAGGCU	777	1155-1173
AD-71102	A-142583	CGCUUCGUGUCGACGGUGA	570	A-142584	UCACCUGCGACACGAAGCG	778	1168-1186
AD-71103	A-142585	UCGCAGGUGGAGAGCGACA	571	A-142586	UGUCCGCUUCCACCUGCGA	779	1177-1195
AD-71104	A-142587	AGCGACACGGGCGACCGCA	572	A-142588	UGCGGUCGCCCCGUGUCGCU	780	1189-1207
AD-71105	A-142589	CGACCGCAAGCAGAUUCUAA	573	A-142590	UUAGAUCUGCUUGCGGUCG	781	1200-1218
AD-71106	A-142591	CAGAUUCUACAACAUCUUGA	574	A-142592	UCAGGAUGUUGUAGAUCUG	782	1210-1228
AD-71107	A-142593	UCCUGAGCACGCUUGGGGCU	575	A-142594	AGCCCCAGCGUGCUCAGGA	783	1223-1241
AD-71108	A-142595	CUGGGGCUUGCGACCCUCGA	576	A-142596	UCGAGGGUUCGACGCCCCAG	784	1234-1252
AD-71109	A-142597	CGACCCUUCGACCAACCGACU	577	A-142598	AGUCGGUGGUCGAGGGUCG	785	1243-1261
AD-71110	A-142599	CACCGACUGCGACAUUCGUA	578	A-142600	UACGAUGUCGACGUCGGUG	786	1254-1272

AD-71111	A-142601	CAUCGUGCGCGCGCCUGA	579	A-142602	UCAGGCGCGGCGCACGAUG	787	1266-1284
AD-71112	A-142603	CGGCGGUGCGAGAGCGUGU	580	A-142604	ACACGCUCUCGACGGCGCG	788	1276-1294
AD-71113	A-142605	AGCGUGUCUACGCGGCUA	581	A-142606	UAGCGCGGUAGACACGCU	789	1288-1306
AD-71114	A-142607	CGGCGUGCGCACAUUGUCU	582	A-142608	AGCACAUUGCGCAGCGCG	790	1300-1318
AD-71115	A-142609	ACAUGUCUCGCGGGGCGU	583	A-142610	AGCCCCGCGGAGCACAUU	791	1310-1328
AD-71116	A-142611	CGGGGUGGGCGGCGUUAU	584	A-142612	AUGACGCCCGCCAGCCCCG	792	1322-1340
AD-71117	A-142613	CGGGGUGUAUACCCGCAU	585	A-142614	AUGCGGUUGAUGACGCCCCG	793	1331-1349
AD-71118	A-142615	AACCGCAUGCGCGAGAGCA	586	A-142616	UGCUCUCGCGCAUGCGGUU	794	1342-1360
AD-71119	A-142617	AGAGCCGCGAGCGAGGACGU	587	A-142618	ACGUCCUCGCGUGGGGCUU	795	1355-1373
AD-71120	A-142619	CGAGGACGUAAUGCGCAUA	588	A-142620	UAUUGCGCAUACGUUCCUCG	796	1365-1383
AD-71121	A-142621	UGCAGCAUCACUGUGGGCGU	589	A-142622	ACGCCACAGUGAUGCGCA	797	1376-1394
AD-71122	A-142623	UGGGGUGGAUGGCUCCGU	590	A-142624	ACGGAGCCAUCCACGCCCA	798	1388-1406
AD-71123	A-142625	UGGCUCCGUGUACAAAGCUA	591	A-142626	UAGCUUGUACACGGAGCCA	799	1398-1416
AD-71124	A-142627	UACAAAGCUGCACCCAGCU	592	A-142628	AGCUGGGGUGCAGCUUGUA	800	1408-1426
AD-71125	A-142629	CCAGCUUCAAAGGAGCGGUU	593	A-142630	AACCGCUCCUUGAAGCUGG	801	1421-1439
AD-71126	A-142631	AGGAGCGGUUCCAUGCCAA	594	A-142632	UUGGCAUGGAACCGCUCCU	802	1430-1448
AD-71127	A-142633	AUGCCAGCGUGCGCAGGCU	595	A-142634	AGCCUGCGCACGCUGGGCAU	803	1442-1460
AD-71128	A-142635	CGCAGGCUGACGCCAGCU	596	A-142636	AGCUGGGCGUCAGCCUGCG	804	1453-1471
AD-71129	A-142637	CCCAGCUGCGAGAUACCCU	597	A-142638	AGGUGAUCUCGACGCUGGG	805	1465-1483
AD-71130	A-142639	GAGAUACCUUUAUCGAGU	598	A-142640	ACUCGAUGAAGGUGAUCUC	806	1474-1492
AD-71131	A-142641	AUCGAGUCGGAGGAGGCA	599	A-142642	UGCCCUCCUCCGACUCGUAU	807	1486-1504
AD-71132	A-142643	AGGAGGGCAGUGGCCGGGA	600	A-142644	UCCCGGCCACUGCCCCUCCU	808	1496-1514
AD-71133	A-142645	CCGGGCGCGGCCCUUGUA	601	A-142646	UACCAAGGGCGCGCCCCCG	809	1509-1527
AD-71134	A-142647	CCUGGUUCUGGGCGGUGGA	602	A-142648	UCCACGCCGAGACCAAGG	810	1520-1538
AD-71135	A-142649	GCGGUGGGCCUGUAAGAAGA	603	A-142650	UCUUCUUAACAGGCCACCGC	811	1531-1549
AD-71136	A-142651	UAAGAAGGCCCUUGAUUGCUA	604	A-142652	UAGCAUACAGGCCCUUCUUA	812	1542-1560
AD-71137	A-142653	CUGUAUGCUGGGGCCAGUGA	605	A-142654	UCACUGGGCCAGCAUACAG	813	1551-1569

AD-71138	A-142655	CAGUGAGAGCAGUGGGCCGA	606	A-142656	UCGGCCACUGCUCACUG	814	1564-1582
AD-71139	A-142657	CAGUGGCCGCAAGCGCAGA	607	A-142658	UCUGCGCUUGCGGCCACUG	815	1573-1591
AD-71140	A-142659	AGCGCAGGGAGGAUGCCAA	608	A-142660	UUGGCAUCCUCCUGCGCU	816	1584-1602
AD-71141	A-142661	UGCCACAGCCCCACAGCAA	609	A-142662	UUGCUGUGGGGCGUGGGCA	817	1597-1615
AD-71142	A-142663	CACAGACCCAGGCUCCAU	610	A-142664	AUGGAGCCUGGGUGGUGUG	818	1608-1626
AD-71143	A-142665	AGGCUCCAUGGGGAAGUGA	611	A-142666	UCACUCCCCAUGGAGCCU	819	1618-1636
AD-71144	A-142667	GGAAGUGCUCCCCACACGU	612	A-142668	ACGUGUGGGGAGCACUUC	820	1629-1647
AD-71145	A-142669	CCACACGUGCUCGACGCCU	613	A-142670	AGGCUGCGAGCACGUGUGG	821	1640-1658
AD-71146	A-142671	UCGACGCCUGGGGGGGCAA	614	A-142672	UUGCCCCGCCAGGCGUGCGA	822	1650-1668
AD-71147	A-142673	CGGGGCAGGAGGCCUGGCA	615	A-142674	UGCCAGGCCUCCUGCCCCG	823	1661-1679
AD-71148	A-142675	CCUGGCCUUGUCAGGACCA	616	A-142676	UGGUCCUGACAAGGCCAGG	824	1673-1691
AD-71149	A-142677	CAGGACCCAGGCCGCCUGA	617	A-142678	UCAGGCGGCCUGGGUCCUG	825	1684-1702
AD-71150	A-142679	CCGCCUGCCAUACCGCUGA	618	A-142680	UCAGCGGAUGGCAGGCGG	826	1695-1713
AD-71151	A-142681	UACCGCUGGGGAACAGAGA	619	A-142682	UCUCUGUCCCCAGCGGUA	827	1705-1723
AD-71152	A-142683	AACAGAGCGGGCCUCUUA	620	A-142684	UGAAGAGGCCCCGCUCUGUU	828	1716-1734
AD-71153	A-142685	CUCUCCCCUCAGUUUUUA	621	A-142686	UGAAAAACUGAGGGAAGAG	829	1728-1746
AD-71154	A-142687	UUUUUCGGUGGGACAGCCA	622	A-142688	UGGCUGUCCCCACCGAAAA	830	1740-1758
AD-71155	A-142689	GGGACAGCCCCAGGGCCCU	623	A-142690	AGGGCCCCUGGGGCGUCC	831	1749-1767
AD-71156	A-142691	AGGGCCCUAACGGGGGUGA	624	A-142692	UCACCCCGUUAAGGGCCCU	832	1760-1778
AD-71157	A-142693	GGGUGCGGCAGGAGCAGGA	625	A-142694	UCCUGCUCUUGCCGCACCC	833	1773-1791
AD-71158	A-142695	AGGAGCAGGAACAGAGACU	626	A-142696	AGUCUCUGUUCUUGCUCU	834	1782-1800
AD-71159	A-142697	AGAGACUCUGGAAGCCCCA	627	A-142698	UGGGGCUUCCAGAGUCUCU	835	1794-1812
AD-71160	A-142699	AAGCCCCCACCUCUUCUA	628	A-142700	UGAGAAAGUGGGGGGCUU	836	1805-1823
AD-71161	A-142701	UUUCUCGCGGAAUCAAUU	629	A-142702	AAUUGAUUCCAGCGAGAAA	837	1817-1835
AD-71162	A-142703	AAUCAAUUUCCAGAGAGGA	630	A-142704	UCCUUCUGGGGAAAUUGAUU	838	1828-1846
AD-71163	A-142705	CCCAGAAAGGAGUUGCUCA	631	A-142706	UGAGCAACUCCCUUCUGGG	839	1837-1855
AD-71164	A-142707	UUGCUCACUCAGGACUUUA	632	A-142708	UAAAGUCCUGAGUGAGCAA	840	1849-1867

AD-71165	A-142709	AGGACUUUGAUGCAUUUCA	633	A-142710	UGAAUUGCAUCAAAGUCCU	841	1859-1877
AD-71166	A-142711	AUUUCCACACUGUCAGAGA	634	A-142712	UCUCUGACAGUGUGGAAAU	842	1872-1890
AD-71167	A-142713	UGUCAGAGCUGUUGGCCUA	635	A-142714	UAGGCCAACAGCUCUGACA	843	1882-1900
AD-71168	A-142715	UUGGCCUCGCCUGGGCCCA	636	A-142716	UGGGCCCAGGCGAGGCCAA	844	1893-1911
AD-71169	A-142717	CUGGGCCCAGGCUCUGGGA	637	A-142718	UCCCAGAGCCUUGGGCCCAG	845	1903-1921
AD-71170	A-142719	CUCUGGGAAGGGUGGCCCU	638	A-142720	AGGGCACCCCUUCCCCAGAG	846	1914-1932
AD-71171	A-142721	UGCCCUUCUGGAUCCUGCUA	639	A-142722	UAGCAGGAUCCAGAGGGCA	847	1927-1945
AD-71172	A-142723	UCCUGCUGUGGCCUCACUU	640	A-142724	AAGUGAGGCCACAGCAGGA	848	1938-1956
AD-71173	A-142725	CCUCACUUCCUGGGGAACU	641	A-142726	AGUCCCCAGGGAAGUGAGG	849	1949-1967
AD-71174	A-142727	CUGGGAACUCAUCCUGUGU	642	A-142728	ACACAGGAUGAGUUUCCCG	850	1959-1977
AD-71175	A-142729	CCUGUGUGGGAGGCAGCU	643	A-142730	AGCUGCCUCCCCACACAGG	851	1971-1989
AD-71176	A-142731	GGAGGCAGCUCCAACAGCU	644	A-142732	AGCUGUUGGAGCUGCCUCC	852	1980-1998
AD-71177	A-142733	CAACAGCUUGACACAGACCU	645	A-142734	AGGUCUGGUAAGCUGUUG	853	1991-2009
AD-71178	A-142735	CCAGACCUAGACCUUGGGCA	646	A-142736	UGCCCAGGUCUAGGUCUGG	854	2002-2020
AD-71179	A-142737	CUGGGCCAAAAGGGCAGCA	647	A-142738	UGCUGCCCUUUUUGGGCCCAG	855	2014-2032
AD-71180	A-142739	AGGGCAGCCAGGGGCGUCU	648	A-142740	AGCAGCCCUUGGCGUGCCCU	856	2024-2042
AD-71181	A-142741	GGGCGUCUCAUACCCAGU	649	A-142742	ACUGGGUGAUGAGCAGCCC	857	2035-2053
AD-71182	A-142743	ACCCAGUCCUGGCCAUUUU	650	A-142744	AAAAUGGCCAGGACUGGGU	858	2047-2065
AD-71183	A-142745	GCCAUUUUCUUGCCUGAGA	651	A-142746	UCUCAGGCAAGAAAUGGC	859	2058-2076
AD-71184	A-142747	CCUGAGGCUCAAGAGGCCA	652	A-142748	UGGCCUCUUGAGCCUUCAGG	860	2070-2088
AD-71185	A-142749	AAGAGGCCAGGGAGCAAU	653	A-142750	AUUGCUCCCUUGGGCCUCUU	861	2080-2098
AD-71186	A-142751	GGAGCAUUGGGAGGGGCGU	654	A-142752	AGCCCCUCCCAUUGCUC	862	2091-2109
AD-71187	A-142753	AGGGGCGUCCAUUGGAGGAA	655	A-142754	UUCUCCCAUGGAGCCCCCU	863	2102-2120
AD-71188	A-142755	GGAGGAGGUGUCCCAAGCU	656	A-142756	AGCUUGGGACACCUCCUCC	864	2114-2132
AD-71189	A-142757	UCCCAAGCUUUUGAAUACCA	657	A-142758	UGGUUUUCAAAGCUUUGGGA	865	2124-2142
AD-71190	A-142759	AAUACCCCCAGAGACCUUU	658	A-142760	AAAGGUCUCUGGGGUAUU	866	2136-2154
AD-71191	A-142761	AGAGACCUUUUUCUCUCCCA	659	A-142762	UGGGAGAGAAAAGGUCUCU	867	2145-2163

AD-71192	A-142763	UCUCCCCAUACCAUCACUGA	660	A-142764	UCAGUGAUGGUAUGGGAGA	868	2157-2175
AD-71193	A-142765	UCACUGAGUGGCUUGUGAU	661	A-142766	AUCACAAGCCACUCAGUGA	869	2169-2187
AD-71194	A-142767	GGCUUGUGAUUCUGGGAUA	662	A-142768	UAUCCAGAAUACACAAGCC	870	2178-2196
AD-71195	A-142769	UGGGAUGGACCCUCGCAGA	663	A-142770	UCUGCGAGGGUCCAUCCCA	871	2190-2208
AD-71196	A-142771	UCGCAGCAGGUGCAAGAGA	664	A-142772	UCUCUUGCACCCUGCUGCGA	872	2202-2220
AD-71197	A-142773	UGCAAGAGACAGAGCCCCA	665	A-142774	UGGGGCUCUGUCUCUUGCA	873	2212-2230
AD-71198	A-142775	AGAGCCCCCAAGCCUCUGA	666	A-142776	UCAGAGGCUUGGGGGCUCU	874	2222-2240
AD-71199	A-142777	CUCUGCCCCAAGGGCCCCA	667	A-142778	UGGGCCCCUUGGGGCAGAG	875	2235-2253
AD-71200	A-142779	AAGGGCCCCACAAAGGGGA	668	A-142780	UCCCCUUGUGGGGCCCCUU	876	2244-2262
AD-71201	A-142781	AAAGGGGAGAAAGGGCCAGA	669	A-142782	UCUGGGCCCUUCUCCCCUUU	877	2255-2273
AD-71202	A-142783	GGGCCAGCCCUACAUUUUA	670	A-142784	UAAGAUGUAGGGCUGGCC	878	2266-2284
AD-71203	A-142785	AUCUUCAGCUCCCAUAGCA	671	A-142786	UGCUAUGGGAGCUGAAGAU	879	2279-2297
AD-71204	A-142787	UCCCAUAGCGCUGGCUCAA	672	A-142788	UUGAGCCAGCGCUAUGGGA	880	2288-2306
AD-71205	A-142789	UGGCUCAGGAAGAAACCCA	673	A-142790	UGGGUUUCUUCUUGAGCCA	881	2299-2317
AD-71206	A-142791	AACCCCAAGCAGCAUUCAA	674	A-142792	UUGAAUGCUGCUUGGGGUU	882	2312-2330
AD-71207	A-142793	CAGCAUUCAGCACACCCCA	675	A-142794	UGGGGUGUGCUGAAUGCUG	883	2321-2339
AD-71208	A-142795	CACCCCAAGGGACAACCCA	676	A-142796	UGGGUUGUCCCUUGGGGUG	884	2333-2351
AD-71209	A-142797	ACAACCCCAUCAUAUGACA	677	A-142798	UGUCAUAUGAUGGGGUUGU	885	2344-2362
AD-71210	A-142801	ACCCUCUCCAUGCCCAACA	678	A-142802	UGUUGGGCAUGGAGAGGGU	886	2367-2385
AD-71211	A-142803	UGCCCAACCUAAGAUUGUA	679	A-142804	UACAAUCUUAGGUUUGGCA	887	2377-2395
AD-71212	A-142805	AAGAUUGUGUGGGUUUUUU	680	A-142806	AAAAAACCCACACAACUUCU	888	2387-2405
AD-71213	A-142807	UUUUUUAAUUAAAAAUGUU	681	A-142808	AACAUUUUUAAUUAAAAAA	889	2400-2418
AD-71214	A-142809	UAAAAAUGUUAAAAAGUUUU	682	A-142810	AAAAACUUUUACAUUUUUUA	890	2409-2427
AD-71215	A-142811	AAAGUUUUAAACAUGAAAA	683	A-142812	UUUUC AUGUUUUAAAAACUUCU	891	2420-2438

Table 7: GCK Modified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5'-3')	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence (5'-3')	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-71009	A-142377	CUGCCAGCCUCAGGCA GCUdTdT	892	A-142378	AGCUGCCUGAGGCUGG CAGdTdT	1100	CUGCCAGCCUCAGGCA GCU	1308
AD-71010	A-142379	UCAGGCAGCUCUCCAU CCAdTdT	893	A-142380	UGGAUGGAGAGCUGCC UGAdTdT	1101	UCAGGCAGCUCUCCAU CCA	1309
AD-71011	A-142381	CCAUCCAAAGCAGCCGU UGAdTdT	894	A-142382	UCAACGGCUGCUUGGA UGGdTdT	1102	CCAUCCAAAGCAGCCGU UGC	1310
AD-71012	A-142383	AGCCGUUGCUGCCACA GGAdTdT	895	A-142384	UCCUGUGGCAGCAACG GCAdTdT	1103	AGCCGUUGCUGCCACA GGC	1311
AD-71013	A-142385	ACAGGCGGGCCUUAACG CUAdTdT	896	A-142386	UAGCGUAAGGCCCGCC UGUdTdT	1104	ACAGGCGGGCCUUAACG CUC	1312
AD-71014	A-142387	UUACGCUCCAAGGCUA CAAdTdT	897	A-142388	UUGUAGCCUUGGAGCG UAAdTdT	1105	UUACGCUCCAAGGCUA CAG	1313
AD-71015	A-142389	AAGGCUACAGCAUGUG CUAdTdT	898	A-142390	UAGCACAUUGCUGUAGC CUUdTdT	1106	AAGGCUACAGCAUGUG CUA	1314
AD-71016	A-142391	UGUGCUAGGCCUCAGC AGAdTdT	899	A-142392	UCUGCUGAGGCCUAGC ACAdTdT	1107	UGUGCUAGGCCUCAGC AGG	1315
AD-71017	A-142393	UCAGCAGGCAGGAGCA UCAdTdT	900	A-142394	AGAUGCUCUCCUGC UGAdTdT	1108	UCAGCAGGCAGGAGCA UCU	1316
AD-71018	A-142395	AGCAUCUCUGCCUCCC AAAdTdT	901	A-142396	UUUGGAGGCAGAGAU GCAdTdT	1109	AGCAUCUCUGCCUCCC AAA	1317
AD-71019	A-142397	CCUCCCAAAGCAUCUA CCAdTdT	902	A-142398	AGGUAAGAUUGCUUUGG AGGdTdT	1110	CCUCCCAAAGCAUCUA CCU	1318
AD-71020	A-142401	UAGCCCCUCGGAGAGA UGAdTdT	903	A-142402	UCAUCUCUCCGAGGGG CUAdTdT	1111	UAGCCCCUCGGAGAGA UGG	1319
AD-71021	A-142403	AGAGAUUGGCGAUGGAU GUAdTdT	904	A-142404	UACAUCCAUCGCCAUC UCAdTdT	1112	AGAGAUUGGCGAUGGAU GUC	1320
AD-71022	A-142405	UGGAUGUCACAAAGGAG CCAdTdT	905	A-142406	UGGCUCCUUGUGACAU CCAdTdT	1113	UGGAUGUCACAAAGGAG CCA	1321
AD-71023	A-142407	AGGAGCCAGGCCCAGA CAAdTdT	906	A-142408	UUGUCUGGGCCUUGGCU CCAdTdT	1114	AGGAGCCAGGCCCAGA CAG	1322

AD-71024	A-142411	ACUCUGGUAGAGCAGA UCAdTdT	907	A-142412	UGAUCUGCUUACCCAG AGUdTdT	1115	ACUCUGGUAGAGCAGA UCC	1323
AD-71025	A-142413	AGCAGAUCCUGGCAGA GUUdTdT	908	A-142414	AACUCUGCCAGGAUCU GCUdTdT	1116	AGCAGAUCCUGGCAGA GUU	1324
AD-71026	A-142415	CAGAGUCCAGCUGCA GGAdTdT	909	A-142416	UCCUGCAGCUGGAACU CUGdTdT	1117	CAGAGUCCAGCUGCA GGA	1325
AD-71027	A-142417	AGCUGCAGGAGGAGGA CCUdTdT	910	A-142418	AGGUCCUCCUCCUGCA GCUdTdT	1118	AGCUGCAGGAGGAGGA CCU	1326
AD-71028	A-142419	AGGACCUGAAGAAGGU GAUdTdT	911	A-142420	AUCACCUUUCUUCAGGU CCUdTdT	1119	AGGACCUGAAGAAGGU GAU	1327
AD-71029	A-142421	AAGGUGAUGAGACGGA UGAdTdT	912	A-142422	UCAUCCGUCUCAUCAC CUUdTdT	1120	AAGGUGAUGAGACGGA UGC	1328
AD-71030	A-142423	CGGAUGCAGAAAGGAGA UGAdTdT	913	A-142424	UCAUCUCCUUCUGCAU CCGdTdT	1121	CGGAUGCAGAAAGGAGA UGG	1329
AD-71031	A-142425	AAGGAGAUGGACCGCG GCAdTdT	914	A-142426	UGCCGCGGUCCAUUCUC CUUdTdT	1122	AAGGAGAUGGACCGCG GCC	1330
AD-71032	A-142427	CGCGGCCUGAGGCUGG AGAdTdT	915	A-142428	UCUCCAGCCUCACAGGCC GCGdTdT	1123	CGCGGCCUGAGGCUGG AGA	1331
AD-71033	A-142429	CUGGAGACCCAUAGAAG AGAdTdT	916	A-142430	UCUCUUAUGGGUUCUC CAGdTdT	1124	CUGGAGACCCAUAGAAG AGG	1332
AD-71034	A-142431	CAUGAAAGAGGCCAGUG UGAdTdT	917	A-142432	UCACACUGGCCUUCUUC AUGdTdT	1125	CAUGAAAGAGGCCAGUG UGA	1333
AD-71035	A-142433	CAGUGUGAAGAUGCUG CCAdTdT	918	A-142434	UGGCAGCAUCUUCACAC CUGdTdT	1126	CAGUGUGAAGAUGCUG CCC	1334
AD-71036	A-142435	UGCUGCCCACCUACGU GCAdTdT	919	A-142436	UGCACGUAGGUGGGCA GCAdTdT	1127	UGCUGCCCACCUACGU GCG	1335
AD-71037	A-142437	UACGUGCGCUCCACCCC AAAdTdT	920	A-142438	UUGGGUGGAGCGCAC GUAdTdT	1128	UACGUGCGCUCCACCCC AG	1336
AD-71038	A-142439	ACCCAGAAAGGCUCAG AAAdTdT	921	A-142440	UUUCUGAGCCUUCUGG GGUdTdT	1129	ACCCAGAAAGGCUCAG AAG	1337
AD-71039	A-142441	UCAGAAAGUCGGGGACU UCAdTdT	922	A-142442	UGAAGUCCCCGACUUC UGAdTdT	1130	UCAGAAAGUCGGGGACU UCC	1338
AD-71040	A-142443	GGGACUUCUUCUCCCC UGAdTdT	923	A-142444	UCAGGAGAGGAAGUUC CCCdTdT	1131	GGGACUUCUUCUCCCC UGG	1339
AD-71041	A-142445	UCCUGGACCUGGGUG GCAdTdT	924	A-142446	UGCCACCCAGGUCCAG GGAdTdT	1132	UCCUGGACCUGGGUG GCA	1340

AD-71042	A-142447	UGGUGGCACUAAACUU CAAdTdT	925	A-142448	UUGAAGUUAGUGCCAC CCAdTdT	1133	UGGUGGCACUAAACUU CAG	1341
AD-71043	A-142449	ACUUCAGGGUGAUGCU GGUdTdT	926	A-142450	ACCAGCAUCACCCUGA AGUdTdT	1134	ACUUCAGGGUGAUGCU GGU	1342
AD-71044	A-142453	AGGUGGGAGAAAGGUGA GGAdTdT	927	A-142454	UCCUCACCUUCUCCCCAC CUdTdT	1135	AGGUGGGAGAAAGGUGA GGA	1343
AD-71045	A-142457	CAGUGGAGCGUGAAGA CCAdTdT	928	A-142458	UGGUCUUCACGCUCCA CUGdTdT	1136	CAGUGGAGCGUGAAGA CCA	1344
AD-71046	A-142461	CCAGAUUGUACUCCAUC CCAdTdT	929	A-142462	UGGGAUGGAGUACAUC UGGdTdT	1137	CCAGAUUGUACUCCAUC CCC	1345
AD-71047	A-142467	ACCGGCACUGCUGAGA UGAdTdT	930	A-142468	UCAUCUCAGCAGUGCC GGUdTdT	1138	ACCGGCACUGCUGAGA UGC	1346
AD-71048	A-142469	AGAUGCUCUUCGACUA CAUdTdT	931	A-142470	AUGUAGUCGAAAGAGCA UCUdTdT	1139	AGAUGCUCUUCGACUA CAU	1347
AD-71049	A-142471	UCGACUACAUCUCUGA GUAdTdT	932	A-142472	UACUCAGAGAUUGUAGU CGAdTdT	1140	UCGACUACAUCUCUGA GUG	1348
AD-71050	A-142473	UCUGAGUGCAUCUCCG ACUdTdT	933	A-142474	AGUCGGAGAUGCACUC AGAdTdT	1141	UCUGAGUGCAUCUCCG ACU	1349
AD-71051	A-142475	UCCGACUUCUCCUGGACA AGAdTdT	934	A-142476	UCUUGUCCAGGAAGUC GGAdTdT	1142	UCCGACUUCUCCUGGACA AGC	1350
AD-71052	A-142477	GACAAAGCAUCAGAUGA AACdTdT	935	A-142478	GUUUCAUUCUGAUGCUU GUCdTdT	1143	GACAAAGCAUCAGAUGA AAC	1351
AD-71053	A-142479	AGAUGAAACACAAAGAA GCUdTdT	936	A-142480	AGCUUCUUGUGUUUCA UCUdTdT	1144	AGAUGAAACACAAAGAA GCU	1352
AD-71054	A-142481	AGAAGCUGCCCCUUGGG CUUdTdT	937	A-142482	AAGCCCAGGGGCAGCU UCUdTdT	1145	AGAAGCUGCCCCUUGGG CUU	1353
AD-71055	A-142483	CCUGGGCUUCACCCUUC UCAdTdT	938	A-142484	UGAGAAAGGUGAAGCCC AGGdTdT	1146	CCUGGGCUUCACCCUUC UCC	1354
AD-71056	A-142485	ACCUUCUCCUUUCCUG UGAdTdT	939	A-142486	UCACAGGAAAGGAGAA GGUdTdT	1147	ACCUUCUCCUUUCCUG UGA	1355
AD-71057	A-142487	CUGAGAGGCACGAAGA CAUdTdT	940	A-142488	AUGUCUUCGUGCCUCA CAGdTdT	1148	CUGAGAGGCACGAAGA CAU	1356
AD-71058	A-142489	GAAGACAUCGAUAAGG GCAdTdT	941	A-142490	UGCCCUUAUCGAUGUC UUCdTdT	1149	GAAGACAUCGAUAAGG GCA	1357
AD-71059	A-142491	GAUAAAGGGCAUCCUUC UCAdTdT	942	A-142492	UGAGAAAGGAUGCCCUU AUCdTdT	1150	GAUAAAGGGCAUCCUUC UCA	1358

AD-71060	A-142493	UUCUAAACUGGACCAA GGAdTdT	943	A-142494	UCCUUGGUCCAGUUGA GAAdTdT	1151	UUCUAAACUGGACCAA GGG	1359
AD-71061	A-142495	ACCAAGGGCUUCAAGG CCUdTdT	944	A-142496	AGCCUUUGAAGCCCUU GGUdTdT	1152	ACCAAGGGCUUCAAGG CCU	1360
AD-71062	A-142497	CAAGCCUCAGGAGCA GAAdTdT	945	A-142498	UUCUGCUCCUGAGGCC UUGdTdT	1153	CAAGCCUCAGGAGCA GAA	1361
AD-71063	A-142499	AGGAGCAGAAAGGGAAC AAUdTdT	946	A-142500	AUUGUCCCUUCUCU CCUdTdT	1154	AGGAGCAGAAAGGGAAC AAU	1362
AD-71064	A-142501	AACA AUGUCUGGGGC UUAdTdT	947	A-142502	UAAGCCCCACGACAUU GUUdTdT	1155	AACA AUGUCUGGGGC UUC	1363
AD-71065	A-142503	UGGGCUUCUCGCGAGA CGAdTdT	948	A-142504	UCGUCUCGCAGAGCC CCAdTdT	1156	UGGGCUUCUCGCGAGA CGC	1364
AD-71066	A-142505	CGAGACGCUAUCAAAC GGAdTdT	949	A-142506	UCCGUUUGAUAGCGUC UCGdTdT	1157	CGAGACGCUAUCAAAC GGA	1365
AD-71067	A-142507	AAACGGAGAGGGGACU UUAdTdT	950	A-142508	UAAAGUCCCCUCUCCG UUUdTdT	1158	AAACGGAGAGGGGACU UUG	1366
AD-71068	A-142509	GGGACUUUGAAAUUGA UGUdTdT	951	A-142510	ACAUCCAUUUCAAGU CCCdTdT	1159	GGGACUUUGAAAUUGA UGU	1367
AD-71069	A-142513	GCAAUGGUGAAUGACA CGAdTdT	952	A-142514	UCGUGCAUUCACCAU UGCdTdT	1160	GCAAUGGUGAAUGACA CGG	1368
AD-71070	A-142515	AAUGACACGGUGGCCA CGAdTdT	953	A-142516	UCGUGGCCACCGUGUC AUUdTdT	1161	AAUGACACGGUGGCCA CGA	1369
AD-71071	A-142517	GCCACGAUGAUCUCCU GCUdTdT	954	A-142518	AGCAGGAGAUCAUCGU GGCdTdT	1162	GCCACGAUGAUCUCCU GCU	1370
AD-71072	A-142519	UCCUGCUACUACGAAG ACAdTdT	955	A-142520	UGUCUUCGUAGUAGCA GGAdTdT	1163	UCCUGCUACUACGAAG ACC	1371
AD-71073	A-142523	AGUGCGAGGUGCGCAU GAUdTdT	956	A-142524	AUCAUGCCGACCUCCG ACUdTdT	1164	AGUGCGAGGUGCGCAU GAU	1372
AD-71074	A-142525	GGCAUGAUCGUGGGCA CGAdTdT	957	A-142526	UCGUGCCACGCAUCAU GCCdTdT	1165	GGCAUGAUCGUGGGCA CGG	1373
AD-71075	A-142527	GUGGGCACGGGCUUGCA AUAdTdT	958	A-142528	UAUUGCAGCCCGUGCC CACdTdT	1166	GUGGGCACGGGCUUGCA AUG	1374
AD-71076	A-142529	UGCAAUGCCUGCUACA UGAdTdT	959	A-142530	UCAUGUAGCAGGCAUU GCAdTdT	1167	UGCAAUGCCUGCUACA UGG	1375
AD-71077	A-142531	UACAUGGAGGAGAUGC AGAdTdT	960	A-142532	UCUGCAUCCUCCUCCAU GUAdTdT	1168	UACAUGGAGGAGAUGC AGA	1376

AD-71078	A-142533	AGAUGCAGAAUUGUGGA GCUdTdT	961	A-142534	AGCUCCACAUCUGCA UCUdTdT	1169	AGAUGCAGAAUUGUGGA GCU	1377
AD-71079	A-142535	UGUGGAGCUGGUGGAG GGAdTdT	962	A-142536	UCCCUCCACCAGCUCCA CAdTdT	1170	UGUGGAGCUGGUGGAG GGG	1378
AD-71080	A-142537	UGGAGGGGACGAGGG CCAdTdT	963	A-142538	UGGCCCCUCGUCCCCCUC CAdTdT	1171	UGGAGGGGACGAGGG CCG	1379
AD-71081	A-142539	GAGGGCCGCAUGUGCG UCAdTdT	964	A-142540	UGACGCACAUGCGGCC CUCdTdT	1172	GAGGGCCGCAUGUGCG UCA	1380
AD-71082	A-142541	UGCUCAAUACCGAGU GGAdTdT	965	A-142542	UCCACUCGGUAUUGAC GCAdTdT	1173	UGCUCAAUACCGAGU GGG	1381
AD-71083	A-142543	CGAGUGGGCGCCUUC GGAdTdT	966	A-142544	UCCGAAGGCGCCCCAC UCGdTdT	1174	CGAGUGGGCGCCUUC GGG	1382
AD-71084	A-142545	GCCUUCGGGGACUCCG GCAdTdT	967	A-142546	UGCCGGAGUCCCCGAA GGCdTdT	1175	GCCUUCGGGGACUCCG GCG	1383
AD-71085	A-142547	UCCGGCGAGCUGGACG AGUdTdT	968	A-142548	ACUCGUCCAGCUCGCC GGAdTdT	1176	UCCGGCGAGCUGGACG AGU	1384
AD-71086	A-142549	GACGAGUCCUCUGCG AGUdTdT	969	A-142550	ACUCCAGCAGGAACUC GUdTdT	1177	GACGAGUCCUCUGCG AGU	1385
AD-71087	A-142551	UGCUGGAGUAUGACCG CCUdTdT	970	A-142552	AGCGGUAUAUCUCCA GCAdTdT	1178	UGCUGGAGUAUGACCG CCU	1386
AD-71088	A-142553	GACCGCCUGGUGGACG AGAdTdT	971	A-142554	UCUCGUCCACCAGGCG GUdTdT	1179	GACCGCCUGGUGGACG AGA	1387
AD-71089	A-142555	GGACGAGAGCUCUGCA AACdTdT	972	A-142556	GUUUGCAGAGCUCUCG UCCdTdT	1180	GGACGAGAGCUCUGCA AAC	1388
AD-71090	A-142557	UCUGCAAACCCCGGUC AGAdTdT	973	A-142558	UCUGACCGGGGUUUGC AGAdTdT	1181	UCUGCAAACCCCGGUC AGC	1389
AD-71091	A-142559	GGUCAGCAGCUGUAUG AGAdTdT	974	A-142560	UCUCAUACAGCUGCUG ACCTdTdT	1182	GGUCAGCAGCUGUAUG AGA	1390
AD-71092	A-142561	UAUGAGAGCUCUAUAG GUAdTdT	975	A-142562	UACCUAUGAGCUCUCUC AUAdTdT	1183	UAUGAGAGCUCUAUAG GUG	1391
AD-71093	A-142563	UCAUAGGUGGCAAGUA CAUdTdT	976	A-142564	AUGUACUUGCCACCUA UGAdTdT	1184	UCAUAGGUGGCAAGUA CAU	1392
AD-71094	A-142565	AAGUACAUGGGCGAGC UGAdTdT	977	A-142566	UCAGCUCGCCCAUGUA CUUdTdT	1185	AAGUACAUGGGCGAGC UGG	1393
AD-71095	A-142567	GCGAGCUGGUGCGGCU UGUdTdT	978	A-142568	ACAAGCCGCACCAGCU CGCdTdT	1186	GCGAGCUGGUGCGGCU UGU	1394

AD-71096	A-142569	GGCUUGUGCUGCUCAG GCUdTdT	979	A-142570	AGCCUGAGCAGCAAA GCCdTdT	1187	GGCUUGUGCUGCUCAG GCU	1395
AD-71097	A-142571	UCAGGCUUGGACGA AAAdTdT	980	A-142572	UUUUCGUCCACGAGCC UGAdTdT	1188	UCAGGCUUGGACGA AAA	1396
AD-69448	A-139832	UGGACGAAAACCUUCU CUUdTdT	981	A-139833	AAGAGCAGGUUUUCGU CCAdTdT	1189	UGGACGAAAACCUUCU CUU	1397
AD-71098	A-142573	UGCUCUCCACGGGA GGAdTdT	982	A-142574	UCCUCCCCGUGGAAGA GCAdTdT	1190	UGCUCUCCACGGGA GGC	1398
AD-71099	A-142575	GGGAGGCCUCCGAGCA GCUdTdT	983	A-142576	AGCUCUCCGAGGCCU CCCTdTdT	1191	GGGAGGCCUCCGAGCA GCU	1399
AD-71100	A-142577	CGAGCAGCUGCGCACA CGAdTdT	984	A-142578	UCGUGUGCGCAGCUGC UCGdTdT	1192	CGAGCAGCUGCGCACA CGC	1400
AD-71101	A-142581	AGCCUUCGAGACGCGC UUAdTdT	985	A-142582	UAAGCGCGUCUCGAAG GCUdTdT	1193	AGCCUUCGAGACGCGC UUC	1401
AD-71102	A-142583	CGCUUCGUGUCGCAGG UGAdTdT	986	A-142584	UCACCUGCGACACGAA GCGdTdT	1194	CGCUUCGUGUCGCAGG UGG	1402
AD-71103	A-142585	UCGCAGGUGGAGAGCG ACAdTdT	987	A-142586	UGUCGCUCCACCUCG CGAdTdT	1195	UCGCAGGUGGAGAGCG ACA	1403
AD-71104	A-142587	AGCGACACGGGCGACC GCAdTdT	988	A-142588	UGCGGUCGCCCGUGUC GCUdTdT	1196	AGCGACACGGGCGACC GCA	1404
AD-71105	A-142589	CGACCGCAAGCAGAUC UAAdTdT	989	A-142590	UUAGAUCUGCUUGCGG UCGdTdT	1197	CGACCGCAAGCAGAUC UAC	1405
AD-71106	A-142591	CAGAUUCUACAACAUC UGAdTdT	990	A-142592	UCAGGAUGUUUAGAU CUGdTdT	1198	CAGAUUCUACAACAUC UGA	1406
AD-71107	A-142593	UCCUGAGCACGCUUGGG GCUdTdT	991	A-142594	AGCCCCAGCGUGCUCA GGAdTdT	1199	UCCUGAGCACGCUUGGG GCU	1407
AD-71108	A-142595	CUGGGGCUUGCGACCCU CGAdTdT	992	A-142596	UCGAGGUGCGACGCC CAGdTdT	1200	CUGGGGCUUGCGACCCU CGA	1408
AD-71109	A-142597	CGACCCUCCGACCACCGA CUdTdT	993	A-142598	AGUCGGUGGUCGAGGG UCGdTdT	1201	CGACCCUCCGACCACCGA CU	1409
AD-71110	A-142599	CACCGACUCCGACAUC GUAdTdT	994	A-142600	UACGAUGUCGACAGUCG GUGdTdT	1202	CACCGACUCCGACAUC GUG	1410
AD-71111	A-142601	CAUCGUGCGCGCGCC UGAdTdT	995	A-142602	UCAGGCGCGCGCACG AUGdTdT	1203	CAUCGUGCGCGCGCC UGC	1411
AD-71112	A-142603	CGCGCCUCCGAGAGCG UGUdTdT	996	A-142604	ACACGCUUCGCAAGGC GCGdTdT	1204	CGCGCCUCCGAGAGCG UGU	1412

AD-71113	A-142605	AGCGUGUCUACGCGCG CUAdTdT	997	A-142606	UAGCGGCGGUAGACAC GCUdTdT	1205	AGCGUGUCUACGCGCG CUG	1413
AD-71114	A-142607	CGCGCUGCGCACAUUGU GCUdTdT	998	A-142608	AGCACAUUGCGGCAGC GCGdTdT	1206	CGCGCUGCGCACAUUGU GCU	1414
AD-71115	A-142609	ACAUGUGCUCGGCGGG GCUdTdT	999	A-142610	AGCCCCGCCGAGCAC UGUdTdT	1207	ACAUGUGCUCGGCGGG GCU	1415
AD-71116	A-142611	CGGGCUGGCGGGCGU CAUdTdT	1000	A-142612	AUGACGCCGCCAGCCCC CGdTdT	1208	CGGGCUGGCGGGCGU CAU	1416
AD-71117	A-142613	CGGGCUGCAUCAACCG CAUdTdT	1001	A-142614	AUGCGUUGAUGACGC CCGdTdT	1209	CGGGCUGCAUCAACCG CAU	1417
AD-71118	A-142615	AACCGCAUGCGCGAGA GCAdTdT	1002	A-142616	UGCUCUCGCGCAUGCG GUUdTdT	1210	AACCGCAUGCGCGAGA GCC	1418
AD-71119	A-142617	AGAGCCGACGCGAGGA CGUdTdT	1003	A-142618	ACGUCCUCGCGCGGC UCUdTdT	1211	AGAGCCGACGCGAGGA CGU	1419
AD-71120	A-142619	CGAGGACGUAAUGCGC AUAdTdT	1004	A-142620	UAUGCGCAUUAACGUCC UCGdTdT	1212	CGAGGACGUAAUGCGC AUC	1420
AD-71121	A-142621	UCCGCAUCACUGUGGG CGUdTdT	1005	A-142622	ACGCCACAGUGAUGC GCAdTdT	1213	UCCGCAUCACUGUGGG CGU	1421
AD-71122	A-142623	UGGGCGUGGAUGGCUC CGUdTdT	1006	A-142624	ACGGAGCCAUCCACGC CCAdTdT	1214	UGGGCGUGGAUGGCUC CGU	1422
AD-71123	A-142625	UGGCUCCGUGUACAAG CUAdTdT	1007	A-142626	UAGCUUGUACACGGAG CCAdTdT	1215	UGGCUCCGUGUACAAG CUG	1423
AD-71124	A-142627	UACAAGCUGCACCCCA GCUdTdT	1008	A-142628	AGCUGGGGUGCAGCUU GUAdTdT	1216	UACAAGCUGCACCCCA GCU	1424
AD-71125	A-142629	CCAGCUUCAAGGAGCG GUUdTdT	1009	A-142630	AACCGCUCCUUGAAGC UGGdTdT	1217	CCAGCUUCAAGGAGCG GUU	1425
AD-71126	A-142631	AGGAGCGGUUCCAUGC CAAdTdT	1010	A-142632	UUGGCAUGGAACCGCU CCUdTdT	1218	AGGAGCGGUUCCAUGC CAG	1426
AD-71127	A-142633	AUGCCAGCGUGCGCAG GCUdTdT	1011	A-142634	AGCCUGCGCACGCUUG CAUdTdT	1219	AUGCCAGCGUGCGCAG GCU	1427
AD-71128	A-142635	CGCAGGCUAGACGCCCA GCUdTdT	1012	A-142636	AGCUGGGCGUCAGCCU GCGdTdT	1220	CGCAGGCUAGACGCCCA GCU	1428
AD-71129	A-142637	CCCAGCUGCGAGAUCA CCUdTdT	1013	A-142638	AGGUAUCUCGCGAGCU GGGdTdT	1221	CCCAGCUGCGAGAUCA CCU	1429
AD-71130	A-142639	GAGAUACCUUCAUCG AGUdTdT	1014	A-142640	ACUCGAUGAAGGUGAU CUCdTdT	1222	GAGAUACCUUCAUCG AGU	1430

AD-71131	A-142641	AUCGAGUCGGAGGAGG GCAAdTdT	1015	A-142642	UGCCCUCCUCCGACUCG AUAdTdT	1223	AUCGAGUCGGAGGAGG GCA	1431
AD-71132	A-142643	AGGAGGGCAGUGGCCCG GGAdTdT	1016	A-142644	UCCCGGCCACUGCCCCUC CUAdTdT	1224	AGGAGGCAGUGGCCCG GGG	1432
AD-71133	A-142645	CCGGGGCGCGGCCCCUG GUAdTdT	1017	A-142646	UACCAAGGGCCCGGCCCC GGAdTdT	1225	CCGGGGCGCGGCCCCUG GUC	1433
AD-71134	A-142647	CCCUGGUCUCGGCGGU GGAdTdT	1018	A-142648	UCCACCGCCGAGACCA GGGAdTdT	1226	CCCUGGUCUCGGCGGU GGC	1434
AD-71135	A-142649	GCGUGGGCCUGUAAGA AGAdTdT	1019	A-142650	UCUUCUACAGGCCAC CGCAdTdT	1227	GCGUGGCCUGUAAGA AGG	1435
AD-71136	A-142651	UAAGAAAGGCCUGUAUG CUAdTdT	1020	A-142652	UAGCAUACAGGCCUUC UUAdTdT	1228	UAAGAAAGGCCUGUAUG CUG	1436
AD-71137	A-142653	CUGUAUGCUGGGCCAG UGAdTdT	1021	A-142654	UCACUGGCCCCAGCAUA CAGAdTdT	1229	CUGUAUGCUGGGCCAG UGA	1437
AD-71138	A-142655	CAGUGAGAGCAGUGGC CGAdTdT	1022	A-142656	UCGGCCACUGCUCUCA CUGAdTdT	1230	CAGUGAGAGCAGUGGC CGC	1438
AD-71139	A-142657	CAGUGGCCCGCAAAGCGC AGAdTdT	1023	A-142658	UCUGCGCUUGCGGCCA CUGAdTdT	1231	CAGUGGCCCGCAAAGCGC AGG	1439
AD-71140	A-142659	AGCGCAGGGAGGAUGC CAAdTdT	1024	A-142660	UUGGCAUCCUCCUCCUGC GCUAdTdT	1232	AGCGCAGGGAGGAUGC CAC	1440
AD-71141	A-142661	UGCCACAGCCCCACAGC AAdTdT	1025	A-142662	UUGCUGUGGGGCGCUGUG GCAdTdT	1233	UGCCACAGCCCCACAGC AC	1441
AD-71142	A-142663	CACAGCACCCAGGCUCC AUAdTdT	1026	A-142664	AUGGAGCCUGGGUGUCU GUGAdTdT	1234	CACAGCACCCAGGCUCC AU	1442
AD-71143	A-142665	AGGCUCCAUGGGGAAG UGAdTdT	1027	A-142666	UCACUUCGCCAUUGGAG CCUAdTdT	1235	AGGCUCCAUGGGGAAG UGC	1443
AD-71144	A-142667	GGAAGUGCUCCCCACA CGUAdTdT	1028	A-142668	ACGUGUGGGGAGCACU UCCAdTdT	1236	GGAAGUGCUCCCCACA CGU	1444
AD-71145	A-142669	CCACACGUGCUCGCAG CCUAdTdT	1029	A-142670	AGGCUGCCAGCACGUG UGGAdTdT	1237	CCACACGUGCUCGCAG CCU	1445
AD-71146	A-142671	UCGCAGCCUGGCGGGG CAAdTdT	1030	A-142672	UUGCCCCGCCAGGCGUG CGAdTdT	1238	UCGCAGCCUGGCGGGG CAG	1446
AD-71147	A-142673	CGGGGCAGGAGGCCUG GCAdTdT	1031	A-142674	UCCAGGCCUCCUUGCCC CGAdTdT	1239	CGGGGCAGGAGGCCUG GCC	1447
AD-71148	A-142675	CCUGGCCUUGUCAGGA CCAdTdT	1032	A-142676	UGGUCCUUGACAAAGGCC AGGAdTdT	1240	CCUGGCCUUGUCAGGA CCC	1448

AD-71149	A-142677	CAGGACCCAGGCGGCC UGAdTdT	1033	A-142678	UCAGGGGGCCUUGGGUC CUGdTdT	1241	CAGGACCCAGGCGGCC UGC	1449
AD-71150	A-142679	CCGCCUGCCAUACCGCU GAdTdT	1034	A-142680	UCAGCGGU AUGGCAGG CGGdTdT	1242	CCGCCUGCCAUACCGCU GG	1450
AD-71151	A-142681	UACCGCUGGGGAACAG AGAdTdT	1035	A-142682	UCUCUGU UCCCCAGCG GUAdTdT	1243	UACCGCUGGGGAACAG AGC	1451
AD-71152	A-142683	AACAGAGCGGGCCUCU UCAdTdT	1036	A-142684	UGAAGAGGCCCCGCUCU GUUdTdT	1244	AACAGAGCGGGCCUCU UCC	1452
AD-71153	A-142685	CUCUCCCCUCAGUUUU UCAdTdT	1037	A-142686	UGAAAAACUCAGGGGAA GAGdTdT	1245	CUCUCCCCUCAGUUUU UCG	1453
AD-71154	A-142687	UUUUUCGGUGGGACAG CCAdTdT	1038	A-142688	UGGCUGUCCCCACCGAA AAAdTdT	1246	UUUUUCGGUGGGACAG CCC	1454
AD-71155	A-142689	GGGACAGCCCCAGGGC CCUdTdT	1039	A-142690	AGGGCCUUGGGGCUGU CCCdTdT	1247	GGGACAGCCCCAGGGC CCU	1455
AD-71156	A-142691	AGGGCCCUAACGGGGG UGAdTdT	1040	A-142692	UCACCCCGUUAAGGGC CCUdTdT	1248	AGGGCCCUAACGGGGG UGC	1456
AD-71157	A-142693	GGGUGCGGCAGGAGCA GGAdTdT	1041	A-142694	UCCUGCUCU GCGGCAC CCdTdT	1249	GGGUGCGGCAGGAGCA GGA	1457
AD-71158	A-142695	AGGAGCAGGAACAGAG ACUdTdT	1042	A-142696	AGUCUCUGU UCCUGCU CCUdTdT	1250	AGGAGCAGGAACAGAG ACU	1458
AD-71159	A-142697	AGAGACUCUGGAAGCC CCAdTdT	1043	A-142698	UGGGCUUCCAGAGUC UCUdTdT	1251	AGAGACUCUGGAAGCC CCC	1459
AD-71160	A-142699	AAGCCCCCACCUCUUUCU CAdTdT	1044	A-142700	UGAGAAAGGUGGGGGG CUUdTdT	1252	AAGCCCCCACCUCUUUCU CG	1460
AD-71161	A-142701	UUUCUGCUGGAAUCA AUUdTdT	1045	A-142702	AAUUGAUUCCAGCGAG AAAdTdT	1253	UUUCUGCUGGAAUCA AUU	1461
AD-71162	A-142703	AAUCAAUUCCCCAGAA GGAdTdT	1046	A-142704	UCCUUCUGGGA AUUG AUUdTdT	1254	AAUCAAUUCCCCAGAA GGG	1462
AD-71163	A-142705	CCCAGAAAGGAGUUGC UCAdTdT	1047	A-142706	UGAGCAACUCCCCUUCU GGGdTdT	1255	CCCAGAAAGGAGUUGC UCA	1463
AD-71164	A-142707	UUGCUCACUCAGGACU UUAdTdT	1048	A-142708	UAAAGUCCUGAGUGAG CAAdTdT	1256	UUGCUCACUCAGGACU UUG	1464
AD-71165	A-142709	AGGACUUUGAUGCAUU UCAdTdT	1049	A-142710	UGAAAUGCAUCA AAGU CCUdTdT	1257	AGGACUUUGAUGCAUU UCC	1465
AD-71166	A-142711	AUUCCACACUGUCAG AGAdTdT	1050	A-142712	UCUCUGACAGUGUGGA AAUdTdT	1258	AUUCCACACUGUCAG AGC	1466

AD-71167	A-142713	UGUCAGAGCUGUUUGGC CUAdTdT	1051	A-142714	UAGGCCAACAGCUCUG ACAdTdT	1259	UGUCAGAGCUGUUUGGC CUC	1467
AD-71168	A-142715	UUGGCCUCCGCCUGGGC CCAdTdT	1052	A-142716	UGGCCCCAGGCGAGGC CAAdTdT	1260	UUGGCCUCCGCCUGGGC CCA	1468
AD-71169	A-142717	CUGGGCCCAGGCUCUG GGAdTdT	1053	A-142718	UCCCAGAGCCUGGGCC CAGdTdT	1261	CUGGGCCCAGGCUCUG GGA	1469
AD-71170	A-142719	CUCUGGGAAGGGGUGC CCUdTdT	1054	A-142720	AGGGCACCCCUUCCCCA GAGdTdT	1262	CUCUGGGAAGGGGUGC CCU	1470
AD-71171	A-142721	UGCCUCUGGAUCCUG CUAdTdT	1055	A-142722	UAGCAGGAUCCAGAGG GCAdTdT	1263	UGCCUCUGGAUCCUG CUG	1471
AD-71172	A-142723	UCCUGCUGUGGCCUCA CUUdTdT	1056	A-142724	AAGUGAGGCCACAGCA GGAdTdT	1264	UCCUGCUGUGGCCUCA CUU	1472
AD-71173	A-142725	CCUCACUCCCCUGGGA ACUdTdT	1057	A-142726	AGUCCCCAGGGAAGUG AGGdTdT	1265	CCUCACUCCCCUGGGA ACU	1473
AD-71174	A-142727	CUGGGAACUCAUCCUG UGUdTdT	1058	A-142728	ACACAGGAUGAGUUC CAGdTdT	1266	CUGGGAACUCAUCCUG UGU	1474
AD-71175	A-142729	CCUGUGUGGGGAGGCA GCUdTdT	1059	A-142730	AGCUGCCUCCCCACACA GGdTdT	1267	CCUGUGUGGGGAGGCA GCU	1475
AD-71176	A-142731	GGAGGCAGCUCCAACA GCUdTdT	1060	A-142732	AGCUGUUGGAGCUGCC UCCdTdT	1268	GGAGGCAGCUCCAACA GCU	1476
AD-71177	A-142733	CAACAGCUUGACCAGA CCUdTdT	1061	A-142734	AGGUCUGGUCUAGCUG UUGdTdT	1269	CAACAGCUUGACCAGA CCU	1477
AD-71178	A-142735	CCAGACCUAGACCUGG GCAdTdT	1062	A-142736	UGCCAGGUCUAGGUC UGGdTdT	1270	CCAGACCUAGACCUGG GCC	1478
AD-71179	A-142737	CUGGGCCAAAAGGGCA GCAdTdT	1063	A-142738	UGCUGCCCUUUUGGCC CAGdTdT	1271	CUGGGCCAAAAGGGCA GCC	1479
AD-71180	A-142739	AGGCAGCCAGGGGCU GCUdTdT	1064	A-142740	AGCAGCCCUUGGCUGC CCUdTdT	1272	AGGCAGCCAGGGGCU GCU	1480
AD-71181	A-142741	GGGUGCUCAUCACCC AGUdTdT	1065	A-142742	ACUGGUGAUGAGCAG CCCdTdT	1273	GGGUGCUCAUCACCC AGU	1481
AD-71182	A-142743	ACCCAGUCCUGGCCAU UUUdTdT	1066	A-142744	AAAAUGGCCAGGACUG GGUdTdT	1274	ACCCAGUCCUGGCCAU UUU	1482
AD-71183	A-142745	GCCAUUUUCUUGCCUG AGAdTdT	1067	A-142746	UCUCAGGCAAGAAAAU GGCdTdT	1275	GCCAUUUUCUUGCCUG AGG	1483
AD-71184	A-142747	CCUGAGGCUCAAGAGG CCAdTdT	1068	A-142748	UGGCCUCUUGAGCCUC AGGdTdT	1276	CCUGAGGCUCAAGAGG CCC	1484

AD-71185	A-142749	AAGAGGCCAGGGAGC AAUdTdT	1069	A-142750	AUUGCUCCCCUGGGCCU CUUdTdT	1277	AAGAGGCCAGGGAGC AAU	1485
AD-71186	A-142751	GGAGCAUUGGGAGGGG GCUdTdT	1070	A-142752	AGCCCCUCCCAUUGCU CCdTdT	1278	GGAGCAUUGGGAGGGG GCU	1486
AD-71187	A-142753	AGGGGCUCCAUGGAG GAAdTdT	1071	A-142754	UCCUCCAUGGAGCCC CCUdTdT	1279	AGGGGCUCCAUGGAG GAG	1487
AD-71188	A-142755	GGAGGAGGUGUCCCAA GCUdTdT	1072	A-142756	AGCUUGGGACACCUCC UCCdTdT	1280	GGAGGAGGUGUCCCAA GCU	1488
AD-71189	A-142757	UCCCAAGCUUUUGAAUA CCAdTdT	1073	A-142758	UGUAUUCAAAAGCUUG GGAdTdT	1281	UCCCAAGCUUUUGAAUA CCC	1489
AD-71190	A-142759	AAUACCCCCAGAGACC UUUdTdT	1074	A-142760	AAAGGUCUCUGGGGGU AUUdTdT	1282	AAUACCCCCAGAGACC UUU	1490
AD-71191	A-142761	AGAGACCUUUUCUCUC CCAdTdT	1075	A-142762	UGGGAGAGAAAAGGUC UCUdTdT	1283	AGAGACCUUUUCUCUC CCA	1491
AD-71192	A-142763	UCUCCCAUACCAUCAC UGAdTdT	1076	A-142764	UCAGUGAUGGUAUGGG AGAdTdT	1284	UCUCCCAUACCAUCAC UGA	1492
AD-71193	A-142765	UCACUGAGUGGCUUGU GAUdTdT	1077	A-142766	AUCACAAAGCCACUCAG UGAdTdT	1285	UCACUGAGUGGCUUGU GAU	1493
AD-71194	A-142767	GGCUUGUGAUUCUGGG AUAdTdT	1078	A-142768	UAUCCCAAGAAUACAAA GCCdTdT	1286	GGCUUGUGAUUCUGGG AUG	1494
AD-71195	A-142769	UGGGAUGGACCCUCGC AGAdTdT	1079	A-142770	UCUGCGAGGGUCCAU CCAdTdT	1287	UGGGAUGGACCCUCGC AGC	1495
AD-71196	A-142771	UCGCAGCAGGUGCAAG AGAdTdT	1080	A-142772	UCUCUUGCACCCUGCUG CGAdTdT	1288	UCGCAGCAGGUGCAAG AGA	1496
AD-71197	A-142773	UGCAAGAGACAGAGCC CCAdTdT	1081	A-142774	UGGGCUCUCUGUCUCUU GCAdTdT	1289	UGCAAGAGACAGAGCC CCC	1497
AD-71198	A-142775	AGAGCCCCCAAGCCUC UGAdTdT	1082	A-142776	UCAGAGGCUUGGGGGC UCUdTdT	1290	AGAGCCCCCAAGCCUC UGC	1498
AD-71199	A-142777	CUCUGCCCCAAGGGGC CCAdTdT	1083	A-142778	UGGGCCCCUUGGGGCA GAGdTdT	1291	CUCUGCCCCAAGGGGC CCA	1499
AD-71200	A-142779	AAGGGGCCACAAAAGG GGAdTdT	1084	A-142780	UCCCCUUUGUGGGGCC CUUdTdT	1292	AAGGGGCCACAAAAGG GGA	1500
AD-71201	A-142781	AAAGGGGAGAAAGGGCC AGAdTdT	1085	A-142782	UCUGGCCCUUCUCCCCU UUdTdT	1293	AAAGGGGAGAAAGGGCC AGC	1501
AD-71202	A-142783	GGCCAGGCCCUACAUC UUAdTdT	1086	A-142784	UAAGAUUAGGGGCUUG CCCdTdT	1294	GGCCAGGCCCUACAUC UUC	1502

AD-71203	A-142785	AUCUUCAGCUCUCCCAUA GCAdTdT	1087	A-142786	UGCUAUGGGAGCUGAA GAUdTdT	1295	AUCUUCAGCUCUCCCAUA GCG	1503
AD-71204	A-142787	UCCCAUAGCGCUGGCU CAAdTdT	1088	A-142788	UUGAGCCAGCGCUAUG GGAdTdT	1296	UCCCAUAGCGCUGGCU CAG	1504
AD-71205	A-142789	UGGCU CAGGAAGAAAC CCAdTdT	1089	A-142790	UGGUUUCUUCUCCUGAG CCAdTdT	1297	UGGCU CAGGAAGAAAC CCC	1505
AD-71206	A-142791	AACCCCAAGCAGCAUU CAAdTdT	1090	A-142792	UUGAAUGCUGCUUUGGG GUUdTdT	1298	AACCCCAAGCAGCAUU CAG	1506
AD-71207	A-142793	CAGCAUUCAGCACACC CCAdTdT	1091	A-142794	UGGGUGUGCUGAAUG CUGdTdT	1299	CAGCAUUCAGCACACC CCA	1507
AD-71208	A-142795	CACCCCAAGGACAAC CCAdTdT	1092	A-142796	UGGUUUGUCCCUUGGG GUGdTdT	1300	CACCCCAAGGACAAC CCC	1508
AD-71209	A-142797	ACAACCCCAUCAUAUG ACAdTdT	1093	A-142798	UGUCAUAUGAUGGGGU UGUdTdT	1301	ACAACCCCAUCAUAUG ACA	1509
AD-71210	A-142801	ACCCUCCCAUGCCCCAA CAdTdT	1094	A-142802	UGUUGGGCAUGGAGAG GGUdTdT	1302	ACCCUCCCAUGCCCCAA CC	1510
AD-71211	A-142803	UGCCCAACCUAAGAUU GUAdTdT	1095	A-142804	UACAAUCUUAGGUUGG GCAdTdT	1303	UGCCCAACCUAAGAUU GUG	1511
AD-71212	A-142805	AAGAUUGUGUGGGUUU UUUdTdT	1096	A-142806	AAAAAACCCACACAAU CUUdTdT	1304	AAGAUUGUGUGGGUUU UUU	1512
AD-71213	A-142807	UUUUUAAUUAAAAAU GUUdTdT	1097	A-142808	AACAUUUUUAAUUAAA AAAdTdT	1305	UUUUUAAUUAAAAAU GUU	1513
AD-71214	A-142809	UAAAAAUGUUAAAAAGU UUUdTdT	1098	A-142810	AAAAUUUUUAAACAUUU UUAdTdT	1306	UAAAAAUGUUAAAAAGU UUU	1514
AD-71215	A-142811	AAAGUUUUAAACAUGA AAAdTdT	1099	A-142812	UUUUC AUGUUUAAAAAC UUUdTdT	1307	AAAGUUUUAAACAUGA AAA	1515

Table 8. GCK Single Dose Screen in Primary *Cynomolgus* Hepatocytes

DuplexID	20nM_AVG	20nM_STDEV
AD-71009	91.7	8.2
AD-71010	85.5	11.8
AD-71011	101.4	19.6
AD-71012	96.3	20.8
AD-71013	102.8	21.7
AD-71014	105.3	47.3
AD-71015	32.6	6.6
AD-71016	98.7	19.1
AD-71017	64.1	16.4
AD-71018	45.9	12.8
AD-71019	39.6	8.5
AD-71020	89.2	23.9
AD-71021	60.4	7.7
AD-71022	100.8	12.7
AD-71023	97.4	33
AD-71024	60.6	18.8
AD-71025	31.1	9
AD-71026	33.6	10.5
AD-71027	46.3	14.6
AD-71028	35.6	9.6
AD-71029	35.6	15.9
AD-71030	30.4	7.9
AD-71031	107.2	29
AD-71032	78.4	15.7
AD-71033	71.8	13.9
AD-71034	39.7	14.3
AD-71035	46.8	11.4
AD-71036	77.6	21.3
AD-71037	37.4	15.2
AD-71038	48.8	19.6
AD-71039	70.1	9.5
AD-71040	65.9	16.2
AD-71041	94.8	18.6
AD-71042	108.4	24.2
AD-71043	35.8	12.2
AD-71044	46.6	10.9
AD-71045	39.4	9.5
AD-71046	35.3	3.9
AD-71047	82	24.4
AD-71048	39.8	10.1
AD-71049	95.3	6.8

AD-71050	151.2	22.4
AD-71051	54	13.5
AD-71052	48.7	8.1
AD-71053	44	10.7
AD-71054	53.4	8.8
AD-71055	39.8	9.4
AD-71056	51.8	34
AD-71057	71	7.1
AD-71058	38.9	3.6
AD-71059	78.1	17.8
AD-71060	54	14.4
AD-71061	108.4	27.2
AD-71062	69.4	6.7
AD-71063	35.1	11
AD-71064	53.1	13.8
AD-71065	94	11.6
AD-71066	149.2	13.3
AD-71067	50.8	15.2
AD-71068	113.4	23.7
AD-71069	44.9	6.4
AD-71070	112.3	24.3
AD-71071	32.7	5.3
AD-71072	40.1	10.2
AD-71073	53	12.5
AD-71074	135.4	25.3
AD-71075	100.8	31.5
AD-71076	35.6	7.3
AD-71077	26.9	4.7
AD-71078	54.4	11.8
AD-71079	48	6.2
AD-71080	96.1	6
AD-71081	105.4	7.9
AD-71082	127	20
AD-71083	117.2	33.2
AD-71084	124.4	20.7
AD-71085	78.5	6.6
AD-71086	30.2	12.7
AD-71087	44.2	2
AD-71088	92.9	21.9
AD-71089	36.1	13.7
AD-71090	40	3.6
AD-71091	58	3.3
AD-71092	62.3	14.9
AD-71093	58.6	18.2

AD-71094	82.7	22.9
AD-71095	116.8	27.6
AD-71096	40.3	11.8
AD-71097	26.6	6.9
AD-69448	34.9	9.3
AD-71098	50.4	7.4
AD-71099	50.7	18.4
AD-71100	23.8	1.7
AD-71101	70.7	18.1
AD-71102	37.2	5.5
AD-71103	98.3	17.6
AD-71104	78.8	22.8
AD-71105	29.6	6.1
AD-71106	26.1	8.1
AD-71107	54.4	9.2
AD-71108	143.2	28.5
AD-71109	116.6	15.9
AD-71110	107.3	21.5
AD-71111	36.8	9
AD-71112	76.4	19.7
AD-71113	71.7	12.9
AD-71114	94.8	22.5
AD-71115	43.3	12.3
AD-71116	58.7	7.9
AD-71117	26.9	7.1
AD-71118	74	15.8
AD-71119	33.8	12.9
AD-71120	30.5	4.3
AD-71121	51.8	7.6
AD-71122	71.8	20.4
AD-71123	45.6	12.4
AD-71124	156.4	6.6
AD-71125	55.4	6.2
AD-71126	102.3	8.5
AD-71127	107.8	18.2
AD-71128	95.6	19
AD-71129	51.6	16.7
AD-71130	28.4	10.3
AD-71131	49	6.5
AD-71132	127.7	25.6
AD-71133	157.5	17.4
AD-71134	38	11.1
AD-71135	62.6	7.6
AD-71136	141.6	20.3

AD-71137	55.9	6.4
AD-71138	37.9	5
AD-71139	125.8	27.6
AD-71140	41.8	2.5
AD-71141	32.8	6.7
AD-71142	40.4	11.5
AD-71143	177.4	27.3
AD-71144	53.5	9.1
AD-71145	41.3	27
AD-71146	105.9	13.9
AD-71147	98.2	27.5
AD-71148	73.6	8.9
AD-71149	159	24.3
AD-71150	157.9	31.1
AD-71151	131.4	4.3
AD-71152	98.2	25.2
AD-71153	66.6	23
AD-71154	134.8	11.9
AD-71155	52.8	4.2
AD-71156	111.4	43.9
AD-71157	37.6	7.7
AD-71158	83.3	16.9
AD-71159	33.7	7.3
AD-71160	44.2	7.2
AD-71161	159.1	28.3
AD-71162	137.2	20.9
AD-71163	23.4	5.4
AD-71164	27.7	2.6
AD-71165	38.1	8
AD-71166	46.4	11.4
AD-71167	53.1	17.6
AD-71168	130.3	14
AD-71169	95.8	24.4
AD-71170	108.8	15.4
AD-71171	57.6	5.3
AD-71172	75	26.8
AD-71173	87.3	24
AD-71174	62.7	24.2
AD-71175	83.6	25.8
AD-71176	26.7	3.5
AD-71177	31	4.7
AD-71178	114.6	20
AD-71179	101.9	25.9
AD-71180	114	12.4

AD-71181	38.2	10.1
AD-71182	32.3	3.2
AD-71183	49.9	7.4
AD-71184	60.6	6.7
AD-71185	30	3
AD-71186	55.7	16.3
AD-71187	72.8	15.2
AD-71188	32.1	2.2
AD-71189	76.4	13.9
AD-71190	34.2	9.1
AD-71191	51.7	9.5
AD-71192	87.5	8.3
AD-71193	110.2	20.9
AD-71194	56.9	12.6
AD-71195	63.1	25.2
AD-71196	38.1	10.3
AD-71197	34	9.8
AD-71198	108.1	12
AD-71199	117.9	11.5
AD-71200	50.1	12.3
AD-71201	38.2	5.8
AD-71202	73.9	3.6
AD-71203	110.3	9.2
AD-71204	140	11
AD-71205	35.8	4.5
AD-71206	28	12.1
AD-71207	22.4	11
AD-71208	54.6	10.9
AD-71209	40.6	14.9
AD-71210	41.5	6.3
AD-71211	54.4	16
AD-71212	122.4	20.7
AD-71213	111.4	13.7
AD-71214	120.1	19.5
AD-71215	98	11.7

We claim:

1. A double stranded ribonucleic acid (RNAi) agent for inhibiting expression of a glucokinase (GCK) gene, wherein said double stranded RNAi agent
5 comprises a sense strand and an antisense strand, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:13.
10
2. A double stranded ribonucleic acid (RNAi) agent for inhibiting expression of a glucokinase (GCK) gene, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing
15 by no more than 3 nucleotides from any one of the antisense sequences listed in any of Tables 2, 3, 6, and 7.
3. The double stranded RNAi agent of claim 1 or 2, wherein said double stranded RNAi agent comprises at least one modified nucleotide.
20
4. The double stranded RNAi agent of claim 1 or 2, wherein no more than four of the nucleotides of the sense strand are unmodified nucleotides.
5. The double stranded RNAi agent of any one of claims 1-4, wherein no
25 more than four of the nucleotides of the antisense strand are unmodified nucleotides.
6. The double stranded RNAi agent of any one of claims 1-5, wherein all of the nucleotides of the sense strand are modified nucleotides.
- 30 7. The double stranded RNAi agent of any one of claims 1-6, wherein all of the nucleotides of the antisense strand are modified nucleotides.
- 35

8. A double stranded ribonucleic acid (RNAi) agent for inhibiting expression of a glucokinase (GCK) gene, wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:13, wherein substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand are modified nucleotides, and wherein said sense strand is conjugated to a ligand attached at the 3'-terminus.

9. The double stranded RNAi agent of claim 8, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

10. The double stranded RNAi agent of any one of claims 3 and 6-9, wherein at least one of said modified nucleotides is selected from the group consisting of a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5'-methylphosphonate group, a nucleotide comprising a 5' phosphate or 5' phosphate mimic, a nucleotide comprising vinyl phosphate, a nucleotide comprising adenosine-glycol nucleic acid (GNA), a nucleotide comprising thymidine-glycol nucleic acid (GNA) S-Isomer, a nucleotide comprising 2-hydroxymethyl-tetrahydrofuran-5-phosphate, a nucleotide comprising 2'-deoxythymidine-3'phosphate, a nucleotide comprising 2'-deoxyguanosine-3'-phosphate, and a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group.

11. The double stranded RNAi agent of any one of claims 3 and 6-10, wherein at least one of said modified nucleotides is selected from the group consisting of 2'-O-methyl and 2'fluoro modifications.

12. The double stranded RNAi agent of any one of claims 1-11, further comprising at least one phosphorothioate internucleotide linkage.

13. The double stranded RNAi agent of claim 12, wherein the dsRNA comprises
5 6-8 phosphorothioate internucleotide linkages.

14. The double stranded RNAi agent of any one of claims 1-13, wherein the region of complementarity is at least 17 nucleotides in length.

10 15. The double stranded RNAi agent of any one of claims 1-13, wherein the region of complementarity is 19-21 nucleotides in length.

16. The double stranded RNAi agent of claim 15, wherein the region of complementarity is 21 nucleotides in length.
15

17. The double stranded RNAi agent of claim 15, wherein the region of complementarity is 19 nucleotides in length.

18. The double stranded RNAi agent of any one of claims 1-16, wherein the
20 region of complementarity is 21-23 nucleotides in length.

19. The double stranded RNAi agent of any one of claims 1-18, wherein each strand is no more than 30 nucleotides in length.

25 20. The double stranded RNAi agent of any one of claims 1-19, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

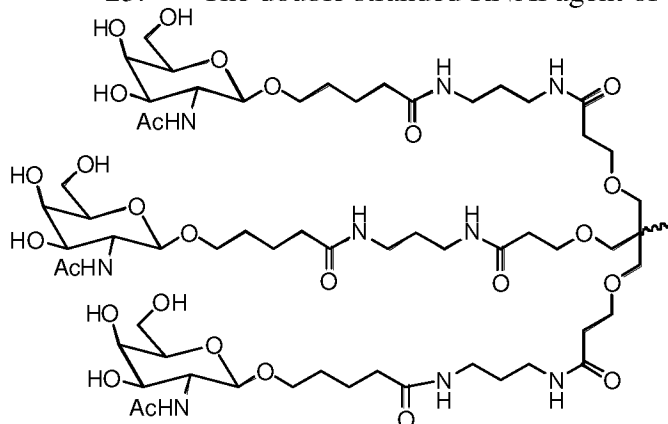
21. The double stranded RNAi agent of any one of claims 1-20, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.
30

22. The double stranded RNAi agent of any one of claims 1-21, further comprising a ligand.

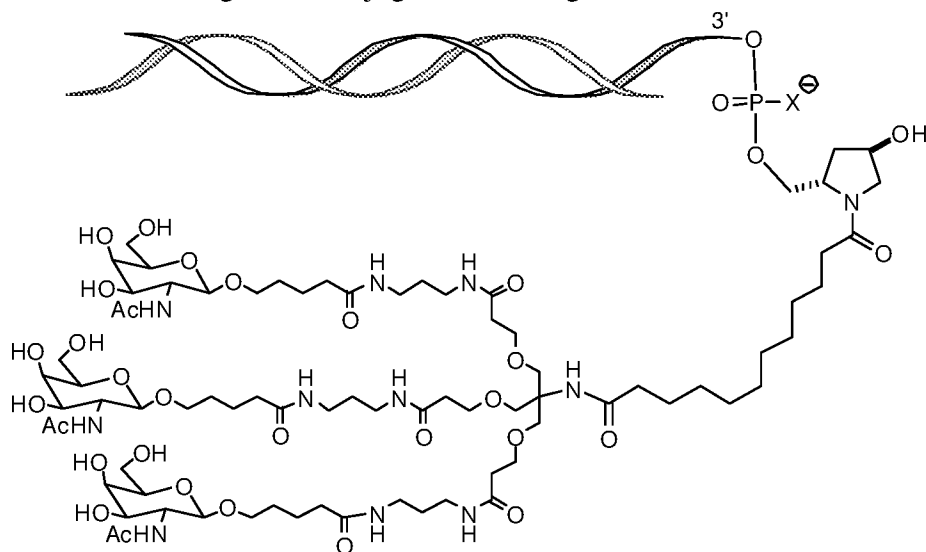
23 The double stranded RNAi agent of claim 22, wherein the ligand is
35 conjugated to the 3' end of the sense strand of the double stranded RNAi agent.

24. The double stranded RNAi agent of claim 22, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

25. The double stranded RNAi agent of claim 24, wherein the ligand is



5 26. The double stranded RNAi agent of claim 23, wherein the double stranded RNAi agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

10 27. The double stranded RNAi agent of claim 26, wherein the X is O.

28. The double stranded RNAi agent of claim 2, wherein the region of complementarity consists of any one of the antisense sequences of any one of Tables 2, 3, 6, and 7.

15

29. A cell containing the double stranded RNAi agent of any one of claims 1-28.

30. A pharmaceutical composition for inhibiting expression of a glucokinase (GCK) gene comprising the double stranded RNAi agent of any one of claims 1-28.

5 31. The pharmaceutical composition of claim 30, further comprising a lipid formulation.

32. A method of inhibiting expression of a glucokinase (GCK) gene in a cell, the method comprising:

- 10 (a) contacting the cell with the double stranded RNAi agent of any one of claims 1-28 or the pharmaceutical composition of claim 30; and
(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a GCK gene, thereby inhibiting expression of the GCK gene in the cell.

15 33. The method of claim 32, wherein said cell is within a subject.

34. The method of claim 33, wherein the subject is a human.

20 35. The method of claim 34, wherein the human subject suffers from a disease or disorder that would benefit from reduction in GCK expression.

36. The method of claim 35, wherein the disease or disorder is a glycogen storage disease (GSD)

25 37. The method of claim 36, wherein the glycogen storage disease (GSD) is type Ia GSD.

38. The method of any one of claims 32-37, wherein the GCK expression is inhibited by at least about 30%.

30 39. A method of treating a subject having a disease or disorder that would benefit from reduction in expression of a glucokinase (GCK) gene, comprising administering to the subject a therapeutically effective amount of the double stranded RNAi agent of any one of claims 1-28 or the pharmaceutical composition of claim 30,
35 thereby treating said subject.

40. A method of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction expression of a glucokinase (GCK) gene, the method comprising administering to the subject a prophylactically effective amount of the double stranded RNAi agent of any one of claims 1-28 or the
5 pharmaceutical composition of claim 30, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in GCK expression.

41. The method of claim 39 or 40, wherein the disease or disorder is a glycogen storage disease (GSD).
10

42. The method of claim 41, wherein the glycogen storage disease (GSD) is type Ia GSD.

43. The method of claim 39 or 40, wherein the administration of the double
15 stranded RNAi agent to the subject causes an increase in serum glucose levels and/or a decrease in GCK protein accumulation.

44. A method of inhibiting the expression of a glucokinase (GCK) gene in a subject, the method comprising
20 administering to said subject a therapeutically effective amount of the double stranded RNAi agent of any one of claims 1-28 or the pharmaceutical composition of claim 30, thereby inhibiting the expression of GCK in said subject.

45. A method of increasing the blood glucose levels in a subject having a
25 disease or disorder that would benefit from reduction in GCK expression, the method comprising

administering to said subject a therapeutically effective amount of the double stranded RNAi agent of any one of claims 1-28 or the pharmaceutical composition of claim 30, thereby increasing the blood glucose levels in said subject.
30

46. A method of decreasing plasma lactate levels in a subject having a disease or disorder that would benefit from reduction in GCK expression, the method comprising

administering to said subject a therapeutically effective amount of the double
35 stranded RNAi agent of any one of claims 1-28 or the pharmaceutical composition of claim 30, thereby decreasing plasma lactate levels in said subject.

47. A method of decreasing plasma uric acid levels in a subject having a disease or disorder that would benefit from reduction in GCK expression, the method comprising

5 administering to said subject a therapeutically effective amount of the double stranded RNAi agent of any one of claims 1-28 or the pharmaceutical composition of claim 30, thereby decreasing plasma uric acid levels in said subject.

48. A method of decreasing plasma triglyceride levels in a subject having a disease or disorder that would benefit from reduction in GCK expression, the method
10 comprising

administering to said subject a therapeutically effective amount of the double stranded RNAi agent of any one of claims 1-28 or the pharmaceutical composition of claim 30, thereby decreasing plasma triglyceride levels in said subject.

49. A method of decreasing total plasma cholesterol levels in a subject having a disease or disorder that would benefit from reduction in GCK expression, the method comprising

15 administering to said subject a therapeutically effective amount of the double stranded RNAi agent of any one of claims 1-28 or the pharmaceutical composition of claim 30, thereby decreasing total plasma cholesterol levels in said subject.
20

50. A method of decreasing hepatomegaly in a subject having a disease or disorder that would benefit from reduction in GCK expression, the method comprising

25 administering to said subject a therapeutically effective amount of the double stranded RNAi agent of any one of claims 1-28 or the pharmaceutical composition of claim 30, thereby decreasing hepatomegaly in said subject.

51. The method of any one of claims 45-50 wherein the disease or disorder is a glycogen storage disease (GSD).
30

52. The method of claim 51, wherein the glycogen storage disease (GSD) is type Ia GSD.

53. The method of any one of claims 39, 40 and 44-50, further comprising
35 administering a sodium-glucose co-transporter 2 (SGLT2) inhibitor to the subject.

54. The method of claim 53, wherein the SGLT2 inhibitor is selected from the group consisting of Dapagliflozin, Canagliflozin, Ipragliflozin (ASP-1941), Tofogliflozin, Empagliflozin, Sergliflozin etabonate, Remogliflozin etabonate (BHV091009), and Ertugliflozin (PF-04971729 / MK-8835).

5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/038616

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:
- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/038616

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

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

B. FIELDS SEARCHED

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

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

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

EPO-Internal, EMBASE, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M. GOLDSWORTHY ET AL.: "Role of the transcription factor Sox4 in insulin secretion and impaired glucose tolerance", DIABETES, vol. 57, no. 8, 1 August 2008 (2008-08-01), pages 2234-2244, XP055304078, US	1-35, 38-40, 43,44
Y	ISSN: 0012-1797, DOI: 10.2337/db07-0337 the whole document ----- -/--	30,31, 33-35, 39,40, 43-45



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

27 September 2016

Date of mailing of the international search report

05/10/2016

Name and mailing address of the ISA/

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Authorized officer

Macchia, Giovanni

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/038616

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GINTAUTAS GRABAUSKAS ET AL.: "Essential elements for glucosensing by gastric vagal afferents: immunocytochemistry and electrophysiology studies in the rat", ENDOCRINOLOGY, vol. 154, no. 1, 1 January 2013 (2013-01-01), pages 296-307, XP055304143, US ISSN: 0013-7227, DOI: 10.1210/en.2012-1382 the whole document	1-54
A	J.R. BAIN ET AL.: "An Adenovirus vector for efficient RNA interference-mediated suppression of target genes in insulinoma cells and pancreatic islets of Langerhans", DIABETES, vol. 53, no. 9, 1 September 2004 (2004-09-01), pages 2190-2194, XP055304096, US ISSN: 0012-1797, DOI: 10.2337/diabetes.53.9.2190 the whole document	1-54
Y	B.E. LEVIN ET AL.: "Ventromedial hypothalamic Glucokinase is an important mediator of the counterregulatory response to insulin-induced hypoglycemia", DIABETES, vol. 57, no. 5, 1 May 2008 (2008-05-01), pages 1371-1379, XP055304080, US ISSN: 0012-1797, DOI: 10.2337/db07-1755 the whole document	30,31, 33-35, 39,40, 43-45
X	L. KANG ET AL: "Glucokinase Is a Critical Regulator of Ventromedial Hypothalamic Neuronal Glucosensing", DIABETES, vol. 55, no. 2, 1 February 2006 (2006-02-01), pages 412-420, XP055304086, US ISSN: 0012-1797, DOI: 10.2337/diabetes.55.02.06.db05-1229 the whole document	1-21, 23-35, 39,40, 43,44
A	US 2005/009129 A1 (RIZZO MARK A. [US]; PISTON DAVID W. [US]) 13 January 2005 (2005-01-13) page 5 - paragraph [0052] the whole document	1-54

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/038616

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2005009129	A1	13-01-2005	NONE
