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(54) **COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF THE ALAS1 GENE**

ZUSAMMENSETZUNGEN UND VERFAHREN ZUR HEMMUNG DER ALAS1-GENEXPRESSION

COMPOSITIONS ET PROCÉDÉS PERMETTANT D'INHIBER L'EXPRESSION DU GÈNE ALAS1

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Description

[0001] The invention is defined by the claims. It relates to the specific inhibition of the expression of the ALAS1 gene.

[0002] The inherited porphyrias are a family of disorders resulting from the deficient activity of specific enzymes in the heme biosynthetic pathway, also referred to herein as the porphyrin pathway. Deficiency in the enzymes of the porphyrin pathway leads to insufficient heme production and to an accumulation of porphyrin precursors and porphyrins, which are toxic to tissue in high concentrations.

[0003] Of the inherited porphyrias, acute intermittent porphyria (AIP, e.g., autosomal dominant AIP), variegate porphyria (VP, e.g., autosomal dominant VP), hereditary coproporphyria (copropophyria or HCP, e.g., autosomal dominant HCP), and 5' aminolevulinic acid (also known as δ- aminolevulinic acid or ALA) dehydratase deficiency porphyria (ADP, e.g., autosomal recessive ADP) are classified as acute hepatic porphyrias and are manifested by acute neurological attacks that can be life threatening. The acute attacks are characterized by autonomic, peripheral, and central nervous symptoms, including severe abdominal pain, hypertension, tachycardias, constipation, motor weakness, paralysis, and seizures. If not treated properly, quadriplegia, respiratory impairment, and death may ensue. Various factors, including cytrochrome P450-inducing drugs, dieting, and hormonoal changes can precipitate acute attacks by increasing the activity of hepatic 5'-aminolevulinic acid synthase 1 (ALAS1), the first and rate-limiting enzyme of the heme biosynthetic pathway. In the acute porphyrias, e.g., AIP, VP, HCP and ADP, the respective enzyme deficiencies result in hepatic production and accumulation of one or more substances (e.g., porphyrins and/or porphyrin precursors, e.g., ALA and/or PBG) that can be neurotoxic and can result in the occurrence of acute attacks. See, e.g., Balwani, M and Desnick, R.J., Blood, 120:4496-4504, 2012.

[0004] The current therapy for the acute neurologic attacks is the intravenous administration of hemin (Panhematin®, Lundbeck or Normosang®, Orphan Europe), which provides exogenous heme for the negative feedback inhibition of ALAS1, and thereby, decreases production of ALA and PBG. Hemin is used for the treatment during an acute attack and for prevention of attacks, particularly in women with the acute porphyrias who experience frequent attacks with the hormonal changes during their menstrual cycles. While patients generally respond well, its effect is slow, typically taking two to four days or longer to normalize urinary ALA and PBG concentrations towards normal levels. As the intravenous hemin is rapidly metabolized, three to four infusions are usually necessary to effectively treat or prevent an acute attack. In addition, repeated infusions may cause iron overload and phlebitis, which may compromise peripheral venous access. Although orthotrophic liver transplantation is curative, this procedure has significant morbidity and mortality and the availability of liver donors is limited. Therefore, an alternative therapeutic approach that is more effective, fast-acting, and safe is needed. It would be particularly advantageous if such treatment could be delivered by subcutaneous administration, as this would preclude the need for infusions and prolonged hospitalization.

[0005] AIP, also referred to as porphobilinogen deaminase (PBGD) deficiency, or hydroxymethylbilane synthase (HMBS) deficiency, is the most common of the acute hepatic prophyrias. It is an autosomal dominant disorder caused by mutations in the *HMBS* gene that result in reduced, e.g., half-normal activity of the enzyme. Previously, a mouse model of AIP that has ~30% of wildtype HMBS activity was generated by homologous recombination. Like human patients, these mice increase hepatic ALAS1 activity and accumulate large quantities of plasma and urinary ALA and PBG when administered porphyrinogenic drugs, such as phenobarbital. Thus, they serve as an excellent model to evaluate the efficacy of novel therapeutics for the acute hepatic porphyrias.

Yin et al. (Science 318, 1786-1789 (2007)) describe Rev-erb, a Heme Sensor That Coordinates Metabolic and Circadian Pathways. Schultz et al. (Silence 2, 3 (2011)) describes that off-target effects dominate a large-scale RNAi screen for modulators of the TGF-[beta] pathway. Estall et al. (PNAS 106, 22510-22515 (2009)) describe that PGC-1 negatively regulates hepatic FGF21 expression. WO 2007/131274 describes short interference ribonucleic acids for treating allergic diseases. EP 1 752 536 describes a polynucleotide causing RNA interference.

The present invention relates to a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1, wherein the dsRNA comprises:

(i) an antisense strand complementary to at least nucleotides 871-889 of SEQ ID NO:1;
(ii) a sense strand comprising at least 15 contiguous nucleotides from SEQ ID NO:1295; and
(iii) a ligand of one or more N-acetylgalactosamine (GalNAc) derivatives.

[0006] The disclosure describes methods and iRNA compositions for modulating the expression of an ALAS1 gene. Expression of an ALAS1 gene is reduced or inhibited using an ALAS1-specific iRNA. Such inhibition can be useful in treating disorders related to ALAS1 expxession, such as porphyrias.

[0007] Accordingly, described herein are compositions and methods that effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of the ALAS1 gene, such as in a cell or in a subject (*e.g.*, in a mammal, such as a human subject). Also described are compositions and methods for treating a disorder related to expression of an ALAS1 gene, such as a porphyria, *e.g.*, X-linked sideroblastic anemia (XLSA), ALA deyhdratase deficiency porphyria

(Doss porphyria or ADP), acute intermittent porphyria (AIP), congenital erythropoietic porphyria (CEP), prophyria cutanea tarda (PCT), hereditary coproporphyria (coproporphyria, or HCP), variegate porphyria (VP), erythropoietic protoporphyria (EPP), or transient erythroporphyria of infancy. The disorder may be an acute hepatic porphyria, e.g., ALA deyhdratase deficiency porphyria (ADP), AIP, HCP, or VP. The disorder may be ALA deyhdratase deficiency porphyria (ADP) or AIP.

[0008] In embodiments, the porphyria is a hepatic porphyria, e.g., a porphyria selected from acute intermittent porphyria (AIP) hereditary coproporphyria (HCP), variegate porphyria (VP), ALA deyhdratase deficiency porphyria (ADP), and hepatoerythropoietic porphyria. The porphyria may be a homozygous dominant hepatic porphyria (e.g., homozygous dominant AIP, HCP, or VP) or hepatoerythropoietic porphyria, The porphyria may be a dual porphyria.

[0009] As used herein, the term "iRNA," "RNAi", "iRNA agent," or "RNAi agent" refers to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript, e.g., via an RNA-induced silencing complex (RISC) pathway. An iRNA as described herein effects inhibition of ALAS1 expression in a cell or mammal.

[0010] The iRNAs included in the compositions featured herein encompass a dsRNA having an RNA strand (the antisense strand) having a region, e.g., a region that is 30 nucleotides or less, generally 19-24 nucleotides in length, that is substantially complementary to at least part of an mRNA transcript of an ALAS 1 gene (*e.g.*, a mouse or human ALAS1 gene) (also referred to herein as an "ALAS1-specific iRNA"). Alternatively, or in combination, iRNAs encompass a dsRNA having an RNA strand (the antisense strand) having a region that is 30 nucleotides or less, generally 19-24 nucleotides in length, that is substantially complementary to at least part of an mRNA transcript of an ALAS 1 gene (*e.g.*, a human variant 1 or 2 of an ALAS1 gene) (also referred to herein as a "ALAS1-specific iRNA").

[0011] The iRNA (e.g, dsRNA) described herein may comprise an antisense strand having a region that is substantially complementary to a region of a human ALAS1. The human ALAS 1 may have the sequence of NM_000688.4 (SEQ ID NO: 1) or NM_000688.5 (SEQ ID NO:382).

[0012] An iRNA may encompass a dsRNA having an RNA strand (the antisense strand) having a region that is substantially complementary to a portion of an ALAS1 mRNA according to any one of Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 or 20. The iRNA may encompass a dsRNA having an RNA strand (the antisense strand) having a region that is substantially complementary to a portion of an ALAS1 mRNA, *e.g.*, a human ALAS 1 mRNA (*e.g.*, a human ALAS 1 mRNA as provided in SEQ ID NO:1 or SEQ ID NO:382).

[0013] An iRNA for inhibiting expression of an ALAS1 gene includes at least two sequences that are complementary to each other. The iRNA includes a sense strand having a first sequence and an antisense strand having a second sequence. The antisense strand includes a nucleotide sequence that is substantially complementary to at least part of an mRNA encoding an ALAS1 transcript, and the region of complementarity is 30 nucleotides or less, and at least 15 nucleotides in length. Generally, the iRNA is 19 to 24 nucleotides in length.

[0014] The iRNA may be 19-21 nucleotides in length. The iRNA may be 19-21 nucleotides in length and is in a lipid formulation, e.g. a lipid nanoparticle (LNP) formulation (e.g., an LNP11 formulation).

[0015] The iRNA may be 21-23 nucleotides in length. The iRNA may be 21-23 nucleotides in length and is in the form of a conjugate, e.g., conjugated to one or more GalNAc derivatives as described herein.

[0016] The iRNA may be from about 15 to about 25 nucleotides in length or from about 25 to about 30 nucleotides in length. An iRNA targeting ALAS1, upon contact with a cell expressing ALAS1, inhibits the expression of an ALAS1 gene by at least 10%, at least 20%, at least 25%, at least 30%, at least 35% or at least 40% or more, such as when assayed by a method as described hereinThe iRNA targeting ALAS1 may be formulated in a stable nucleic acid lipid particle (SNALP).

[0017] An iRNA (e.g., a dsRNA) featured herein includes a first sequence of a dsRNA that is selected from the group consisting of the sense sequences of Tables 2, 3, 6, 7, 8, 9, 14, and 15 and a second sequence that is selected from the group consisting of the corresponding antisense sequences of Tables 2, 3, 6, 7, 8, 9, 14 and 15.

[0018] An iRNA (e.g., a dsRNA) featured herein includes a first sequence of a dsRNA that is selected from the group consisting of the sense sequences of Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20 and a second sequence that is selected from the group consisting of the corresponding antisense sequences of Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20. An iRNA (e.g., a dsRNA) featured herein may have sense and/or antisense sequences selected from those of AD-58882, AD-58878, AD-58886, AD-58877, AD-59115, AD-58856, AD-59129, AD-59124, AD-58874, AD-59125, AD-59105, AD-59120, AD-59122, AD-59106, AD-59126, and AD-59107 as disclosed herein in the Examples. The iRNA (e.g., dsRNA) may have sense and/or antisense sequences selected from those of AD-58882, AD-58878, AD-58886, AD-58877, AD-59115, AD-58856, and AD-59129.

[0019] The iRNA molecules featured herein can include naturally occurring nucleotides or can include at least one modified nucleotide, including, but not limited to a 2'-O-methyl modified nucleotide, a nucleotide having a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative. Alternatively, the modified nucleotide may be chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide. Such a modified sequence can be based, e.g., on a first sequence

of said iRNA selected from the group consisting of the sense sequences of Table 2, and a second sequence selected from the group consisting of the corresponding antisense sequences of Table 2.

[0020] An iRNA (e.g., a dsRNA) featured herein may comprise a sense strand comprising a sequence selected from the group consisting of SEQ ID NO:330, SEQ ID NO:334, SEQ ID NO:342, SEQ ID NO:344, SEQ ID NO:346, SEQ ID NO:356, SEQ ID NO:358, SEQ ID NO:362, SEQ ID NO:366, SEQ ID NO:376, and SEQ ID NO:380.

[0021] An iRNA (e.g., a dsRNA) featured herein may comprise an antisense strand comprising a sequence selected from the group consisting of SEQ ID NO:331, SEQ ID NO:335, SEQ ID NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:363, SEQ ID NO:367, SEQ ID NO:377, and SEQ ID NO:381.

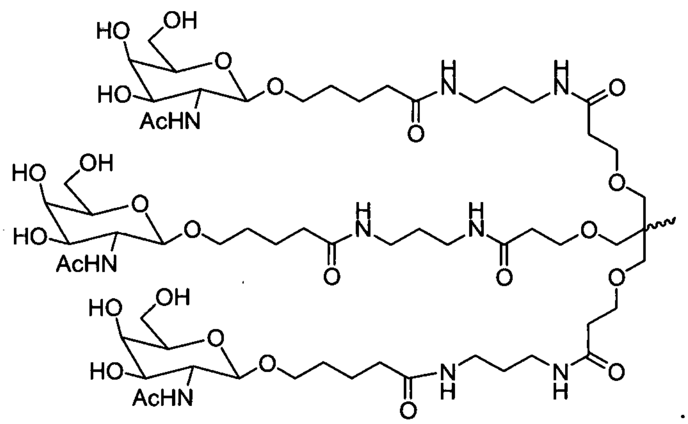
[0022] An iRNA (e.g., a dsRNA) featured herein may comprise a sense strand comprising a sequence selected from the group consisting of SEQ ID NO: 140, SEQ ID NO: 144, SEQ ID NO:152, SEQ ID NO:154, SEQ ID NO:156, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:172, SEQ ID NO:176, SEQ ID NO:186, and SEQ ID NO:190. An iRNA (e.g., a dsRNA) featured herein may comprise an antisense strand comprising a sequence selected from the group consisting of SEQ ID NO:141, SEQ ID NO:145, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO: 157, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO: 173, SEQ ID NO: 177, SEQ ID NO: 187, and SEQ ID NO:191.

[0023] An iRNA as described herein may target a wildtype ALAS1 RNA transcript variant, and in another embodiment, the iRNA targets a mutant transcript (*e.g.*, an ALAS1 RNA carrying an allelic variant). For example, an iRNA can target a polymorphic variant, such as a single nucleotide polymorphism (SNP), of ALAS 1. The iRNA may target both a wildtype and a mutant ALAS1 transcript. The iRNA may target a particular transcript variant of ALAS1 (*e.g.*, human ALAS1 variant 1). The iRNA agent may target multiple transcript variants (*e.g.*, both variant 1 and variant 2 of human ALAS1).

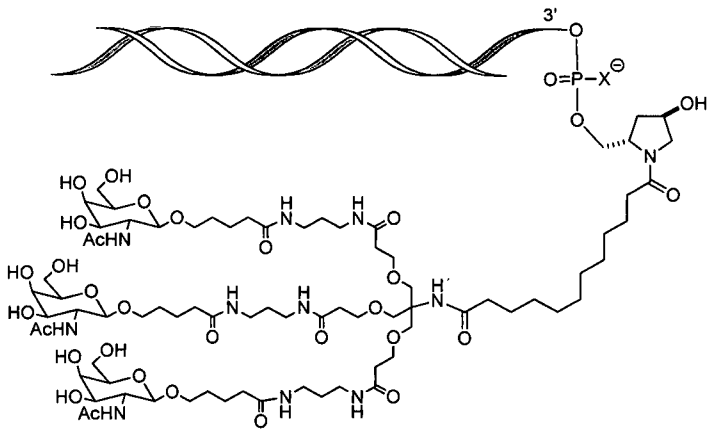
[0024] An iRNA may target a non-coding region of an ALAS 1 RNA transcript, such as the 5' or 3' untranslated region of a transcript.

[0025] An iRNA as described herein is in the form of a conjugate, e.g., a carbohydrate conjugate, which may serve as a targeting moiety and/or ligand, as described herein. The conjugate may be attached to the 3' end of the sense strand of the dsRNA. The conjugate may be attached via a linker, e.g., via a bivalent or trivalent branched linker.

[0026] The conjugate may comprise one or more N-acetylgalactosamine (GalNAc) derivatives. Such a conjugate is also referred to herein as a GalNAc conjugate. The conjugate may target the RNAi agent to a particular cell, e.g., a liver cell, e.g., a hepatocyte. The GalNAc derivatives can be attached via a linker, e.g., a bivalent or trivalent branched linker. The conjugate may be

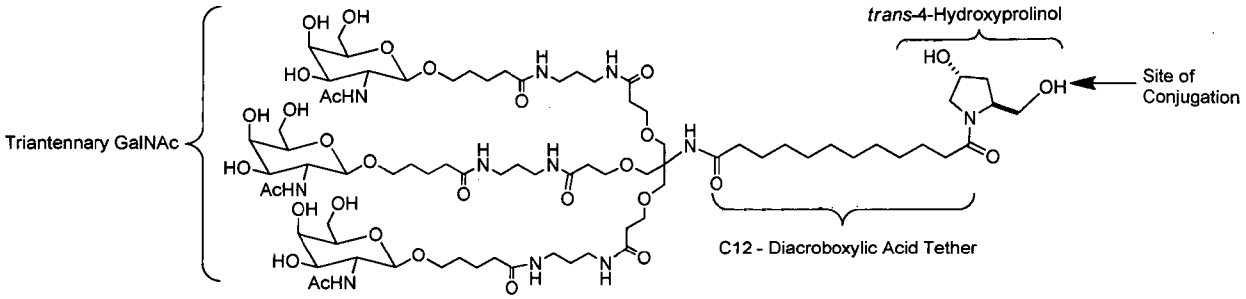


[0027] The RNAi agent may be attached to the carbohydrate conjugate via a linker, e.g., a linker as shown in the following schematic, wherein X is O or S



[0028] X may be O. X may be S.

[0029] In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below



[0030] In an aspect provided herein is a pharmaceutical composition for inhibiting the expression of an ALAS1 gene in an organism, generally a human subject. The composition typically includes one or more of the iRNAs described herein and a pharmaceutically acceptable carrier or delivery vehicle. The composition can be used for treating a porphyria, *e.g.*, AIP.

[0031] Described is a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1, wherein said dsRNA comprises a sense strand and an antisense strand 15-30 base pairs in length and the antisense strand is complementary to at least 15 contiguous nucleotides of SEQ ID NO: 1 or 382.

[0032] Described is a double stranded RNAi (dsRNA) comprising a sense strand complementary to an antisense strand, wherein said antisense strand comprises a region of complementarity to an ALAS1 1 RNA transcript, wherein each strand has about 14 to about 30 nucleotides, wherein said double stranded RNAi agent is represented by formula (III):

$$\text{sense: } 5' \; n_p\text{-}N_a\text{-}(X\,X\,X)_i\text{-}N_b\text{-}Y\,Y\,Y\text{-}N_b\text{-}(Z\,Z\,Z)j\text{-}N_a\text{-}n_q\; 3' \text{ antisense: } 3' \; n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_1\text{-}N_a'\text{-}n_q'\; 5' \qquad \text{(III)}$$

wherein:

i, j, k, and 1 are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

each n_p, n_p', n_q, and n_q' independently represents an overhang nucleotide;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'.

[0033] The sense strand may be conjugated to at least one ligand.

[0034] Described is that i is 1; j is 1; or both i and j are 1.

[0035] Described is that k is 1; 1 is 1; or both k and 1 are 1.

[0036] Described is that XXX is complementary to X'X'X', YYY is complementary to Y'Y'Y', and ZZZ is complementary to Z'Z'Z'.

[0037] Described is that the Y'Y'Y' motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end.

[0038] Described is that the Y' is 2'-O-methyl.

[0039] Described is that the duplex region is 15-30 nucleotide pairs in length.

[0040] Described is that the duplex region is 17-23 nucleotide pairs in length.

[0041] Described is that the duplex region is 19-21 nucleotide pairs in length.

[0042] Described is that the duplex region is 21-23 nucleotide pairs in length.

[0043] Described is that the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl, 2'-C- allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, and combinations thereof.

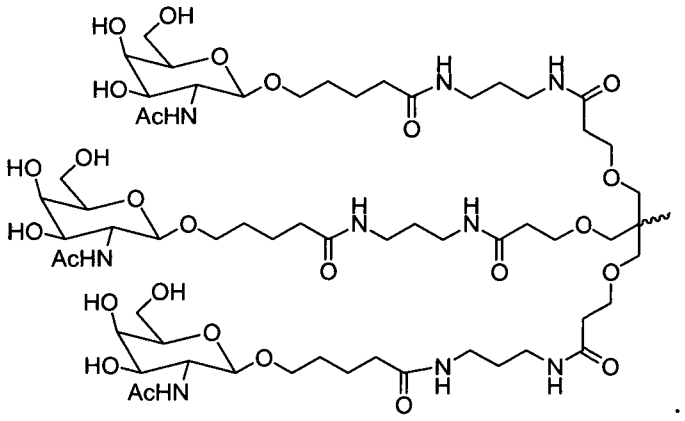
[0044] Described is that the modifications on the nucleotides are 2'-O-methyl, 2'-fluoro or both.

[0045] Described is that the ligand comprises a carbohydrate.

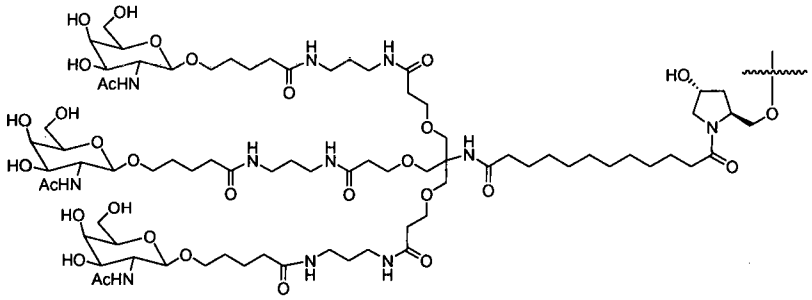
[0046] Described is that the ligand is attached via a linker.

[0047] Described is that the linker is a bivalent or trivalent branched linker.

[0048] Described is that the ligand is



[0049] Described is that the ligand and linker are as shown in Formula XXIV:



[0050] Described is that the ligand is attached to the 3' end of the sense strand.

[0051] The dsRNA may have (e.g., comprise) a nucleotide sequence selected from the group of sequences provided in Tables 2 and 3. The dsRNA may have a nucleotide sequence selected from the group of sequences provided in Tables 2, 3, 6, 7, 8 and 9. The dsRNA may have a nucleotide sequence selected from the group of sequences provided in Tables 2, 3, 6, 7, 8, 9, 14, and 15. The dsRNA may have a nucleotide sequence selected from the group of sequences provided in Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20. The dsRNA may have a nucleotide sequence disclosed in Table

18. The dsRNA may have a nucleotide sequence selected from the group of sequences provided in Tables 14 and 15.

[0052] The dsRNA may have a nucleotide sequence selected from the group of sequences provided in Tables 3 and 8.

[0053] In a further aspect, an iRNA provided herein is a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS 1, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to an ALAS1 RNA transcript, which antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense sequences listed in any one of Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 or 20. The sense and antisense sequences may be selected from those of the duplexes AD-58882, AD-58878, AD-58886, AD-58877, AD-59115, AD-58856, AD-59129, AD-59124, AD-58874, AD-59125, AD-59105, AD-59120, AD-59122, AD-59106, AD-59126, and AD-59107 as disclosed herein in the Examples. The sense and antisense sequences may be selected from those of the duplexes AD-58882, AD-58878, AD-58886, AD-58877, AD-59115, AD-58856, and AD-59129. The sense and antisense sequences may be those of the duplex AD-58632. The sense and antisense sequences may be selected from those of the duplexes AD-59453, AD-59395, AD-59477, and AD-59492. The sense and antisense sequences may be those of a duplex disclosed herein that suppresses ALAS 1 mRNA expression by at least 50%, 60%, 70%, 80%, 85% or 90%, e.g., as assessed using an assay disclosed in the Examples provided herein.

[0054] In some embodiments, the dsRNA comprises at least one modified nucleotide.

[0055] In some embodiments, at least one of the modified nucleotides is chosen from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group.

[0056] In some embodiments, the modified nucleotide is chosen from the group consisting of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

[0057] Described is that the region of complementarity is at least 17 nucleotides in length.

[0058] Described is that the region of complementarity is between 19 and 21 nucleotides in length.

[0059] Described is that the region of complementarity is 19 nucleotides in length.

[0060] Described is that each strand is no more than 30 nucleotides in length.

[0061] Described is that at least one strand comprises a 3' overhang of at least 1 nucleotide.

[0062] Described is that at least one strand comprises a 3' overhang of at least 2 nucleotides.

[0063] Described is that a dsRNA described herein further comprises a ligand.

[0064] Described is that the ligand is a GalNAc ligand.

[0065] Described is that the ligand targets the dsRNA to hepatocytes.

[0066] Described is that the ligand is conjugated to the 3' end of the sense strand of the dsRNA.

[0067] Described is that the region of complementarity consists of an antisense sequence selected from Table 2 or Table 3. Described is that the region of complementarity consists of an antisense sequence selected from Tables 2, 3, 6, 7, 8, 9, 14, or 15. Described is that the region of complementarity consists of an antisense sequence selected from Tables 2, 3, 6, 7, 8, 9, 14, 15, 18, or 20. Described is that the region of complementarity consists of an antisense sequence selected from that of AD-58882, AD-58878, AD-58886, AD-58877, AD-59115, AD-58856, AD-59129, AD-59124, AD-58874, AD-59125, AD-59105, AD-59120, AD-59122, AD-59106, AD-59126, or AD-59107 as disclosed herein in the Examples. Described is that the region of complementarity consists of the antisense sequence of the duplex AD-58632. In embodiments, the region of complementarity consists of an antisense sequence selected from that of AD-59453, AD-59395, AD-59477, and AD-59492. Described is that the region of complementarity consists of an antisense sequence selected from a duplex disclosed herein that suppresses ALAS 1 mRNA expression by at least 50%, 60%, 70%, 80%, 85% or 90%, e.g., as assessed using an assay disclosed in the Examples provided herein.

[0068] Described is that the dsRNA comprises a sense strand consisting of a sense strand sequence selected from Table 2 or Table 3, and an antisense strand consisting of an antisense sequence selected from Table 2 or Table 3.

[0069] Described is that the dsRNA comprises a sense strand consisting of a sense strand sequence selected from Tables 2, 3, 6, 7, 8, 9, 14, or 15, and an antisense strand consisting of an antisense sequence selected from Tables 2, 3, 6, 7, 8, 9, 14, or 15. In embodiments, the dsRNA comprises a pair of corresponding sense and antisense sequences selected from those of the duplexes disclosed in Tables 2, 3, 6, 7, 8, 9, 14, and 15.

[0070] Described is that the dsRNA comprises a sense strand consisting of a sense strand sequence selected from Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 or 20, and an antisense strand consisting of an antisense sequence selected from Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 or 20. Described is that the dsRNA comprises a pair of corresponding sense and antisense sequences selected from those of the duplexes disclosed in Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20.

[0071] In one aspect, the invention provides a cell containing at least one of the dsRNAs of the invention. The cell is generally a mammalian cell, such as a human cell. The cell may be an erythroid cell. The cell may be a liver cell (*e.g.*, a hepatocyte).

[0072] In an aspect provided herein is a pharmaceutical composition for inhibiting expression of an ALAS 1 gene, the

composition comprising a dsRNA of the invention.

[0073] Related to the pharmaceutical compositions described herein, the iRNA (e.g., dsRNA) may be administered in an unbuffered solution. The unbuffered solution may be saline or water.

[0074] Related to the pharmaceutical compositions described herein, the iRNA (e.g., dsRNA) may be administered with a buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof. The buffer solution may be phosphate buffered saline (PBS).

[0075] Related to the pharmaceutical compositions described herein, the iRNA (e.g., dsRNA) may be targeted to hepatocytes.

[0076] Related to the pharmaceutical compositions described herein, the composition may be administered intravenously.

[0077] Related to the pharmaceutical compositions described herein, the composition may be administered subcutaneously.

[0078] A pharmaceutical composition may comprise an iRNA (e.g., a dsRNA) described herein that comprises a ligand (e.g., a GalNAc ligand) that targets the iRNA (e.g., dsRNA) to hepatocytes.

[0079] A pharmaceutical composition may comprise an iRNA (e.g., a dsRNA) described herein that comprises a ligand (e.g., a GalNAc ligand), and the pharmaceutical composition is administered subcutaneously. The ligand may target the iRNA (e.g., dsRNA) to hepatocytes.

[0080] A pharmaceutical composition, e.g., a composition described herein, may include a lipid formulation. The RNAi agent may be in a LNP formulation, e.g., a MC3 formulation. The LNP formulation may target the RNAi agent to a particular cell, e.g., a liver cell, e.g., a hepatocyte. The lipid formulation may be a LNP 11 formulation. The composition may be administered intravenously.

[0081] The pharmaceutical composition may be formulated for administration according to a dosage regimen described herein, *e.g.*, not more than once every four weeks, not more than once every three weeks, not more than once every two weeks, or not more than once every week. The administration of the pharmaceutical composition can be maintained for a month or longer, *e.g.*, one, two, three, or six months, or one year or longer.

[0082] A composition containing an iRNA featured in the invention, i.e., a dsRNA targeting ALAS 1, may be administered with a non-iRNA therapeutic agent, such as an agent known to treat a porphyria (e.g., AIP), or a symptom of a porphyria (e.g., pain). A composition containing an iRNA featured in the invention, i.e. a dsRNA targeting AIP, may be administered along with a non-iRNA therapeutic regimen, such as hemin or glucose (*e.g.*, glucose infusion (e.g., IV glucose)). For example, an iRNA featured in the invention can be administered before, after, or concurrent with glucose, dextrose, or a similar treatment that serves to restore energy balance (*e.g.*, total parenteral nutrition). An iRNA featured in the invention can also be administered before, after, or concurrent with the administration of a heme product (*e.g.*, hemin, heme arginate, or heme albumin), and optionally also in combination with a glucose (e.g. IV glucose) or the like.

[0083] Typically, glucose administered for the treatment of a porphyria is administered intravenously (IV). Administration of glucose intravenously is referred to herein as "IV glucose." However, alternative options in which glucose is administered by other means are also encompassed.

[0084] An ALAS1 iRNA ma be administered to a patient, and then the non-iRNA agent or therapeutic regimen (*e.g.*, glucose and/or a heme product) is administered to the patient (or vice versa). An ALAS1 iRNA and the non-iRNA therapeutic agent or therapeutic regimen may be administered at the same time.

[0085] Described herein is a method of inhibiting ALAS1 expression in a cell, the method comprising: (a) introducing into the cell an iRNA (e.g. a dsRNA) described herein and (b) maintaining the cell of step (a) for a time sufficient to obtain degradation of the mRNA transcript of an ALAS 1 gene, thereby inhibiting expression of the ALAS 1 gene in the cell.

[0086] Described herein is a method for reducing or inhibiting the expression of an ALAS1 gene in a cell (*e.g.*, an erythroid cell or a liver cell, such as, *e.g.*, a hepatocyte). The method includes:

(a) introducing into the cell a double-stranded ribonucleic acid (dsRNA), wherein the dsRNA includes at least two sequences that are complementary to each other. The dsRNA has a sense strand having a first sequence and an antisense strand having a second sequence; the antisense strand has a region of complementarity that is substantially complementary to at least a part of an mRNA encoding ALAS 1, and where the region of complementarity is 30 nucleotides or less, *i.e.*, 15-30 nucleotides in length, and generally 19-24 nucleotides in length, and where the dsRNA upon contact with a cell expressing ALAS 1, inhibits expression of an ALAS 1 gene by at least 10%, *e.g.,* at least 20%, at least 30%, at least 40% or more; and

(b) maintaining the cell of step (a) for a time sufficient to obtain degradation of the mRNA transcript of the ALAS 1 gene, thereby reducing or inhibiting expression of an ALAS 1 gene in the cell.

[0087] Related to the foregoing methods of inhibiting ALAS1 expression in a cell, the cell is treated *ex vivo*, *in vitro*, or *in vivo.* The cell may be a hepatocyte.

[0088] The cell may be present in a subject in need of treatment, prevention and/or management of a disorder related

to ALAS1 expression.

[0089] The disorder may be a porphyria. The porphyria may be acute intermittent porphyria or ALA-dehydratase deficiency porphyria.

[0090] In embodiments, the porphyria is a hepatic porphyria, e.g., a porphyria selected from acute intermittent porphyria (AIP) hereditary coproporphyria (HCP), variegate porphyria (VP), ALA deyhdratase deficiency porphyria (ADP), and hepatoerythropoietic porphyria. The porphyria may be a homozygous dominant hepatic porphyria (e.g., homozygous dominant AIP, HCP, or VP) or hepatoerythropoietic porphyria. The porphyria may be a dual porphyria.

[0091] The expression of ALAS1 may be inhibited by at least 30%.

[0092] The iRNA (*e.g.*, dsRNA) may have an IC_{50} in the range of 0.01-1nM.

[0093] The cell (*e.g.*, the hepatocyte) may be a mammalian cell (*e.g.*, a human, non-human primate, or rodent cell).

[0094] The cell may be treated *ex vivo*, *in vitro*, *or in vivo* (*e.g.*, the cell is present in a subject (*e.g.*, a patient in need of treatment, prevention and/or management of a disorder related to ALAS1 expression).

[0095] The subject may be a mammal (*e.g.*, a human) at risk, or diagnosed with a porphyria, *e.g.*, X-linked sideroblastic anemia (XLSA), ALA deyhdratase deficiency porphyria (ADP or Doss porphyria), acute intermittent porphyria (AIP), congenital erythropoietic porphyria (CEP), prophyria cutanea tarda (PCT), hereditary coproporphyria (coproporphyria, or HCP), variegate porphyria (VP), erythropoietic protoporphyria (EPP), or transient erythroporphyria of infancy. The disorder may be an acute hepatic porphyria, *e.g.*, ALA deyhdratase deficiency porphyria (ADP), AIP, HCP, or VP. The disorder may be ALA deyhdratase deficiency porphyria (ADP) or AIP.

[0096] In embodiments, the porphyria is a hepatic porphyria, e.g., a porphyria selected from acute intermittent porphyria (AIP) hereditary coproporphyria (HCP), variegate porphyria (VP), ALA deyhdratase deficiency porphyria (ADP), and hepatoerythropoietic porphyria. The porphyria may be a homozygous dominant hepatic porphyria (e.g., homozygous dominant AIP, HCP, or VP) or hepatoerythropoietic porphyria, The porphyria may be a dual porphyria.

[0097] The dsRNA introduced may reduce or inhibit expression of an ALAS 1 gene in the cell.

[0098] The dsRNA introduced may reduce or inhibit expression of an ALAS 1 gene, or the level of one or more porphyrins or porphyrin precursors (e.g., δ-aminolevulinic acid (ALA), porphopilinogen (PBG), hydroxymethylbilane (HMB), uroporphyrinogen I or III, coproporphyrinogen I or III, protoporphrinogen IX, and protoporphyrin IX) or porphyrin products or metabolites, by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% or more compared to a reference, (*e.g.*, an untreated cell or a cell treated with a non-targeting control dsRNA). Without being bound by theory, ALAS1 is the first enzyme of the porphyrin pathway. Thus, reducing expression of the ALAS 1 gene is likely to reduce the level of one or more porphyrin precursors, porphyrins or porphyrin products or metabolites.

[0099] In other aspects, the disclosure provides methods for treating, preventing or managing pathological processes related to ALAS1 expression (*e.g.*, pathological processes involving porphyrins, porphyrin precuorsors, or defects in the porphyrin pathway, such as, for example, porphyrias). The method may include administering to a subject, *e.g.*, a patient in need of such treatment, prevention or management, an effective (*e.g.*, a therapeutically or prophylactically effective) amount of one or more of the iRNAs featured herein.

[0100] Described herein is a method of treating and/or preventing a disorder related to ALAS 1 expression comprising administering to a subject in need of such treatment a therapeutically effective amount of an iRNA (e.g., a dsRNA) described herein, or a composition comprising an iRNA (e.g., a dsRNA) described herein.

[0101] Described herein is a method of treating and/or preventing a porphyria comprising administering to a subject in need of such treatment a double-stranded ribonucleic acid (dsRNA), wherein said dsRNA comprises a sense strand and an antisense strand 15-30 base pairs in length and the antisense strand is complementary to at least 15 contiguous nucleotides of SEQ ID NO: 1 or SEQ ID NO:382.

[0102] The subject (e.g., the patient) may have a porphyria. The subject (e.g., patient) may be at risk for developing a porphyria. Administration of the iRNA targeting ALAS1 may alleviate or relieve the severity of at least one symptom of a disorder related to ALAS 1 in the patient.

[0103] The subject may be a mammal (*e.g.*, a human) at risk, or that has been diagnosed with, a disorder related to ALAS1 expression, e.g., a porphyria, *e.g.*, X-linked sideroblastic anemia (XLSA), ALA deyhdratase deficiency porphyria (Doss porphyria), acute intermittent porphyria (AIP), congenital erythropoietic porphyria (CEP), prophyria cutanea tarda (PCT), hereditary coproporphyria (coproporphyria, or HCP), variegate porphyria (VP), erythropoietic protoporphyria (EPP), or transient erythroporphyria of infancy. The porphyria may be an acute hepatic porphyria, *e.g.*, ALA deyhdratase deficiency porphyria (ADP), AIP, HCP, or VP. The disorder may be ALA deyhdratase deficiency porphyria (ADP) or AIP.

[0104] The subject may have, or may be at risk for developing, a porphyria. The porphyria may be a hepatic porphyria, e.g., a porphyria selected from acute intermittent porphyria (AIP) hereditary coproporphyria (HCP), variegate porphyria (VP), ALA deyhdratase deficiency porphyria (ADP), and hepatoerythropoietic porphyria. The porphyria may be a homozygous dominant hepatic porphyria (e.g., homozygous dominant AIP, HCP, or VP) or hepatoerythropoietic porphyria. The porphyria may be a dual porphyria.

[0105] A porphyria, a symptom of porphyria, a prodrome, or an attack of porphyria may be induced by exposure to a precipitating factor, as described herein. The precipitating factor may be a chemical exposure. The precipitating factor

may be a drug, e.g., a prescription drug or an over the counter drug. The precipitating factor may be the menstrual cycle, e.g., a particular phase of the menstrual cycle, e.g., the luteal phase.

[0106] Described is that the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered after an acute attack of porphyria.

[0107] Described is that the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered during an acute attack of porphyria.

[0108] Described is that the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered prophylactically to prevent an acute attack of porphyria.

[0109] Described is that the iRNA (e.g., dsRNA) is formulated as an LNP formulation.

[0110] Described is that the iRNA (e.g., dsRNA) is in the form of a GalNAc conjugate.

[0111] Described is that iRNA (e.g., dsRNA) is administered at a dose of 0.05-50 mg/kg.

[0112] Described is that the iRNA (e.g., dsRNA) is administered at a concentration of 0.01 mg/kg-5 mg/kg bodyweight of the subject.

[0113] Described is that the iRNA (e.g., dsRNA) is formulated as an LNP formulation and is administered at a dose of 0.05-5 mg/kg.

[0114] Described is that the iRNA (e.g., dsRNA) is in the form of a GalNAc conjugate and is administered at a dose of 0.5-50 mg/kg.

[0115] Described is that the method decreases a level of a porphyrin or a porphyrin precursor in the subject.

[0116] Described is that the level is decreased by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%. In an embodiment, the level is decreased by at least 30%.

[0117] Described is that the porphyrin precursor is δ-aminolevulinic acid (ALA) or porphopilinogen (PBG).

[0118] Described is that the iRNA (e.g., dsRNA) has an IC_{50} in the range of 0.01-1nM.

[0119] A method described herein

(i) ameliorates a symptom associated with an ALAS1 related disorder (e.g., a porphyria)
(ii) inhibits ALAS1 1 expression in the subject,
(iii) decreases a level of a porphyrin precursor (e.g., ALA or PBG) or a porphyrin in the subject,
(iv) decreases frequency of acute attacks of symptoms associated with a porphyria in the subject, or
(v) decreases incidence of acute attacks of symptoms associated with a porphyria in the subject when the subject is exposed to a precipitating factor (e.g., the premenstrual phase or the luteal phase).

[0120] Described is that the method ameliorates pain and/or progressive neuropathy.

[0121] Described is that the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered according to a dosing regimen.

[0122] Described is that the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered before or during an acute attack of porphyria. Described is that the iRNA is administered before an acute attack of porphyria.

[0123] Described is that the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered during a prodrome. Described is that the prodrome is characterized by abdominal pain, nausea, psychological symptoms (e.g., anxiety), restlessness and/or insomnia.

[0124] Described is that the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered during a particular phase of the menstrual cycle, e.g., during the luteal phase. Described is that the method ameliorates or prevents cyclical attacks of porphyria, e.g., by reducing the severity, duration, or frequency of attacks. Described is that the cyclical attacks are associated with a precipitating factor. Described is that the precipitating factor is the menstrual cycle, e.g., a particular phase of the menstrual cycle, e.g., the luteal phase.

[0125] Described is that the subject has an elevated level of ALA and/or PBG. Described is that the subject has or is at risk for developing a porphyria, e.g., a hepatic porphyria. Described is that the subject is asymptomatic. Described is that the subject carries a genetic alteration (e.g., a gene mutation) associated with a porphyria, as described herein.

[0126] Described is that the subject has or is at risk for developing a porphyria and suffers from pain (e.g., chronic pain, e.g., chronic neuropathic pain) and/or neuropathy (e.g., progressive neuropathy). Described is that the subject does not suffer from acute attacks but suffers from pain (e.g., chronic pain, e.g., chronic neuropathic pain) and/or neuropathy (e.g., progressive neuropathy). Described is that the pain is abdominal pain.

[0127] Described is that the subject (a) has an elevated level of ALA and/or PBG and (b) suffers from pain (e.g., chronic pain, e.g., chronic neuropathic pain) and/or neuropathy (e.g., progressive neuropathy). Described is that the pain is abdominal pain.

[0128] Described is that the subject has a plasma level and/ or a urine level of ALA and/or PBG that is elevated. Described is that the elevated level of ALA and/or PBG is accompanied by other symptoms, e.g., pain (e.g., chronic pain, e.g., chronic neuropathic pain) or neuropathy (e.g., progressive neuropathy). Described is that the pain is abdominal pain. Described is that the subject is asymptomatic. Described is that the subject has a genetic mutation associated with

a porphyria, e.g., a mutation as described herein.

[0129] Described is that the subject has a level (e.g., a plasma level or a urine level) of a porphyrin precursor, e.g., ALA and/or PBG, that is elevated, e.g., the level is greater than, or greater than or equal to, a reference value. Described is that the level is greater than the reference value. Described is that the reference value is two standard deviations above the mean level in a sample of healthy individuals. Described is that the reference value is an upper reference limit.

[0130] Described is that the subject has a plasma level and/or a urine level of ALA and/or PBG that is greater than, or greater than or or equal to, 2 times, 3 times, 4 times, or 5 times that of an upper reference limit. As used herein, an "upper reference limit" refers to a level that is the upper limit of the 95% confidence interval for a reference sample, e.g., a sample of normal (e.g., wild type) or healthy individuals, e.g., individuals who do not carry a genetic mutation associated with a porphyria and/or individuals who do not suffer from a porphyria. Described is that the subject has a urine level of ALA and/or PBG that is greater than 2 to 4 times that of an upper reference limit. Described is that the subject has a urine level of ALA and/or PBG that is greater than 4 times that of an upper reference limit.

[0131] Described is that the reference value for plasma PBG is 0.12 μmol/L. Described is that the subject is a human and has a plasma PBG level that is greater than, or greater than or equal to, 0.12 μmol/L, 0.24 μmol/L, 0.36 μmol/L, 0.48 μmol/L, or 0.60 μmol/L. Described is that the subject is a human and has a plasma level of PBG that is greater than, or greater than or equal to, 0.48 μmol/L.

[0132] Described is that the reference value for urine PBG is 1.2 mmol/mol creatinine. Described is that the subject is a human and has a urine PBG level that is greater than, or greater than or equal to, 1.2 mmol/mol creatinine, 2.4 mmol/mol creatinine, 3.6 mmol/mol creatinine, 4.8 mmol/mol creatinine, or 6.0 mmol/mol creatinine. Described is that the subject is a human and has a urine level of PBG that is greater than, or greater than or equal to, 4.8 mmol/mol creatinine.

[0133] Described is that the reference value for plasma ALA is 0.12 μmol/L. Described is that the subject is a human and has a plasma ALA level that is greater than, or greater than or equal to, 0.12 μmol/L, 0.24 μmol/L, 0.36 μmol/L, 0.48 μmol/L, or 0.60 μmol/L. Described is that the subject is a human and has a plasma ALA level that is greater than, or greater than or equal to 0.48 μmol/L.

[0134] Described is that the reference value for urine ALA is 3.1 mmol/mol creatinine. Described is that the subject is a human and has a urine ALA level that is greater than, or greater than or equal to, 3.1 mmol/mol creatinine, 6.2 mmol/mol creatinine, 9.3 mmol/mol creatinine, 12.4 mmol/mol creatinine, or 15.5 mmol/mol creatinine.

[0135] Described is that the method decreases an elevated level of ALA and/or PBG. Described is that the method decreases pain (e.g., chronic pain, e.g. chronic neuropathic pain) and/or neuropathy (e.g., progressive neuropathy). Described is that the pain is abdominal pain. In embodiments, the pain is neuropathic pain (e.g., pain associated with the progressive neuropathy of acute porphyrias). The decrease in pain can include, e.g., prevention of pain, delay in the onset of pain, reduction in the frequency of pain, and/or reduction in severity of pain.

[0136] Described is that the method ameliorates or prevents acute attacks of porphyria, e.g., by reducing the severity, duration, or frequency of attacks.

[0137] Described is that the method decreases or prevents nerve damage.

[0138] Described is that the method prevents deterioration (e.g., prevents development of abnormalities) of or results in an improvement of clinical measures, e.g., clinical measures of muscle and/or nerve function, e.g., EMG and/or nerve conduction velocities,

[0139] Described is that the method is effective to reduce a level of ALA and/or PBG (e.g., a plasma or urine level of ALA and/or PBG). Described is that the method is effective to produce a predetermined reduction in the elevated level of ALA and/or PBG.

[0140] Described is that the predetermined reduction is a reduction to a value that is less than or equal to a reference value. Described is that the reference value is an upper reference limit. Described is that the reference value is the value that is two standard deviations above the mean level in a reference sample.

[0141] Described is that the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered repeatedly, e.g., according to a dosing regimen.

[0142] Described is that the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered prophylactically to a subject who is at risk for developing a porphyria. Described is that the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered prophylactically beginning at puberty. Described is that the subject carries a genetic mutation associated with a porphyria and/or has an elevated level of ALA and/or PBG (e.g., an elevated plasma or urine level of ALA and/or PBG). Described is that the mutation makes an individual susceptible to an acute attack (e.g., upon exposure to a precipitating factor, e.g., a drug, dieting or other precipitating factor, e.g., a precipitating factor as disclosed herein). Described is that the mutation is associated with elevated levels of a porphyrin or a porphyrin precursor (e.g., ALA and/or PBG). Described is that the mutation is associated with chronic pain (e.g., chronic neuropathic pain) and/or neuropathy (e.g., progressive neuropathy).

[0143] Described is that the mutation is a mutation in the ALAS 1 gene. Described is that the mutation is a mutation in the ALAS 1 gene promoter, or in regions upstream or downstream from the ALAS 1 gene. Described is that the mutation is a mutation in transcription factors or other genes that interact with ALAS1. Described is that the mutation is

a mutation in a gene that encodes an enzyme in the heme biosynthetic pathway.

[0144] Described is that the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered subcutaneously. Described is that the iRNA is in the form of a GalNAc conjugate. In embodiments, the iRNA (e.g., the dsRNA) is administered at a dose of 0.5-50 mg/kg.

[0145] Described herein is a method of treating a subject with an elevated level of ALA and/or PBG, the method comprising administering to the subject a double-stranded ribonucleic acid (dsRNA), wherein said dsRNA comprises a sense strand and an antisense strand 15-30 base pairs in length and the antisense strand is complementary to at least 15 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:382.

[0146] Described herein is a method of treating a subject with an elevated level of ALA and/or PBG, the method comprising administering to the subject a therapeutically effective amount of an dsRNA or a composition comprising a dsRNA, as described herein.

[0147] Described is that the methods described herein are effective to decrease the level of ALA and/or PBG. Described is that the level of ALA and/or PBG is decreased such that it is less than, or less than or equal to, a reference value, e.g., an upper reference limit. In another aspect, the invention provides methods for decreasing a level of a porphyrin or a porphyrin precursor in a cell *(e.g.,* an erythroid cell or a liver cell, such as, *e.g.*, a hepatocyte). Described is that the cell is treated *ex vivo*, *in vitro*, or *in vivo* (*e.g.*, the cell is present in a subject (*e.g.*, a patient in need of treatment, prevention and/or management of a disorder related to ALAS1 expression). The method includes contacting the cell with an effective amount of one or more of the iRNAs targeting ALAS 1, *e.g.,* one or more of the iRNAs disclosed herein, thereby decreasing the level of a porphyrin or a porphyrin precursor in the cell; or decreasing the level of a porphyrin or a porphyrin precursor in other cells, tissues, or fluids within a subject in which the cell is located; relative to the level prior to contacting. Such methods can be used to treat (e.g., ameliorate the severity) of disorders related to ALAS 1 expression, such as porphyrias, e.g., AIP or ALA dehydratase deficiency porphyria.

[0148] Described is that the contacting step is effected *ex vivo*, *in vitro*, or *in vivo.* For example, the cell can be present in a subject, *e.g.,* a mammal (*e.g.*, a human) at risk, or that has been diagnosed with, a porphyria. Described is that the porphyria is an acute hepatic porphyria. Described is that the porphyria is a hepatic porphyria, e.g., a porphyria selected from acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP), ALA deyhdratase deficiency porphyria (ADP), and hepatoerythropoietic porphyria. Described is that the porphyria is a homozygous dominant hepatic porphyria (e.g., homozygous dominant AIP, HCP, or VP) or hepatoerythropoietic porphyria. Described is that the porphyria is a dual porphyria.

[0149] Described is a method for decreasing a level of a porphyrin or a porphyrin precursor (e.g., ALA or PBG) in a cell, comprising contacting the cell with an iRNA (e.g. a dsRNA), as described herein, in an amount effective to decrease the level of the porphyrin or the porphyrin precursor in the cell. Described is that the cell is a hepatocyte. Described is that the porphyrin or porphyrin precursor is δ-aminolevulinic acid (ALA), porphopilinogen (PBG), hydroxymethylbilane (HMB), uroporphyrinogen I or III, coproporphyrinogen I or III, protoporphrinogen IX, or protoporphyrin IX. Described is that the porphyrin precursor is ALA or PBG.

[0150] Described is that the cell is an erythroid cell. Described is that the cell is a liver cell (*e.g.*, a hepatocyte).

[0151] Described is a vector encoding at least one strand of an iRNA (e.g., a dsRNA) as described herein.

[0152] Described is a vector encoding at least one strand of a dsRNA, wherein said dsRNA comprises a region of complementarity to at least a part of an mRNA encoding ALAS 1, wherein said dsRNA is 30 base pairs or less in length, and wherein said dsRNA targets said mRNA for cleavage.

[0153] Described is that the region of complementarity is at least 15 nucleotides in length.

[0154] Described is that the region of complementarity is 19 to 21 nucleotides in length.In one aspect, the invention provides a vector for inhibiting the expression of an ALAS 1 gene in a cell. Described is that the vector comprises an iRNA as described herein. Described is that the vector includes at least one regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of an iRNA as described herein. Described is that the vector comprises at least one strand of an ALAS1 iRNA.

[0155] Described is a cell comprising a vector as described herein. Described herein is a cell containing a vector for inhibiting the expression of an ALAS 1 gene in a cell. The vector includes a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the iRNAs as described herein. The cell may be a liver cell (*e.g.*, a hepatocyte). The cell may be an erythroid cell.

[0156] The invention is set forth in the claims.

FIG. 1 depicts the heme biosynthetic pathway.

FIG. 2 summarizes certain porphyrias associated with genetic errors in heme metabolism.

FIG. 3 depicts a human ALAS 1 mRNA sequence transcript variant 1 (Ref. Seq. NM_000688.4 (GI:40316942, record dated November 19, 2011), SEQ ID NO: 1).

FIG. 4 depicts a human ALAS 1 mRNA sequence transcript variant 2 (Ref. Seq. NM_000688.5 (GI: 362999011, record dated April 1, 2012), SEQ ID NO: 382).

FIG. 5 shows the dose-response of the siRNA AD-53558 in suppressing mouse ALAS1 (mALAS1) mRNA relative to a PBS control. Results for a luciferase (LUC) AD-1955 control are also shown.

FIG. 6 shows the dose-response of the siRNA AD-53558 in suppressing ALAS1 mRNA in rats relative to a PBS control. Results for a luciferase (LUC) AD-1955 control are also shown.

FIG. 7 shows the durability of suppression of mouse ALAS1 (mALAS1) mRNA by the siRNA AD-53558 relative to a PBS control.

FIG. 8 shows means ± standard deviations of plasma ALA levels (in μM) at baseline, and after phenobarbital treatment in the experimental (ALAS1 siRNA) and control (LUC siRNA) groups.

FIG. 9 shows shows the plasma ALA levels (in μM) of individual animals at baseline, and after phenobarbital treatment in animals that received ALAS1 siRNA and control (LUC siRNA) treatment.

FIG. 10 shows means ± standard deviations of plasma PBG levels (in μM) at baseline, and after phenobarbital treatment in animals that received ALAS1 siRNA and control (LUC siRNA) treatment.

FIG. 11 shows shows the plasma PBG levels (in μM) of individual animals at baseline, and after phenobarbital treatment in animals that received ALAS 1 siRNA and control (LUC siRNA) treatment.

FIG. 12 shows the relative mALAS1mRNA level in liver at baseline, and after phenobarbital treatment in select representative experimental (ALAS1 siRNA) and control (PBS) animals.

FIG. 13 shows the effects of three GalNAc conjugated mALAS1 siRNAs on mALAS1 expression (relative to a PBS control) in mouse liver tissue.

FIG. 14 shows plasma ALA and PBG levels over time after phenobarbital administration and treatment with ALAS 1 siRNA or control LUC siRNA.

FIG. 15 shows the effects of a GalNAc conjugated ALAS1 siRNA on plasma ALA and plasma PBG levels in the mouse AIP phenobarbital induction model.

[0157] iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). Described herein are iRNAs and methods of using them for inhibiting the expression of an ALAS 1 gene in a cell or a mammal where the iRNA targets an ALAS 1 gene. Also provided are compositions and methods for disorders related to ALAS 1 expression, such as porphyrias (*e.g.*, ALA deyhdratase deficiency porphyria (ADP or Doss porphyria), acute intermittent porphyria, congenital erythropoietic porphyria, prophyria cutanea tarda, hereditary coproporphyria (coproporphyria), variegate porphyria, erythropoietic protoporphyria (EPP), X-linked sideroblastic anemia (XLSA), and and transient erythroporphyria of infancy).

[0158] Porphyrias are inherited or acquired disorders that can be caused by decreased or enhanced activity of specific enzymes in the heme biosynthetic pathway, also referred to herein as the porphyrin pathway (See FIG. 1). Porphyrins are the main precursors of heme. Porphyrins and porphyrin precursors include δ-aminolevulinic acid (ALA), porphopilinogen (PBG), hydroxymethylbilane (HMB), uroporphyrinogen I or III, coproporphyrinogen I or III, protoporphrinogen IX, and protoporphyrin IX. Heme is an essential part of hemoglobin, myoglobin, catalases, peroxidases, and cytochromes, the latter including the respiratory and P450 liver cytochromes. Heme is synthesized in most or all human cells. About 85% of heme is made in erythroid cells, primarily for hemoglobin. Most of the remaining heme is made in the liver, 80% of which is used for the synthesis of cytochromes. Deficiency of specific enzymes in the porphyrin pathway leads to insufficient heme production and also to an accumulation of porphyrin precursors and/or porphyrins, which can be toxic to cell or organ function in high concentrations.

[0159] Porphyrias may manifest with neurological complications ("acute"), skin problems ("cutaneous") or both. Porphyrias may be classified by the primary site of the overproduction and accumulation of porphyrins or their precursors. In hepatic porphyrias, porphyrins and porphyrin precursors are overproduced predominantly in the liver, whereas in erythropoietic porphyrias, porphyrins are overproduced in the erythroid cells in the bone. The acute or hepatic porphyrias lead to dysfunction of the nervous system and neurologic manifestations that can affect both the central and peripheral nervous system, resulting in symptoms such as, for example, pain (e.g., abdominal pain and/or chronic neuropathic pain), vomiting, neuropathy (e.g., acute neuropathy, progressive neuropathy), muscle weakness, seizures, mental disturbances (e.g., hallucinations, depression anxiety, paranoia), cardiac arrhythmias, tachycardia, constipation, and diarrhea. The cutaneous or erythropoietic porphyrias primarily affect the skin, causing symptoms such as photosensitivity that can be painful, blisters, necrosis, itching, swelling, and increased hair growth on areas such as the forehead. Subsequent infection of skin lesions can lead to bone and tissue loss, as well as scarring, disfigurement, and loss of digits (e.g., fingers, toes). Most porphyrias are caused by mutations that encode enzymes in the heme biosynthetic pathway. A summary of porphyrias associated with genetic errors in heme metabolism is provided in FIG. 2.

[0160] Not all porphyrias are genetic. For example, patients with liver disease may develop porphyria as a result of liver dysfunction, and a transient form of erythroporphria (transient erythroporphyria of infancy) has been described in infancy (see Crawford, R.I. et al, J Am Acad Dermatol. 1995 Aug; 33(2 Pt 2):333-6.) Patients with PCT can acquire the deficient activity of uroporphyrinogen decarboxylase (URO-D), due to the formation of a ORO-D enzyme with lower than normal enzymatic activity (see Phillips et al. Blood, 98:3179-3185, 2001.)

[0161] Acute intermittent porphyria (AIP) (also be referred to as porphobilinogen (PBG) deaminase deficiency, or hydroxymethylbilane synthase (HMBS) deficiency), is the most common type of acute hepatic porphyria. Other types of acute hepatic porphyrias include hereditary coproporphyria (HCP), variegate porphyria (VP), and ALA deyhdratase deficiency porphyria (ADP). Acute hepatic porphyrias are described, e.g., in Balwani, M and Desnick, R.J., *Blood*, 120:4496-4504,2012.

[0162] AIP is typically an autosomal dominant disease that is characterized by a deficiency of the enzyme porphobilinogen deaminase (PBG deaminase); this enzyme is also known as hydroxymethylbilane synthase (HMB synthase or HMBS). PBG deaminase is the third enzyme of the heme biosynthetic pathway (see FIG. 1) and catalyzes the head to tail condensation of four porphobilinogen molecules into the linear tetrapyrrole, hydroxymethylbilane (HMB). Alternatively spliced transcript variants encoding different isoforms of PBG deaminase have been described. Mutations in the PBG deaminase gene are associated with AIP. Such mutations may lead to decreased amounts of PBG deaminase and/or decreased activity of PBG deaminase (affected individuals typically have a ~50% reduction in PBG deaminase activity).

[0163] There are at least two different models of the pathophysiology of AIP and other acute hepatic porphyrias (see, *e.g.*, Lin CS-Y et al., Clinical Neurophysiology, 2011; 122:2336-44). According to one model, the decreased heme production resulting from PBG deaminase deficiency causes energy failure and axonal degeneration. According to the other, currently more favored model, the buildup of porphyrin precursors (e.g., ALA and PBG) results in neurotoxicity.

[0164] AIP has been found to have a prevalence as high as 1 in 10,000 in certain populations (*e.g.*, in Northern Sweden; see Floderus Y, et al. Clin Genet. 2002;62:288-97). The prevalence in the general population in United States and Europe, excluding the U.K., is estimated to be about 1 in 10,000 to 1 in 20,000. Clinical disease manifests itself in only approximately 10-15% of individuals who carry mutations that are known to be associated with AIP. However, the penetrance is as high as 40% in individuals with certain mutations (*e.g.*, the W198X mutation). AIP is typically latent prior to puberty. Symptoms are more common in females than in males. The prevalence of the disease is probably underestimated due to its incomplete penetrance and long periods of latency. In the United States, it is estimated that there are about 2000 patients who have suffered at least one attack. It is estimated that there are about 150 active recurrent cases in France, Sweden, the U.K., and Poland; these patients are predominantly young women, with a median age of 30. See, e.g., Elder et al, J Inherit Metab Dis., published online Nov 1, 2012.

[0165] AIP affects, for example, the visceral, peripheral, autonomic, and central nervous systems. Symptoms of AIP are variable and include gastrointestinal symptoms (*e.g.*, severe and poorly localized abdominal pain, nausea/vomiting, constipation, diarrhea, ileus), urinary symptoms (dysuria, urinary retention/incontinence, or dark urine), neurologic symptoms (*e.g.*, sensory neuropathy, motor neuropathy (e.g., affecting the cranial nerves and/or leading to weakness in the arms or legs), seizures, neuropathic pain (e.g., pain associated with progressive neuropathy, e.g., chronic neuropathic pain), neuropsychiatric symptoms (*e.g.*, mental confusion, anxiety, agitation, hallucination, hysteria, delirium, apathy, depression, phobias, psychosis, insomnia, somnolence, coma), autonomic nervous system involvement (resulting *e.g.*, in cardiovascular sysmptoms such as tachycardia, hypertension, and/or arrhythmias, as well as other symptoms, such as, *e.g.*, increased circulating catecholamine levels, sweating, restlessness, and/or tremor), dehydration, and electrolyte abnormalities. The most common symptoms are abdominal pain and tachycardia. In addition, patients frequently have chronic neuropathic pain and develop a progressive neuropathy. Patients with recurring attacks often have a prodrome. Permanent paralysis may occur after a severe attack. Recovery from severe attacks that are not promptly treated may take weeks or months. An acute attack may be fatal, for example, due to paralysis of respiratory muscles or cardiovascular failure from electrolyte imbalance. (See, *e.g.*, Thunell S. Hydroxymethylbilane Synthase Deficiency. 2005 Sep 27 [Updated 2011 Sep 1]. In: Pagon RA, Bird TD, Dolan CR, et al., editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993- (hereinafter Thunell (1993)).) Prior to the availability of Hemin treatments, up to 20% of patients with AIP died from the disease.

[0166] In individuals who carry genes for AIP, the risk of hepatocellular cancer is increased. In those with recurrent attacks, the risk of hepatocellular cancer is particularly grave: after the age of 50, the risk is nearly 100-fold greater than in the general population.

[0167] Attacks of acute porphyria may be precipitated by endogenous or exogenous factors. The mechanisms by which such factors induce attacks may include, for example, increased demand for hepatic P450 enzymes and/or induction of ALAS1 activity in the liver. Increased demand for hepatic P450 enzymes results in decreased hepatic free heme, thereby inducing the synthesis of hepatic ALAS 1.

[0168] Precipitating factors include fasting (or other forms of reduced or inadequate caloric intake, due to crash diets, long-distance athletics, etc.), metabolic stresses (*e.g.*, infections, surgery, international air travel, and psychological stress), endogenous hormones (e.g., progesterone), cigarette smoking, lipid-soluble foreign chemicals (including, *e.g.*, chemicals present in tobacco smoke, certain prescription drugs, organic solvents, biocides, components in alcoholic beverages), endocrine factors (e.g., reproductive hormones (women may experience exacerbations during the premenstrual period), synthetic estrogens, progesterones, ovulation stimulants, and hormone replacement therapy). See, for example, Thunell (1993).

[0169] Over 1000 drugs are contraindicated in the acute hepatic porphyrias (e.g., AIP, HCP, ADP, and VP) including, for example, alcohol, barbiturates, Carbamazepine, Carisoprodol, Clonazepam (high doses), Danazol, Diclofenac and possibly other NSAIDS, Ergots, estrogens, Ethyclorvynol, Glutethimide, Griseofulvin, Mephenytoin, Meprobamate (also mebutamate and tybutamate), Methyprylon, Metodopramide, Phenytoin, Primidone, progesterone and synthetic progestins, Pyrazinamide, Pyrazolones (aminopyrine and antipyrine), Rifampin, Succinimides (ethosuximide and methsuximide), sulfonamide antibiotics, and Valproic acid.

[0170] Objective signs of AIP include discoloration of the urine during an acute attack (the urine may appear red or red-brown), and increased concentrations of PBG and ALA in urine during an acute attack. Molecular genetic testing identifies mutations in the PBG deaminase (also known as HMBS) gene in more than 98% of affected individuals. Thunell (1993).

[0171] The differential diagnosis of porphyrias may involve determining the type of porphyria by measuring individual levels of porphyrins or porphyrin precursors (e.g., ALA, PBG) in the urine, feces, and/or plasma (e.g., by chromatography and fluorometry) during an attack. The diagnosis of AIP can be confirmed by establishing that erythrocyte PBG deaminase activity is at 50% or less of the normal level. DNA testing for mutations may be carried out in patients and at-risk family members. The diagnosis of AIP is typically confirmed by DNA testing to identify a specific caustative gene mutation (e.g., an HMBS mutation).

[0172] Treatment of acute attacks typically requires hospitalization to control and treat acute sysmptoms, including, *e.g.*, abdominal pain, seizures, dehydration/hyponatremia, nausea/vomiting, tachycardia/hypertension, urinary retention/ileus. For example, abdominal pain may be treated, e.g., with narcotic analgesics, seizures may be treated with seizure precautions and possibly medications (although many anti-seizure medications are contraindicated), nausea/vomiting may be treated, e.g., with phenothiazines, and tachycardia/hypertension may be treated, e.g., with beta blockers. Treatment may include withdrawal of unsafe medications, monitoring of respiratory function, as well as muscle strength and neurological status. Mild attacks (e.g., those with no paresis or hyponatremia) may be treated with at least 300 g intravenous 10% glucose per day, although increasingly hemin is provided immediately. Severe attacks should be treated as soon as possible with intravenous hemin (3-4 mg/kg daily for 4-14 days) and with IV glucose while waiting for the IV hemin to take effect. Typically, attacks are treated with IV hemin for 4 days and with IV glucose while waiting for administration of the IV hemin.

[0173] Hemin (Panhematin® or hemin for injection, previously known as hematin) is the only heme product approved for use in the United States and was the first drug approved under the Orphan Drug Act. Panhematin® is hemin derived from processed red blood cells (PRBCs), and is Protoporphyrin IX containing a ferric iron ion (Heme B) with a chloride ligand. Heme acts to limit the hepatic and/or marrow synthesis of porphyrin. The exact mechanism by which hemin produces symptomatic improvement in patients with acute episodes of the hepatic porphyrias has not been elucidated; however, its action is likely due to the (feedback) inhibition of δ-aminolevulinic acid (ALA) synthase, the enzyme which limits the rate of the porphyrin/heme biosynthetic pathway. See Panhematin® product label, Lundbeck, Inc., October 2010. Inhibition of ALA synthase should result in reduced production of ALA and PBG as well as porphyrins and porphyrin intermediates.

[0174] Drawbacks of hemin include its delayed impact on clinical symptoms and its failure to prevent the recurrence of attacks. Adverse reactions associated with hemin administration may include thrombophlebitis, anticoagulation, thrombocytopenia, renal shut down, or iron overload, which is particularly likely in patients requiring multiple courses of hemin treatment for recurrent attacks. To prevent phlebitis, an indwelling venous catheter is needed for access in patients with recurrent attacks. Uncommonly reported side effects include fever, aching, malaise, hemolysis, anaphalaxis, and circulatory collapse. See Anderson, K.E., Approaches to Treatment and Prevention of Human Porphyrias, in The Porphyrin Handbook: Medical Aspects of Porphyrins, Edited by Karl M. Kadish, Kevin M. Smith, Roger Guilard (2003) (hereinafter Anderson).

[0175] Heme is difficult to prepare in a stable form for intravenous administration. It is insoluble at neutral pH but can be prepared as heme hydroxide at pH 8 or higher. Anderson. Panhematin is a lyophilized hemin preparation. When lyophilized hemin is solubilized for intravenous administration, degradation products form rapidly; these degradation products are responsible for a transient anticoagulant effect and for phlebitis at the site of infusion. Anderson. Heme albumin and heme arginate (Normosang, the European version of hemin) are more stable and may potentially cause less thrombophlebitis. However, heme arginate is not approved for use in the United States. Panhemin may be stabilized by solubilizing it for infusion in 30% human albumin rather than in sterile water; however, albumin adds intravascular volume-expanding effects and increases the cost of treatment as well as risk of pathogens since it is isolated from human blood. See, e.g., Anderson.

[0176] The successful treatment of an acute attack does not prevent or delay recurrence. There is a question of whether hemin itself can trigger recurring attacks due to induction of heme oxygenase. Nonetheless, in some areas (especially France), young women with multiply recurrent attacks are being treated with weekly hemin with the goal of achieving prophylaxis.

[0177] Limited experience with liver transplantation suggests that if successful, it is an effective treatment for AIP.

There have been approximately 12 transplants in Europe in human patients, with curative or varying effects. Liver transplantation can restore normal excretion of ALA and PBG and prevent acute attacks. See, *e.g.,* Dar, F.S. et al. Hepatobiliary Pancreat. Dis. Int., 9(1):93-96 (2010). Furthermore, if the liver of a patient with AIP is transplanted into another patient ("domino transplant"), the patient receiving the transplant may develop AIP.

[0178] Among the long-term clinical effects of acute porphyrias is chronic neuropathic pain that may result from a progressive neuropathy due to neurotoxic effects, e.g., of elevated porphyrin precursors (e.g., ALA and/or PBG). Patients may suffer from neuropathic pain prior to or during an acute attack. Older patients may experience increased neuropathic pain with age for which various narcotic drugs are typically prescribed. Electromyogram abnormalities and decreased conduction times have been documented in patients with acute hepatic porphyrias. Of note, untreated, uninduced mice with AIP (PBG deaminase deficiency) develop a progressive motor neuropathy that has been shown to cause progressive quadriceps nerve axon degeneration and loss presumably due to constitutively elevated porphyrin precursor (ALA & PBG) levels, porphyrins and/or heme deficiency (Lindberg et al., J. Clin. Invest., 103(8): 1127-1134, 1999). In patients with acute porphyria (e.g., ADP, AIP, HCP, or VP), levels of porphyrin precursors (ALA & PBG) are often elevated in asymptomatic patients and in symptomatic patients between attacks. Thus, reduction of the porphyrin precursors and resumption of normal heme biosynthesis by reducing the level of ALAS 1 expression and/or activity is expected to prevent and/or minimize development of chronic and progressive neuropathy. Treatment, e.g., chronic treatment (e.g., periodic treatment with iRNA as described herein, e.g., treatment according to a dosing regimen as described herein, e.g., weekly or biweekly treatment) can continuously reduce the ALAS 1 expression in acute porphyria patients who have elevated levels of porphyrin precursors, porphyrins, porphyrin products or their metabolites. Such treatment may be provided as needed to prevent or reduce the frequency or severity of an individual patient's symptoms (e.g., pain and/or neuropathy) and/or to reduce a level of a porphyrin precursor, porphyrin, porphyrin product or metabolite.

[0179] The need exists for identifying novel therapeutics that can be used for the treatment of porphyrias. As discussed above, existing treatments such as hemin have numerous drawbacks. For example, the impact of hemin on clinical symptoms is delayed, it is expensive, and it may have side effects (e.g., thrombophlebitis, anticoagulation, thrombocytopenia, iron overload, renal shutdown). Novel therapeutics such as those described herein can address these drawbacks and the unmet needs of patients by, for example, acting faster, not inducing phlebitis, providing the convenience of subcutaneous administration, successfully preventing recurrent attacks, preventing or ameliorating pain (e.g., chronic neuropathic pain) and/or progressive neuropathy, and/or not causing certain adverse effects associated with hemin (e.g., iron overload, increased risk of hepatocellular cancer).

[0180] The present disclosure provides methods and iRNA compositions for modulating the expression of an ALAS1 gene. In certain embodiments, expression of ALAS1 is reduced or inhibited using an ALAS1-specific iRNA, thereby leading to a decreased expression of an ALAS1 gene. Reduced expression of an ALAS1 gene may reduce the level of one or more porphyrin precursors, porphyrins, or porphyrin products or metabolites. Decreased expression of an ALAS1 gene, as well as related decreases in the level of one or more porphyrin precursors and/or porphyrins, can be useful in treating disorders related to ALAS 1 expression, e.g., porphyrias.

[0181] The iRNAs of the compositions featured herein include an RNA strand (the antisense strand) having a region which is 30 nucleotides or less in length, *i.e.*, 15-30 nucleotides in length, generally 19-24 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of an ALAS 1 gene (also referred to herein as an "ALAS1-specific iRNA"). The use of such an iRNA enables the targeted degradation of mRNAs of genes that are implicated in pathologies associated with ALAS 1 expression in mammals, *e.g.*, porphyrias such as ALA dehydratase deficiency porphyria (Doss porphyria) or acute intermittent porphyria. Very low dosages of ALAS1-specific iRNAs can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of an ALAS1 gene. iRNAs targeting ALAS1 can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of an ALAS1 gene, *e.g.*, in cell based assays. Thus, methods and compositions including these iRNAs are useful for treating pathological processes related to ALAS1 expression, such as porphyrias (*e.g.*, X-linked sideroblastic anemia (XLSA), ALA deyhdratase deficiency porphyria (Doss porphyria), acute intermittent porphyria (AIP), congenital erythropoietic porphyria, prophyria cutanea tarda, hereditary coproporphyria (coproporphyria), variegate porphyria, erythropoietic protoporphyria (EPP), and transient erythroporphyria of infancy).

[0182] The following description discloses how to make and use compositions containing iRNAs to inhibit the expression of an ALAS1 gene, as well as compositions and methods for treating diseases and disorders caused by or modulated by the expression of this gene. Embodiments of the pharmaceutical compositions featured in the invention include an iRNA having an antisense strand comprising a region which is 30 nucleotides or less in length, generally 19-24 nucleotides in length, which region is substantially complementary to at least part of an RNA transcript of an ALAS1 gene, together with a pharmaceutically acceptable carrier. Embodiments of compositions featured in the invention also include an iRNA having an antisense strand having a region of complementarity which is 30 nucleotides or less in length, generally 19-24 nucleotides in length, and is substantially complementary to at least part of an RNA transcript of an ALAS1 gene.

[0183] Accordingly, in some aspects, pharmaceutical compositions containing an ALAS1 iRNA and a pharmaceutically acceptable carrier, methods of using the compositions to inhibit expression of an ALAS1 gene, and methods of using

the pharmaceutical compositions to treat disorders related to ALAS1 expression are described.

I. Definitions

[0184] For convenience, the meaning of certain terms and phrases used in the specification, examples, and appended claims, are provided below. If there is an apparent discrepancy between the usage of a term in other parts of this specification and its definition provided in this section, the definition in this section shall prevail.

[0185] "G," "C," "A," "T" and "U" each generally stand for a nucleotide that contains 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 may be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base may base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine may be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another 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.

[0186] As used herein, "ALAS1" (also known as ALAS-1; δ-aminolevulinate synthase 1; δ-ALA synthase 1; 5'-aminolevulinic acid synthase 1; ALAS-H; ALASH; ALAS-N; ALAS3; EC2.3.1.37; 5-aminolevulinate synthase, nonspecific, mitochondrial; ALAS; MIG4; OTTHUMP00000212619; OTTHUMP00000212620; OTTHUMP00000212621; OTTHUMP00000212622; migration-inducing protein 4; EC 2.3.1) refers to a nuclear-encoded mitochondrial enzyme that is the first and typically rate-limiting enzyme in the mammalian heme biosynthetic pathway. ALAS 1 catalyzes the condensation of glycine with succinyl-CoA to form δ-aminolevulinic acid (ALA). The human ALAS1 gene is expressed ubiquitously, is found on chromosome 3p21.1 and typically encodes a sequence of 640 amino acids. In contrast, the ALAS-2 gene, which encodes an isozyme, is expressed only in erythrocytes, is found on chromoxome X11.21, and typicallyencodes a sequence of 550 amino acids. As used herein an "ALAS1 protein" means any protein variant of ALAS1 from any species (*e.g.*, human, mouse, non-human primate), as well as any mutants and fragments thereof that retain an ALAS 1 activity. Similarly, an "ALAS1 transcript" refers to any transcript variant of ALAS1, from any species (*e.g.*, human, mouse, non-human primate). A sequence of a human ALAS1 variant 1 mRNA transcript can be found at NM_000688.4 (FIG. 3; SEQ ID NO: 1). Another version, a human ALAS1 variant 2 mRNA transcript, can be found at NM_000688.5 (FIG. 4; SEQ ID NO:382). The level of the mature encoded ALAS1 protein is regulated by heme: high levels of heme down-regulate the mature enzyme in mitochondria while low heme levels up-regulate. Multiple alternatively spliced variants, encoding the same protein, have been identified.

[0187] As used herein, the term "iRNA," "RNAi", "iRNA agent," or "RNAi agent" refers to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript, e.g., via an RNA-induced silencing complex (RISC) pathway. An iRNA as described herein effects inhibition of ALAS1 expression. Inhibition of ALAS1 expression may be assessed based on a reduction in the level of ALAS1 mRNA or a reduction in the level of the ALAS1 protein. As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an ALAS 1 gene, including mRNA that is a product of RNA processing of a primary transcription product. The target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion. For example, the target sequence will generally be from 9-36 nucleotides in length, *e.g.*, 15-30 nucleotides in length, including all sub-ranges therebetween. As non-limiting examples, the target sequence can be from 15-30 nucleotides, 15-26 nucleotides, 15-23 nucleotides, 15-22 nucleotides, 15-21 nucleotides, 15-20 nucleotides, 15-19 nucleotides, 15-18 nucleotides, 15-17 nucleotides, 18-30 nucleotides, 18-26 nucleotides, 18-23 nucleotides, 18-22 nucleotides, 18-21 nucleotides, 18-20 nucleotides, 19-30 nucleotides, 19-26 nucleotides, 19-23 nucleotides, 19-22 nucleotides, 19-21 nucleotides, 19-20 nucleotides, 20-30 nucleotides, 20-26 nucleotides, 20-25 nucleotides, 20-24 nucleotides,20-23 nucleotides, 20-22 nucleotides, 20-21 nucleotides, 21-30 nucleotides, 21-26 nucleotides, 21-25 nucleotides, 21-24 nucleotides, 21-23 nucleotides, or 21-22 nucleotides.

[0188] 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.

[0189] 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 may 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. Other conditions, such as physiologically relevant conditions as may 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.

[0190] 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 may 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, may yet be referred to as "fully complementary" for the purposes described herein.

[0191] "Complementary" sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs includes, but are not limited to, G:U Wobble or Hoogstein base pairing.

[0192] The terms "complementary," "fully complementary" and "substantially complementary" herein may 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.

[0193] 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 an ALAS1 protein). For example, a polynucleotide is complementary to at least a part of an ALAS 1 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding ALAS1. As another example, a polynucleotide is complementary to at least a part of an ALAS1 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding ALAS1.

[0194] The term "double-stranded RNA" or "dsRNA," as used herein, refers to an iRNA that includes an RNA molecule or complex of molecules having a hybridized duplex region that comprises two anti-parallel and substantially complementary nucleic acid strands, which will be referred to as having "sense" and "antisense" orientations with respect to a target RNA. The duplex region can be of any length that permits specific degradation of a desired target RNA, e.g., through a RISC pathway, but will typically range from 9 to 36 base pairs in length, *e.g.,* 15-30 base pairs in length. Considering a duplex between 9 and 36 base pairs, the duplex can be any length in this range, for example, 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, or 36 and any sub-range therein between, including, but not limited to 15-30 base pairs, 15-26 base pairs, 15-23 base pairs, 15-22 base pairs, 15-21 base pairs, 15-20 base pairs, 15-19 base pairs, 15-18 base pairs, 15-17 base pairs, 18-30 base pairs, 18-26 base pairs, 18-23 base pairs, 18-22 base pairs, 18-21 base pairs, 18-20 base pairs, 19-30 base pairs, 19-26 base pairs, 19-23 base pairs, 19-22 base pairs, 19-21 base pairs, 19-20 base pairs, 20-30 base pairs, 20-26 base pairs, 20-25 base pairs, 20-24 base pairs, 20-23 base pairs, 20-22 base pairs, 20-21 base pairs, 21-30 base pairs, 21-26 base pairs, 21-25 base pairs, 21-24 base pairs, 21-23 base pairs, or 21-22 base pairs. dsRNAs generated in the cell by processing with Dicer and similar enzymes are generally in the range of 19-22 base pairs in length. One strand of the duplex region of a dsDNA comprises a sequence that is substantially complementary to a region of a target RNA. The two strands forming the duplex structure can be from a single RNA molecule having at least one self-complementary region, or can be formed from two or more separate RNA molecules. Where the duplex region is formed from two strands of a single molecule, the molecule can have a duplex region separated by a single stranded chain of nucleotides (herein referred to as a "hairpin loop") between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure. The hairpin loop can comprise at least one unpaired nucleotide; the hairpin loop can comprise at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23 or more unpaired 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. Where the two strands are connected covalently by means other than a hairpin loop, the connecting structure is referred to as a "linker." The term "siRNA" is also used herein to refer to a dsRNA as described above.

[0195] The iRNA agent may be a "single-stranded siRNA" 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 modified. The design and testing of single-stranded siRNAs 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 (e.g., sequences provided in Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20) 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;:883-894.

[0196] In another aspect, the RNA agent is a "single-stranded antisense RNA molecule". An single-stranded antisense RNA molecule is complementary to a sequence within the target mRNA. Single-stranded antisense RNA molecules can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. et al., (2002) Mol Cancer Ther 1:347-355. Alternatively, the single-stranded antisense molecules inhibit a target mRNA by hydridizing to the target and cleaving the target through an RNaseH cleavage event. The single-stranded antisense RNA molecule may be about 10 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense RNA molecule may comprise a sequence that is at least about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense nucleotide sequences described herein, e.g., sequences provided in any one of Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20.

[0197] The skilled artisan will recognize that the term "RNA molecule" or "ribonucleic acid molecule" encompasses not only RNA molecules as expressed or found in nature, but also analogs and derivatives of RNA comprising one or more ribonucleotide/ribonucleoside analogs or derivatives as described herein or as known in the art. Strictly speaking, a "ribonucleoside" includes a nucleoside base and a ribose sugar, and a "ribonucleotide" is a ribonucleoside with one, two or three phosphate moieties. However, the terms "ribonucleoside" and "ribonucleotide" can be considered to be equivalent as used herein. The RNA can be modified in the nucleobase structure or in the ribose-phosphate backbone structure, *e.g.*, as described herein below. However, the molecules comprising ribonucleoside analogs or derivatives must retain the ability to form a duplex. As non-limiting examples, an RNA molecule can also include at least one modified ribonucleoside including but not limited to a 2'-O-methyl modified nucleostide, a nucleoside comprising a 5' phosphorothioate group, a terminal nucleoside linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a locked nucleoside, an abasic nucleoside, a 2'-deoxy-2'-fluoro modified nucleoside, a 2'-amino-modified nucleoside, 2'-alkyl-modified nucleoside, morpholino nucleoside, a phosphoramidate or a non-natural base comprising nucleoside, or any combination thereof. Alternatively, an RNA molecule can comprise at least two modified ribonucleosides, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20 or more, up to the entire length of the dsRNA molecule. The modifications need not be the same for each of such a plurality of modified ribonucleosides in an RNA molecule. In one embodiment, modified RNAs contemplated for use in methods and compositions described herein are peptide nucleic acids (PNAs) that have the ability to form the required duplex structure and that permit or mediate the specific degradation of a target RNA, e.g., via a RISC pathway.

[0198] In one aspect, a modified ribonucleoside includes a deoxyribonucleoside. In such an instance, an iRNA agent can comprise one or more deoxynucleosides, including, for example, a deoxynucleoside overhang(s), or one or more deoxynucleosides within the double stranded portion of a dsRNA. However, it is self evident that under no circumstances is a double stranded DNA molecule encompassed by the term "iRNA."

[0199] In one aspect, an RNA interference agent includes a single stranded RNA that interacts with a target RNA sequence to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp et al., Genes Dev. 2001, 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 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 cleaves the target to induce silencing (Elbashir, et al., (2001) Genes Dev. 15:188). Thus, in one aspect the disclosure relates to a single stranded RNA that promotes the formation of a RISC complex to effect silencing of the target gene.

[0200] 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 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 analog, including a deoxynucleotide/nucleoside. The overhang(s) may 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.

[0201] The antisense strand of a dsRNA may have a 1-10 nucleotide overhang at the 3' end and/or the 5' end. The sense strand of a dsRNA may have a 1-10 nucleotide overhang at the 3' end and/or the 5' end. One or more of the nucleotides in the overhang may be replaced with a nucleoside thiophosphate.

[0202] 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.

[0203] 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. 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, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches may 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.

[0204] 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.

[0205] As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle. A SNALP represents a vesicle of lipids coating a reduced aqueous interior comprising a nucleic acid such as an iRNA or a plasmid from which an iRNA is transcribed. SNALPs are described, *e.g.,* in U.S. Patent Application Publication Nos. 20060240093, 20070135372, and in International Application No. WO 2009082817.

[0206] "Introducing into a cell," when referring to an iRNA, means facilitating or effecting uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of an iRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells *in vitro*; an iRNA may also be "introduced into a cell," wherein the cell is part of a living organism. In such an instance, introduction into the cell will include the delivery to the organism. For example, for *in vivo* delivery, iRNA can be injected into a tissue site or administered systemically. *In vivo* delivery can also be by a β-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. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or known in the art.

[0207] As used herein, the term "modulate the expression of," refers to at an least partial "inhibition" or partial "activation" of an ALAS1 gene expression in a cell treated with an iRNA composition as described herein compared to the expression of ALAS1 in a control cell. A control cell includes an untreated cell, or a cell treated with a non-targeting control iRNA.

[0208] The terms "activate," "enhance," "up-regulate the expression of," "increase the expression of," and the like, in so far as they refer to an ALAS1 gene, herein refer to the at least partial activation of the expression of an ALAS1 gene, as manifested by an increase in the amount of ALAS1 mRNA, which may be isolated from or detected in a first cell or group of cells in which an ALAS1 gene is transcribed and which has or have been treated such that the expression of an ALAS1 gene is increased, 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).

[0209] Expression of an ALAS1 1 gene may be activated by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by administration of an iRNA as described herein. An ALAS1 gene may be activated by at least about 60%, 70%, or 80% by administration of an iRNA featured in the invention. Expression of an ALAS 1 gene may be activated by at least about 85%, 90%, or 95% or more by administration of an iRNA as described herein. The ALAS1 gene expression may be increased by at least 1-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1000 fold or more in cells treated with an iRNA as described herein compared to the expression in an untreated cell. Activation of expression by small dsRNAs is described, for example, in Li et al., 2006 Proc. Natl. Acad. Sci. U.S.A. 103:17337-42, and in US20070111963 and US2005226848.

[0210] The terms "silence," "inhibit expression of," "down-regulate expression of," "suppress expression of," and the like, in so far as they refer to an ALAS1 gene, herein refer to the at least partial suppression of the expression of an ALAS1 gene, as assessed, e.g., based on on ALAS1 mRNA expression, ALAS 1 protein expression, or another parameter functionally linked to ALAS 1 gene expression (e.g., ALA or PBG concentrations in plasma or urine). For example, inhibition of ALAS 1 expression may be manifested by a reduction of the amount of ALAS1 mRNA which may be isolated from or detected in a first cell or group of cells in which an ALAS 1 gene is transcribed and which has or have been treated such that the expression of an ALAS 1 gene is inhibited, as compared to a control. The control may be a second cell or group of cells substantially identical to the first cell or group of cells, except that the second cell or group of cells have not been so treated (control cells). The degree of inhibition is usually expressed as a percentage of a control level, *e.g.*,

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

[0211] Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to ALAS1 gene expression, e.g., the amount of protein encoded by an ALAS1 gene, or the level of one or more porphyrins. The reduction of a parameter functionally linked to ALAS1 gene expression may similarly be expressed as

a percentage of a control level. In principle, ALAS1 gene silencing may be determined in any cell expressing ALAS1, either constitutively or by genomic engineering, and by any appropriate assay. However, when a reference is needed in order to determine whether a given iRNA inhibits the expression of the ALAS1 gene by a certain degree and therefore is encompassed by the instant invention, the assays provided in the Examples below shall serve as such reference.

[0212] For example, in certain instances, expression of an ALAS1 gene is suppressed by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by administration of an iRNA featured in the invention. Described is that an ALAS 1 gene is suppressed by at least about 60%, 65%, 70%, 75%, or 80% by administration of an iRNA featured in the invention. Described is that an ALAS 1 gene is suppressed by at least about 85%, 90%, 95%, 98%, 99%, or more by administration of an iRNA as described herein.

[0213] As used herein in the context of ALAS 1 expression, the terms "treat," "treating," "treatment," and the like, refer to relief from or alleviation of pathological processes related to ALAS 1 expression (*e.g.*, pathological processes involving porphyrins or defects in the porphyrin pathway, such as, for example, porphyrias). In the context of the present invention insofar as it relates to any of the other conditions recited herein below (other than pathological processes related to ALAS 1 expression), the terms "treat," "treatment," and the like mean to prevent, relieve or alleviate at least one symptom associated with such condition, or to slow or reverse the progression or anticipated progression of such condition. For example, the methods featured herein, when employed to treat porphyria, may serve to reduce or prevent one or more symptoms associated with porphyria (e.g., pain), to reduce the severity or frequency of attacks associated with porphyria, to reduce the likelihood that an attack of one or more symptoms associated with porphyria will occur upon exposure to a precipitating condition, to shorten an attack associated with porphyria, and/or to reduce the risk of developing conditions associated with porphyria (*e.g.*, hepatocellular cancer or neuropathy (e.g., progressive neuropathy),). Thus, unless the context clearly indicates otherwise, the terms "treat," "treatment," and the like are intended to encompass prophylaxis, e.g., prevention of disorders and/or symptoms of disorders related to ALAS1 expression.

[0214] By "lower" in the context of a disease marker or symptom is meant a statistically or clinically 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, and is typically down to a level accepted as within the range of normal for an individual without such disorder.

[0215] As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of pathological processes related to ALAS 1 expression. The specific amount that is therapeutically effective can be readily determined by an ordinary medical practitioner, and may vary depending on factors known in the art, such as, for example, the type of pathological process, the patient's history and age, the stage of pathological process, and the administration of other agents.

[0216] As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of an iRNA and a pharmaceutically acceptable carrier. As used herein, "pharmacologically effective amount," "therapeutically effective amount" or simply "effective amount" refers to that amount of an iRNA effective to produce the intended pharmacological, therapeutic or preventive result. For example, in a method of treating a disorder related to ALAS 1 expression (e.g., in a method of treating a porphyria), an effective amount includes an amount effective to reduce one or more symptoms associated with a porphyria, an amount effective to reduce the frequency of attacks, an amount effective to reduce the likelihood that an attack of one or more symptoms associated with porphyria will occur upon exposure to a precipitating factor, or an amount effective to reduce the risk of developing conditions associated with porphyria (*e.g.*, neuropathy (e.g., progressive neuropathy), hepatocellular cancer). For example, if a given clinical treatment is considered effective when there is at least a 10% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to effect at least a 10% reduction in that parameter. For example, a therapeutically effective amount of an iRNA targeting ALAS1 can reduce ALAS1 protein levels by any measurable amount, e.g., by at least 10%, 20%, 30%, 40% or 50%.

[0217] The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The term specifically excludes cell culture medium. For drugs administered orally, pharmaceutically acceptable carriers include, but are not limited to pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract. Agents included in drug formulations are described further herein below.

[0218] The term "about" when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary from, for example, between 1% and 15% of the stated number or numerical range.

II. Double-stranded ribonucleic acid (dsRNA)

[0219] Described herein are iRNA agents that inhibit the expression of an ALAS1 gene. The iRNA agent may include double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of an ALAS1 1 gene in a cell or in a subject (*e.g.*, in a mammal, *e.g.*, in a human having a porphyria), where the dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of an ALAS 1 gene, and where the region of complementarity is 30 nucleotides or less in length, generally 19-24 nucleotides in length, and where the dsRNA, upon contact with a cell expressing the ALAS 1 gene, inhibits the expression of the ALAS 1 gene by at least 10% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by Western blot. The iRNA agent may activate the expression of an ALAS1 gene in a cell or mammal. Expression of an ALAS1 gene in cell culture, such as in COS cells, HeLa cells, primary hepatocytes, HepG2 cells, primary cultured cells or in a biological sample from a subject can be assayed by measuring ALAS1 mRNA levels, such as by bDNA or TaqMan assay, or by measuring protein levels, such as by immunofluorescence analysis, using, for example, Western Blotting or flow cytometric techniques.

[0220] A dsRNA includes two RNA strands that are sufficiently complementary to 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, derived from the sequence of an mRNA formed during the expression of an ALAS 1 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. Generally, the duplex structure is between 15 and 30 inclusive, more generally between 18 and 25 inclusive, yet more generally between 19 and 24 inclusive, and most generally between 19 and 21 base pairs in length, inclusive. Similarly, the region of complementarity to the target sequence is between 15 and 30 inclusive, more generally between 18 and 25 inclusive, yet more generally between 19 and 24 inclusive, and most generally between 19 and 21 nucleotides in length, inclusive. The dsRNA may be between 15 and 20 nucleotides in length or between 25 and 30 nucleotides in length, inclusive. As the ordinarily skilled person will recognize, the targeted 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 be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway). dsRNAs having duplexes as short as 9 base pairs can, under some circumstances, mediate RNAi-directed RNA cleavage. Most often a target will be at least 15 nucleotides in length, *e.g.,* 15-30 nucleotides in length.

[0221] 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 9 to 36, *e.g.,* 15-30 base pairs. Thus, 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, then, an miRNA is a dsRNA. A dsRNA may not be a naturally occurring miRNA. An iRNA agent useful to target ALAS 1 expression may not be generated in the target cell by cleavage of a larger dsRNA.

[0222] A dsRNA as described herein may further include one or more single-stranded nucleotide overhangs. The 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, Biosearch, Applied Biosystems, Inc. An ALAS 1 gene may be a human ALAS 1 gene. The ALAS1 gene may be a mouse or a rat ALAS1 gene. The first sequence may be a sense strand of a dsRNA that includes a sense sequence from Table 2 or Table 3, and the second sequence is an antisense strand of a dsRNA that includes an antisense sequence from Table 2 or Table 3. The first sequence may be a sense strand of a dsRNA that includes a sense sequence from Table 2, 3, 6, 7, 8, 9, 14, or 15, and the second sequence may be an antisense strand of a dsRNA that includes an antisense sequence from Table 2, 3, 6, 7, 8, 9, 14, or 15. The first sequence may be a sense strand of a dsRNA that includes a sense sequence from Table 2, 3, 6, 7, 8, 9, 14, 15, 18 or 20, and the second sequence may be an antisense strand of a dsRNA that includes an antisense sequence from Table 2, 3, 6, 7, 8, 9, 14, 15, 18 or 20. Alternative dsRNA agents that target sequences other than those of the dsRNAs disclosed herein (e.g. in Table 2 or Table 3) can readily be determined using the target sequence and the flanking ALAS1 sequence.

[0223] In one aspect, a dsRNA will include at least sense and antisense nucleotide sequences, whereby the sense strand is selected from the groups of sequences provided in Tables 2 and 3, and the corresponding antisense strand of the sense strand is selected from Tables 2 and 3. In a further aspect, a dsRNA will include at least sense and antisense nucleotide sequences, whereby the sense strand is selected from the groups of sequences provided in Tables 2, 3, 6, 7, 8, 9, 14, and 15, and the corresponding antisense strand of the sense strand is selected from Tables 2, 3, 6, 7, 8, 9, 14, and 15. In a further aspect, a dsRNA will include at least sense and antisense nucleotide sequences, whereby the sense strand is selected from the groups of sequences provided in Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20, and the corresponding antisense strand of the sense strand is selected from Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20. In these aspects, 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 by the expression of an ALAS 1 gene gene. As such, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in Table 2, 3, 6, 7, 8, 9, 14, 15, 18 or 20, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand from 2, 3, 6, 7, 8, 9, 14, 15, 18 or 20. 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 to being on separate oligonucleotides.

[0224] The skilled person is well aware that dsRNAs having a duplex structure of between 20 and 23, but specifically 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir et al., EMBO 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can be effective as well. In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having one of the sequences of Table 2, 3, 6, 7, 8, 9, 14, 15, 18 or 20 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Table 2, 3, 6, 7, 8, 9, 14, 15, 18 or 20, and differing in their ability to inhibit the expression of an ALAS 1 gene by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated.

[0225] In addition, the RNAs provided in Tables 2 and 3, as well as the RNAs provided in Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20, identify a site in an ALAS1 transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within one of such sequences. 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 15 contiguous nucleotides from one of the sequences provided in Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in an ALAS 1 gene.

[0226] While a target sequence is generally 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 may 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, for example, in Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20, 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.

[0227] Further, it is contemplated that for any sequence identified, *e.g.*, in Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 and 20, further optimization can be achieved by systematically either adding or removing nucleotides to generate longer or shorter sequences and testing those and 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 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, *etc.*) as an expression inhibitor.

[0228] An iRNA 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 not be 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 RNA strand which is complementary to a region of an ALAS 1 gene, the RNA strand 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 an ALAS 1 gene. Consideration of the efficacy of iRNAs with mismatches in

inhibiting expression of an ALAS1 gene is important, especially if the particular region of complementarity in an ALAS1 gene is known to have polymorphic sequence variation within the population.

[0229] At least one end of a dsRNA may have a single-stranded nucleotide overhang of 1 to 4, generally 1 or 2 nucleotides. dsRNAs having at least one nucleotide overhang have unexpectedly superior inhibitory properties relative to their blunt-ended counterparts. The RNA of an iRNA, *e.g.,* a dsRNA, may be chemically modified to enhance stability or other beneficial characteristics. The nucleic acids featured in the invention may 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. Modifications include, for example, (a) end modifications, *e.g.,* 5' end modifications (phosphorylation, conjugation, inverted linkages, *etc.*) 3' end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*), (b) 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, (c) sugar modifications (*e.g.*, at the 2' position or 4' position) or replacement of the sugar, as well as (d) backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of RNA compounds useful in this invention 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. The modified RNA may have a phosphorus atom in its internucleoside backbone.

[0230] 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.

[0231] Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. 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.

[0232] 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 CH_2 component parts.

[0233] Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. 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.

[0234] In other RNA mimetics suitable or contemplated for use in iRNAs, 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. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262. Further teaching of PNA compounds can be found, for example, in Nielsen et al., Science, 1991, 254, 1497-1500.

[0235] Described are RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH2--NH--CH2--, --CH_2--N(CH_3)--O--CH_2--[known as a methylene (methylimino) or MMI backbone], --CH_2--O--N(CH_3)--CH_2--, --CH_2--N(CH_3)-N(CH_3)--CH_2-- and --N(CH_3)--CH_2--CH_2--[wherein the native phosphodiester backbone is represented as --O--P--O--CH_2--] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

[0236] Modified RNAs may 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 may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. dsRNAs may include one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an iRNA, or a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. The modification may include a 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also know 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--CH_2--O--CH_2--$N(CH_2)_2$, also described in examples herein below.

[0237] Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may 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 may 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.

[0238] An iRNA may 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 anal 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., Angewandte Chemie, International Edition, 1991, 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-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 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 substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

[0239] 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. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,30; 5,134,066; 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; 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, and U.S. Pat. No. 5,750,692.

[0240] 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 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).

[0241] Representative U.S. Patents that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Pat. Nos. 6,268,490; 6,670,461; 6,794,499; 6,998,484; 7,053,207; 7,084,125; and 7,399,845.

[0242] Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hy-

droxyprolinol (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.

iRNA Motifs

[0243] Described is that the sense strand sequence may be represented by formula (I):

$$5' \; n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3' \qquad (I)$$

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

each N_a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p and n_q independently represent an overhang nucleotide;

wherein Nb and Y do not have the same modification; and

XXX, YYY and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. Preferably YYY is all 2'-F modified nucleotides.

[0244] Described is that the N_a and/or N_b comprise modifications of alternating pattern.

[0245] Described is that the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at positions 6, 7, 8; 7, 8, 9; 8, 9, 10; 9, 10, 11; 10, 11,12 or 11, 12, 13) of - the sense strand, the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end.

[0246] Described is that i is 1 and j is 0, or i is 0 and j is 1, or both i and j are 1. The sense strand can therefore be represented by the following formulas:

$$5' \; n_p\text{-}N_a\text{-}YYY\text{-}N_b\text{-}ZZZ\text{-}N_a\text{-}n_q\ 3' \qquad (Ib);$$

$$5' \; n_p\text{-}N_a\text{-}XXX\text{-}N_b\text{-}YYY\text{-}N_a\text{-}n_q\ 3' \qquad (Ic);$$

or

$$5' \; n_p\text{-}N_a\text{-}XXX\text{-}N_b\text{-}YYY\text{-}N_b\text{-}ZZZ\text{-}N_a\text{-}n_q\ 3' \qquad (Id).$$

[0247] When the sense strand is represented by formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0248] When the sense strand is represented as formula (Ic), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0249] When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0250] Each of X, Y and Z may be the same or different from each other.

[0251] Described is that i is 0 and j is 0, and the sense strand may be represented by the formula:

5' n_p-N_a-YYY-N_a-n_q 3' (Ia).

[0252] When the sense strand is represented by formula (Ia), each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0253] Described is that the antisense strand sequence of the RNAi may be represented by formula (II):

5' $n_{q'}$-N_a'-(Z'Z'Z')$_k$-N_b'-Y'Y'Y'-N_b'-(X'X'X')$_1$-N'$_a$-n_p' 3' (II)

wherein:

k and 1 are each independently 0 or 1;

p' and q' are each independently 0-6;

each N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p' and n_q' independently represent an overhang nucleotide;

wherein N_b' and Y' do not have the same modification;

and

X'X'X', Y'Y'Y' and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

[0254] Described is that the N_a' and/or N_b' comprise modifications of alternating pattern.

[0255] The Y'Y'Y' motif occurs at or near the cleavage site of the antisense strand. For example, when the RNAi agent has a duplex region of 17-23nucleotidein length, the Y'Y'Y' motif can occur at positions 9, 10, 11; 10, 11, 12; 11, 12, 13; 12, 13, 14 ; or 13, 14, 15 of the antisense strand, with the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end. Preferably, the Y'Y'Y' motif occurs at positions 11, 12, 13.

[0256] Described is that Y'Y'Y' motif is all 2'-OMe modified nucleotides.

[0257] Described is that k is 1 and 1 is 0, or k is 0 and 1 is 1, or both k and 1 are 1.

[0258] The antisense strand can therefore be represented by the following formulas:

5' n_q-N_a'-Z'Z'Z'-N_b'-Y'Y'Y'-N_a'-$n_{p'}$ 3' (IIb);

5' n_q'-N_a'-Y'Y'Y'-N_b'-X'X'X'-$n_{p'}$ 3' (IIc);

or

5' $n_{q'}$-N_a'- Z'Z'Z'-N_b'-Y'Y'Y'-N_b'- X'X'X'-N_a'-$n_{p'}$ 3' (IId).

[0259] When the antisense strand is represented by formula (IIb), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0260] When the antisense strand is represented as formula (IIc), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0261] When the antisense strand is represented as formula (IId), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6.

[0262] Described is that k is 0 and 1 is 0 and the antisense strand may be represented by the formula:

5' $n_{p'}$-$N_{a'}$-Y'Y'Y'- $N_{a'}$-$n_{q'}$ 3' (Ia).

[0263] When the antisense strand is represented as formula (IIa), each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0264] Each of X', Y' and Z' may be the same or different from each other.

[0265] Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X, Y, Z, X', Y' and Z', in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

[0266] Described is that the sense strand of the RNAi agent may contain YYY motif occurring at 9, 10 and 11 positions of the strand when the duplex region is 21 nt, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end; and Y represents 2'-F modification. The sense strand may additionally contain XXX motif or ZZZ motifs as wing modifications at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-OMe modification or 2'-F modification.

[0267] Described is that the antisense strand may contain Y'Y'Y' motif occurring at positions 11, 12, 13 of the strand, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end; and Y' represents 2'-O-methyl modification. The antisense strand may additionally contain X'X'X' motif or Z'Z'Z' motifs as wing modifications at the opposite end of the duplex region; and X'X'X' and Z'Z'Z' each independently represents a 2'-OMe modification or 2'-F modification.

[0268] The sense strand represented by any one of the above formulas (Ia), (Ib), (Ic), and (Id) forms a duplex with a antisense strand being represented by any one of formulas (IIa), (IIb), (IIc), and (IId), respectively.

[0269] Accordingly, the RNAi agents for use in the described methods may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the RNAi duplex represented by formula (III):

$$\text{sense: } 5' \; n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}\ Y\ Y\ Y\ \text{-}N_b\ \text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3' \text{ antisense: } 3' \; n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_1\text{-}N_a'\text{-}n_q'\ 5' \qquad \text{(III)}$$

wherein:

i, j, k, and 1 are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

wherein

each n_p', n_p, n_q', and n_q, each of which may or may not be present, independently represents an overhang nucleotide; and

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

[0270] Described is that i is 0 and j is 0; or i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 0; or both i and j are 1. Described is that k is 0 and 1 is 0; or k is 1 and 1 is 0; k is 0 and 1 is 1; or both k and 1 are 0; or both k and 1 are 1.

[0271] Exemplary combinations of the sense strand and antisense strand forming a RNAi duplex include the formulas below:

$$5' \; n_p\text{-}\ N_a\ \text{-}Y\ Y\ Y\ \text{-}N_a\text{-}n_q\ 3'\ 3'\ n_p'\text{-}N_a'\text{-}Y'Y'Y'\ \text{-}N_a'n_q'\ 5' \qquad \text{(IIIa)}$$

$$5' \; n_p\text{-}N_a\text{-}Y\ Y\ Y\ \text{-}N_b\ \text{-}Z\ Z\ Z\ \text{-}N_a\text{-}n_q\ 3'\ 3'\ n_p'\text{-}N_a'\text{-}Y'Y'Y'\text{-}N_b'\text{-}Z'Z'Z'\text{-}N_a'n_q'\ 5' \qquad \text{(IIIb)}$$

$$5' \; n_p\text{-}N_a\text{-}\ X\ X\ X\ \text{-}N_b\ \text{-}Y\ Y\ Y\ \text{-}\ N_a\text{-}n_q\ 3'\ 3'\ n_p'\text{-}N_a'\text{-}X'X'X'\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_a'\text{-}n_q'\ 5' \qquad \text{(IIIc)}$$

$$5' \; n_p\text{-}N_a\text{-}X\ X\ X\ \text{-}N_b\text{-}Y\ Y\ Y\ \text{-}N_b\text{-}\ Z\ Z\ Z\ \text{-}N_a\text{-}n_q\ 3'\ 3'\ n_p'\text{-}N_a'\text{-}X'X'X'\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}Z'Z'Z'\text{-}N_a\text{-}n_q'\ 5' \qquad \text{(IIId)}$$

[0272] When the RNAi agent is represented by formula (IIIa), each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0273] When the RNAi agent is represented by formula (IIIb), each N_b independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5 or 1-4 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0274] When the RNAi agent is represented as formula (IIIc), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

[0275] When the RNAi agent is represented as formula (IIId), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0modified nucleotides. Each N_a, N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of N_a, N_a', N_b and N_b' independently comprises modifications of alternating pattern.

[0276] Each of X, Y and Z in formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) may be the same or different from each other.

[0277] When the RNAi agent is represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), at least one of the Y nucleotides may form a base pair with one of the Y' nucleotides. Alternatively, at least two of the Y nucleotides form base pairs with the corresponding Y' nucleotides; or all three of the Y nucleotides all form base pairs with the corresponding Y' nucleotides.

[0278] When the RNAi agent is represented by formula (IIIb) or (IIId), at least one of the Z nucleotides may form a base pair with one of the Z' nucleotides. Alternatively, at least two of the Z nucleotides form base pairs with the corresponding Z' nucleotides; or all three of the Z nucleotides all form base pairs with the corresponding Z' nucleotides.

[0279] When the RNAi agent is represented as formula (IIIc) or (IIId), at least one of the X nucleotides may form a base pair with one of the X' nucleotides. Alternatively, at least two of the X nucleotides form base pairs with the corresponding X' nucleotides; or all three of the X nucleotides all form base pairs with the corresponding X' nucleotides.

[0280] Described is that the modification on the Y nucleotide is different than the modification on the Y' nucleotide, the modification on the Z nucleotide is different than the modification on the Z' nucleotide, and/or the modification on the X nucleotide is different than the modification on the X' nucleotide.

[0281] Described is that when the RNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications. Described is that when the RNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications and n_p' >0 and at least one n_p' is linked to a neighboring nucleotide a via phosphorothioate linkage. Described is that when the RNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications, n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker. Described is that when the RNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications , n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

[0282] Described is that when the RNAi agent is represented by formula (IIIa), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications , n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

[0283] Described is that the RNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

[0284] Described is that the RNAi agent is a multimer containing three, four, five, six or more duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

[0285] Described is that two RNAi agents represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends and are optionally conjugated to to a ligand. Each of the agents can target the same gene or two different genes; or each of the agents can target same gene at two different target sites.

iRNA Conjugates

[0286] The iRNA agents disclosed herein can be in the form of conjugates. The conjugate may be attached at any suitable location in the iRNA molecule, e.g., at the 3' end or the 5' end of the sense or the antisense strand. The conjugates are optionally attached via a linker.

[0287] Described is that an iRNA agent described herein is chemically linked to one or more ligands, moieties or

conjugates, which may confer functionality, e.g., by affecting (e.g., enhancing) 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., Proc. Natl. Acid. Sci. USA, 1989, 86: 6553-6556), cholic acid (Manoharan et al., Biorg. Med. Chem. Let., 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660:306-309; Manoharan et al., Biorg. Med. Chem. Let., 1993, 3:2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J, 1991, 10:1111-1118; Kabanov et al., FEBS Lett., 1990, 259:327-330; Svinarchuk et al., Biochimie, 1993, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36:3651-3654; Shea et al., Nucl. Acids Res., 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14:969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36:3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277:923-937).

[0288] Described is that a ligand alters the distribution, targeting or lifetime of an iRNA agent into which it is incorporated. Described is that 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. Typical ligands will not take part in duplex pairing in a duplexed nucleic acid.

[0289] 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 or hyaluronic acid); or a lipid. The ligand may 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-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic 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 α helical peptide.

[0290] 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, multivalent galactose, N-acetylgalactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, biotin, or an RGD peptide or RGD peptide mimetic.

[0291] Described is that the ligand is a GalNAc ligand that comprises one or more N-acetylgalactosamine (GalNAc) derivatives. Additional description of GalNAc ligands is provided in the section titled Carbohydrate Conjugates.

[0292] 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,03-(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.

[0293] 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 cancer cell, endothelial cell, or bone cell. Ligands may 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-κB.

[0294] 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.

[0295] Described is that 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.

[0296] 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.

[0297] 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 Biosystems (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.

[0298] 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.

[0299] 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.

Lipid Conjugates

[0300] The ligand may be a lipid or lipid-based molecule. Such a lipid or lipid-based molecule can typically bind a serum protein, such as 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 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.

[0301] A lipid based ligand can be used to modulate, *e.g.*, control (*e.g.*, inhibit) the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA 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.

[0302] The lipid based ligand may bind HSA. For example, the ligand can bind HSA with a sufficient affinity such that distribution of the conjugate to a non-kidney tissue is enhanced. However, the affinity is typically not so strong that the HSA-ligand binding cannot be reversed.

[0303] The lipid based ligand may bind HSA weakly or not at all, such that distribution of the conjugate to the kidney is enhanced. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid based ligand.

[0304] 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 exemplary vitamins include are B vitamin, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by cancer cells. Also included are HSA and low density lipoprotein (LDL).

Cell Permeation Agents

[0305] In another aspect, the ligand is a cell-permeation agent, such as a helical cell-permeation agent. In one embodiment, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. 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 typically an α-helical agent, and can have a lipophilic and a lipophobic phase.

[0306] 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 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.

[0307] 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 MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO:3367). An RFGF analogue (e.g., amino acid sequence AALLPVLLAAP (SEQ ID NO:3368)) 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 membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO:3369)) and the *Drosophila Antennapedia* protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 3370)) 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) combinatorial library (Lam et al., Nature, 354:82-84, 1991). Typically, the peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit is a cell targeting peptide such as 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 direct conformational properties. Any of the structural modifications described below can be utilized.

[0308] 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 peptidiomimemtics may 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.

[0309] An RGD peptide moiety can be used to target a particular cell type, e.g., a tumor cell, such as an endothelial tumor cell or a breast cancer tumor cell (Zitzmann et al., Cancer Res., 62:5139-43, 2002). An RGD peptide can facilitate targeting of an dsRNA agent to tumors of a variety of other tissues, including the lung, kidney, spleen, or liver (Aoki et al., Cancer Gene Therapy 8:783-787, 2001). Typically, the RGD peptide will facilitate targeting of an iRNA agent to the kidney. The RGD peptide can be linear or cyclic, and can be modified, *e.g.*, glycosylated or methylated to facilitate targeting to specific tissues. For example, a glycosylated RGD peptide can deliver a iRNA agent to a tumor cell expressing $\alpha_V\beta_3$ (Haubner et al., Jour. Nucl. Med., 42:326-336, 2001).

[0310] 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, an 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).

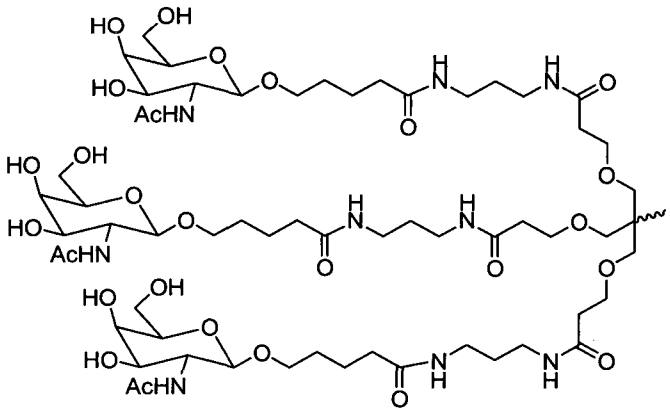
Carbohydrate Conjugates

[0311] Related to the compositions and methods of the invention, an iRNA oligonucleotide may further comprise 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).

[0312] Described is that a carbohydrate conjugate comprises a monosaccharide. Described is that the monosaccharide is an N-acetylgalactosamine (GalNAc). GalNAc conjugates are described, for example, in U.S. Patent No. 8,106,022. Described is that the GalNAc conjugate serves as a ligand that targets the iRNA to particular cells. In some embodiments, the GalNAc conjugate targets the iRNA to liver cells, e.g., by serving as a ligand for the asialoglycoprotein receptor of liver cells (e.g., hepatocytes).

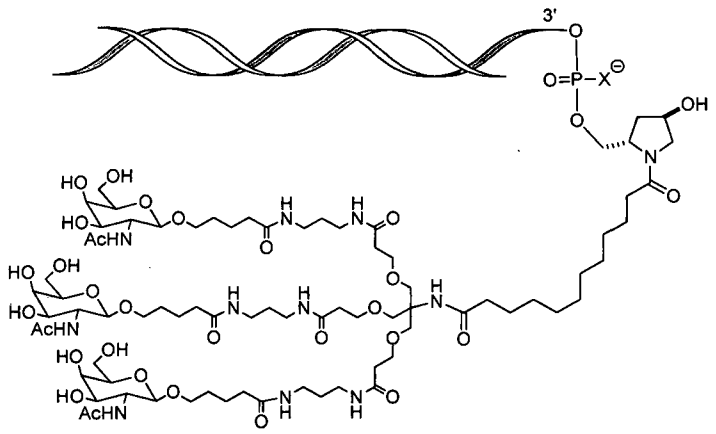
[0313] Described is that the carbohydrate conjugate comprises one or more GalNAc derivatives. The GalNAc derivatives may be attached via a linker, e.g., a bivalent or trivalent branched linker. Described is that the GalNAc conjugate is conjugated to the 3' end of the sense strand. Described is that the GalNAc conjugate is conjugated to the iRNA agent (e.g., to the 3' end of the sense strand) via a linker, e.g., a linker as described herein.

[0314] Described is that the GalNAc conjugate is

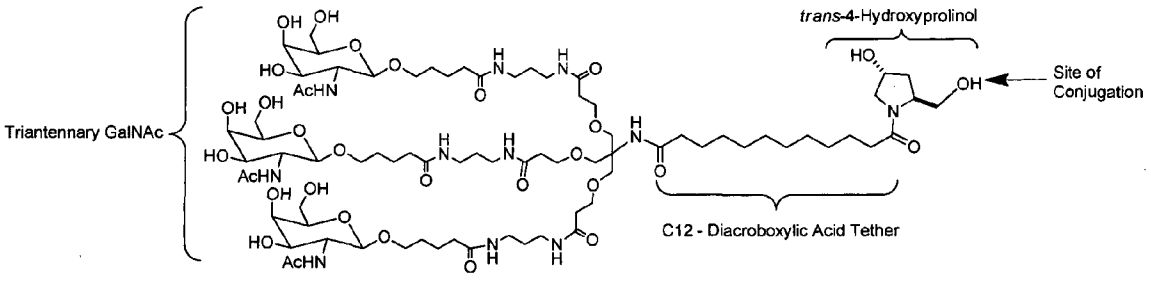


Formula II.

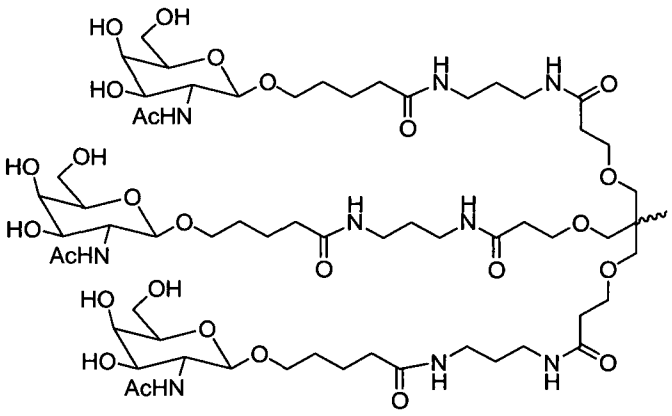
[0315] Described is that the RNAi agent is attached to the carbohydrate conjugate via a linker as shown in the following schematic, wherein X is O or S



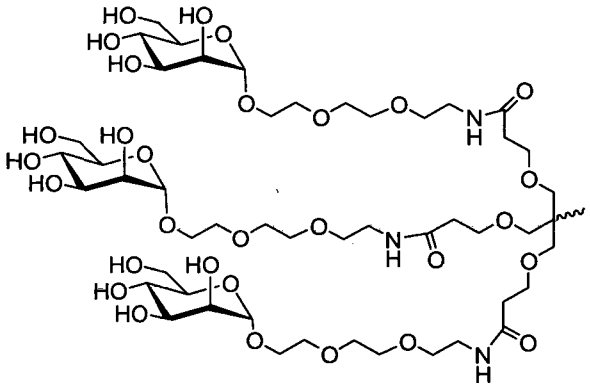
[0316] In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below



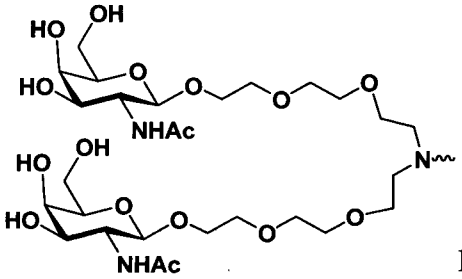
[0317] A carbohydrate conjugate for use in the compositions and methods may be selected from the group consisting of:



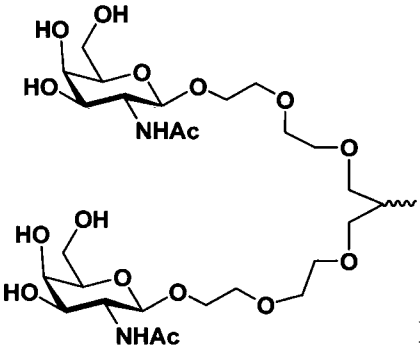
Formula II,



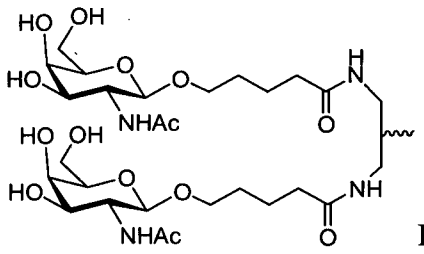
Formula III,



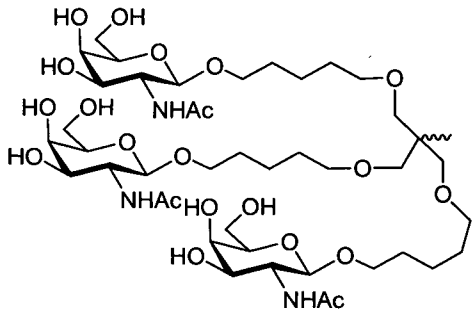
Formula IV,



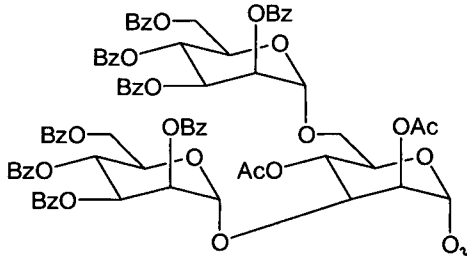
Formula V,



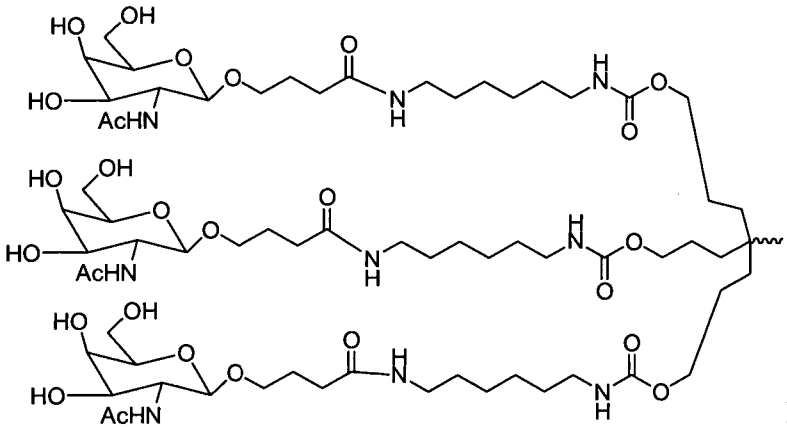
Formula VI,



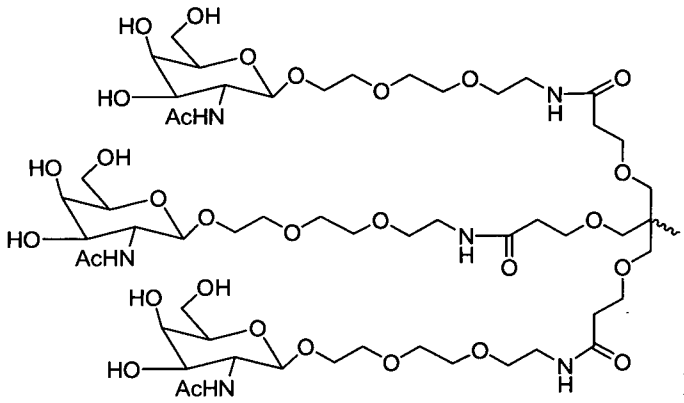
Formula VII,



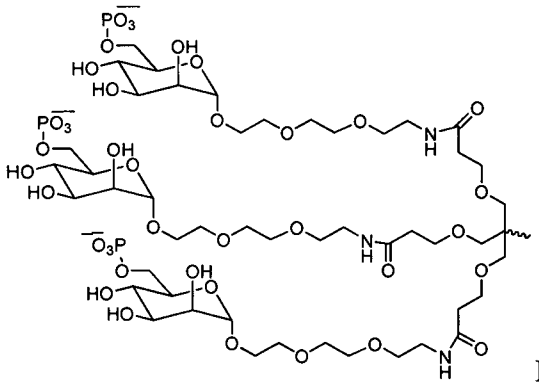
Formula VIII,



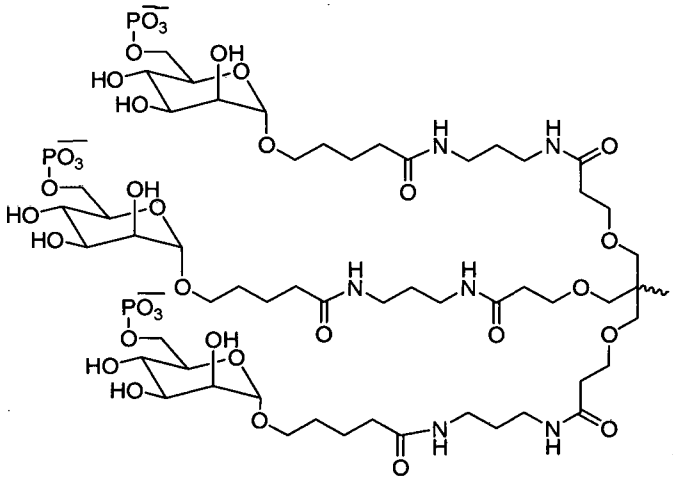
Formula IX,



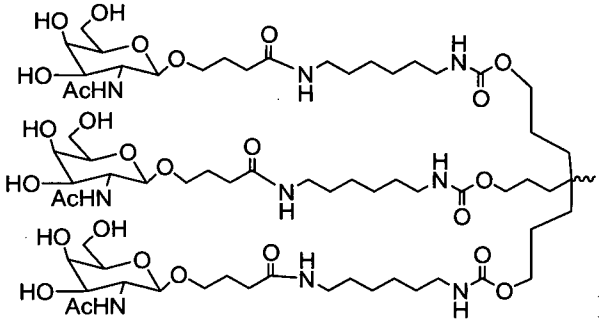
Formula X,



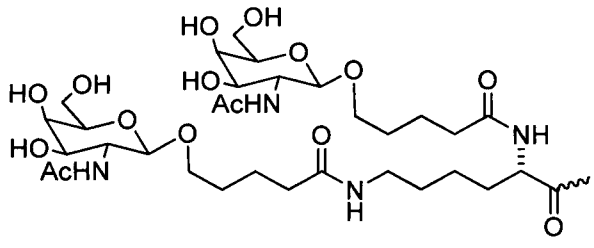
Formula XI,



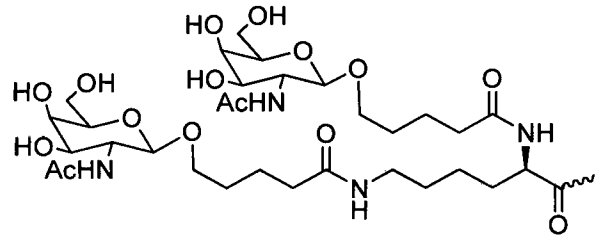
Formula XII,



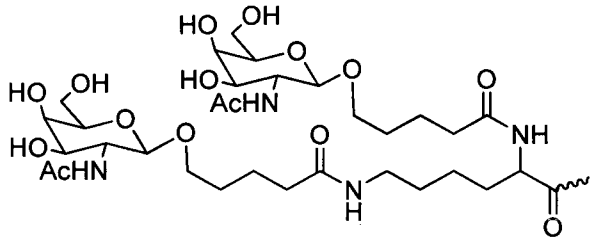
Formula XIII,



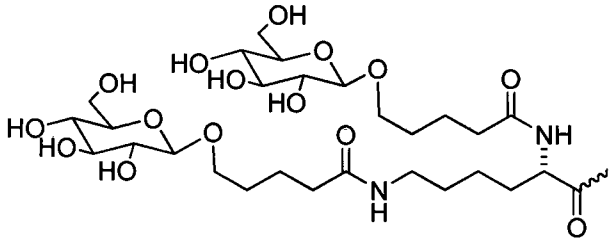
Formula XIV,



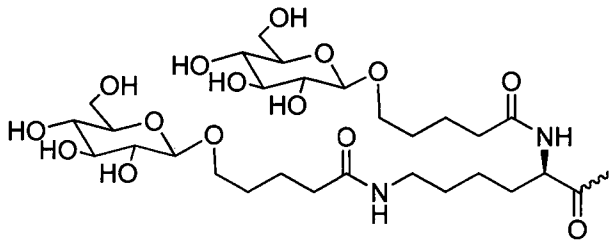
Formula XV,



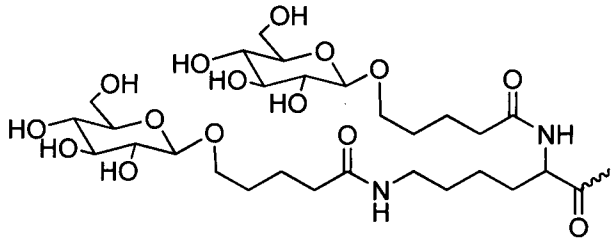
Formula XVI,



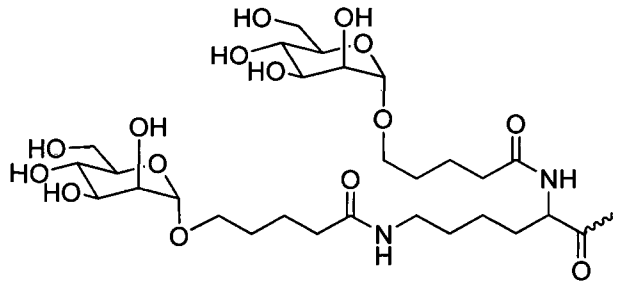
Formula XVII,



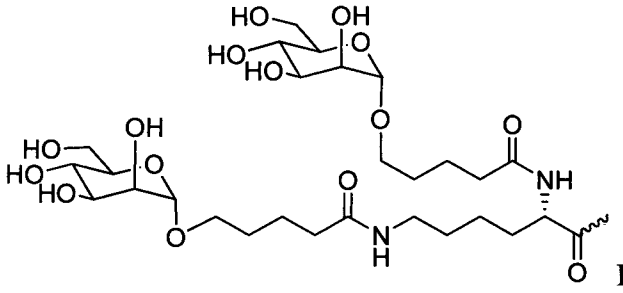
Formula XVIII,



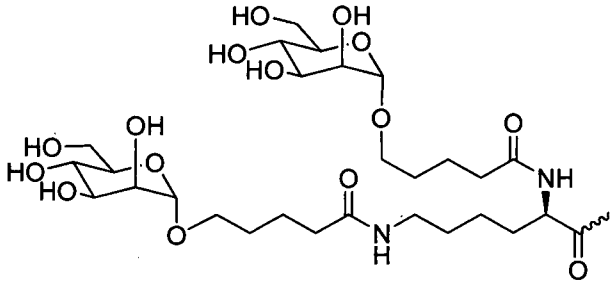
Formula XIX,



Formula XX,

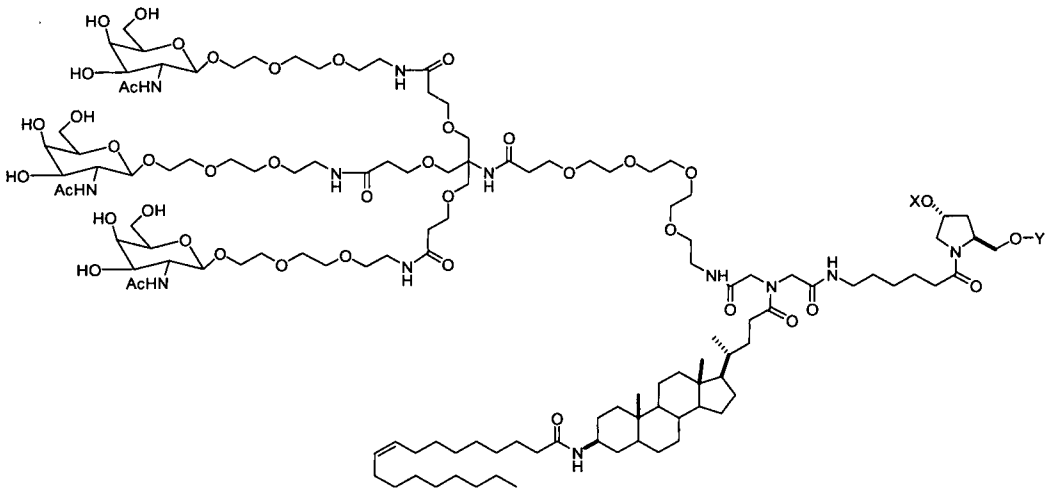


Formula XXI,



Formula XXII.

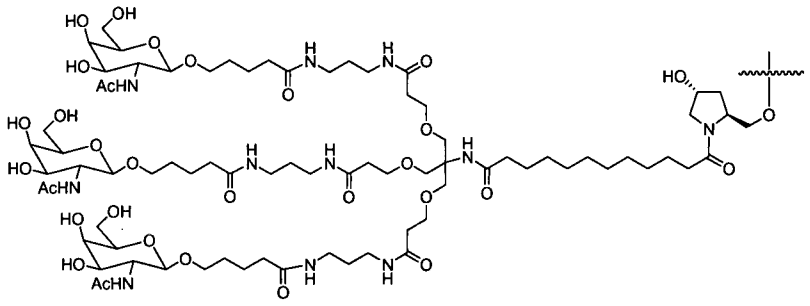
[0318] Another representative carbohydrate conjugate includes, but is not limited to,



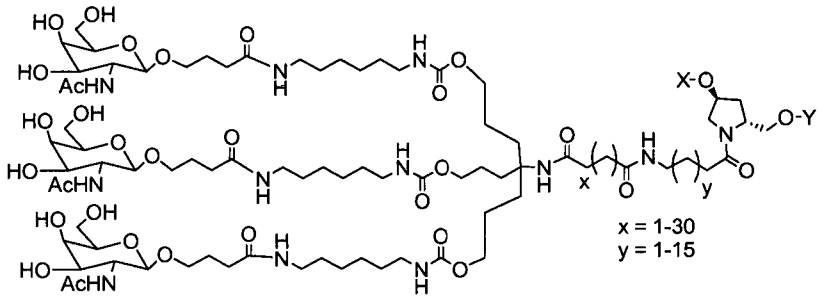
(Formula XXIII), when one of X or Y is an oligonucleotide, the other is a hydrogen.

[0319] Described is that 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.

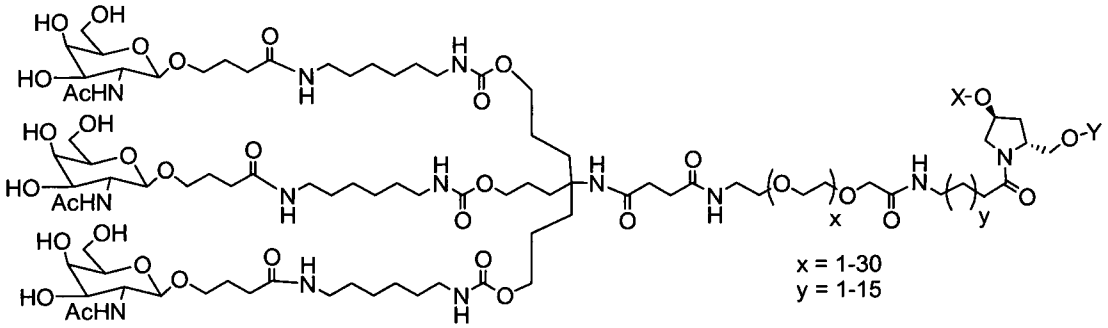
[0320] Described is that an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



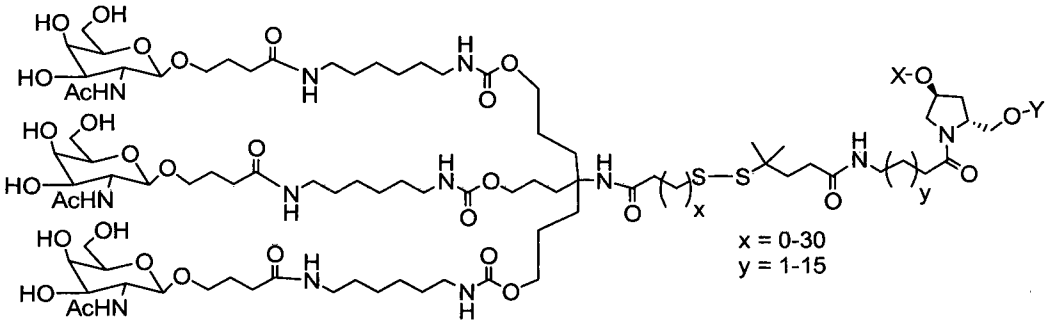
(Formula XXIV),



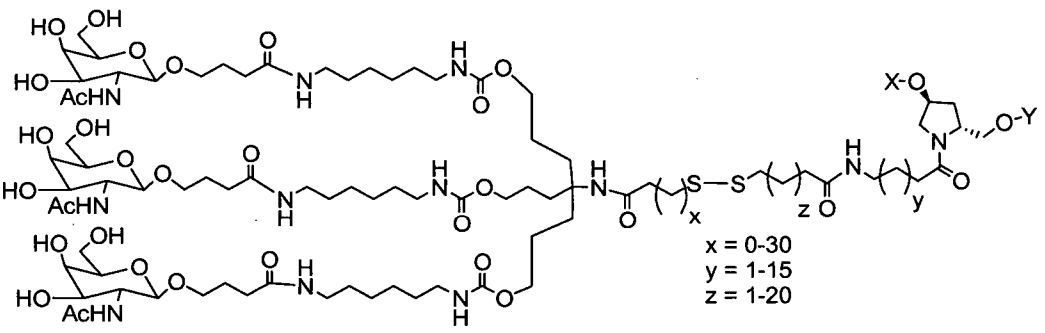
(Formula XXV),



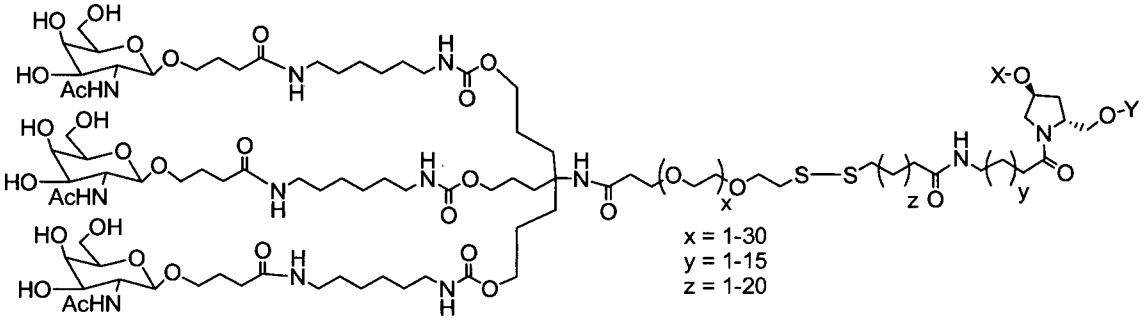
(Formula XXVI),



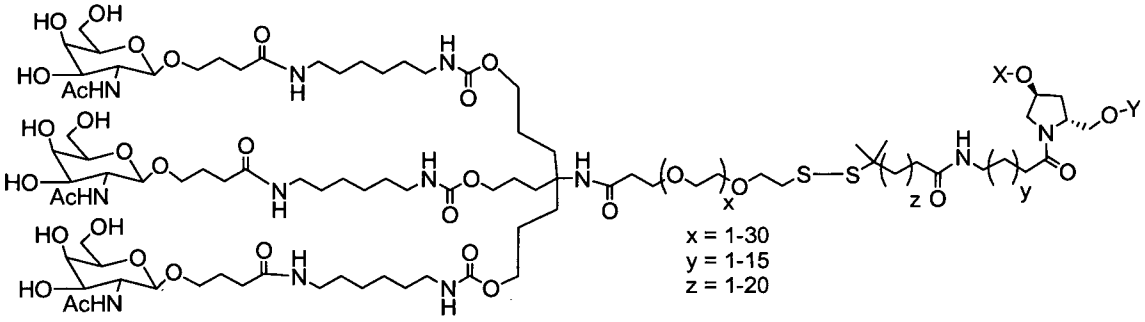
(Formula XXVII),



(Formula XXVIII),



(Formula XXIX), and



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

Linkers

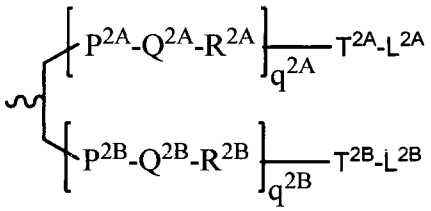
[0321] Described is that the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

[0322] 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, SO_2, SO_2NH 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, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynyl-

heterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, 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. Described is that 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, 7-16, or 8-16 atoms.

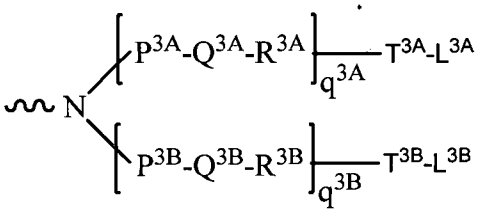
[0323] Described is that a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XXXI) - (XXXIV):

Formula XXXI

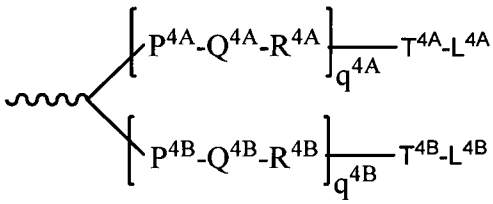


,

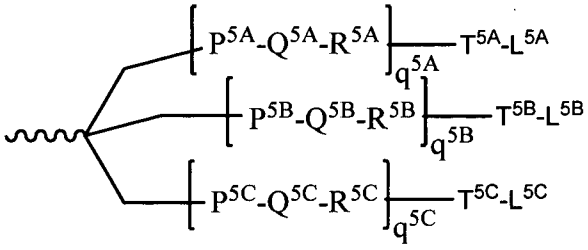
Formula XXXII



,



Formula XXXIII



;

Formula XXXIV

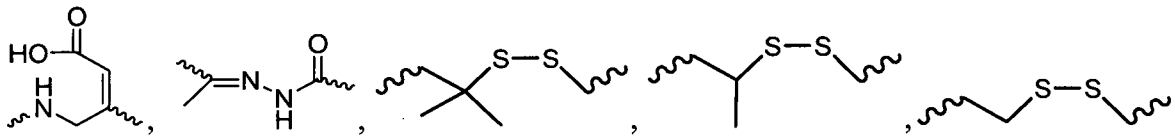
wherein:

q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C 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^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or CH_2O;

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 wherin one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^N)$, C(R')=C(R"), C=C or C(O);

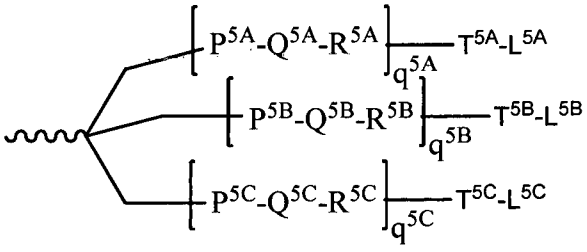
R^{2A}, R^{2B}, R^{3a}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, -C(O)-$CH(R^a)$-NH-, CO, CH=N-O,



or heterocyclyl;

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 (XXXV):

Formula XXXV



,

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

[0324] 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.

[0325] 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).

[0326] 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 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.

[0327] 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 lipid from the ligand inside the cell, or into the desired compartment of the cell.

[0328] 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 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.

[0329] Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

[0330] 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 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).

Redox cleavable linking groups

[0331] Described is that 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 know 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. Candidate compounds may be cleaved by at most about 10% in the blood. Described is that 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.

Phosphate-based cleavable linking groups

[0332] Described is that 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-. Preferred is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

Acid cleavable linking groups

[0333] Described is that a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. Described is that 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.

Ester-based cleavable linking groups

[0334] Described is that 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.

Peptide-based cleavable linking groups

[0335] Described is that 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 alkynelene. 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 - NHCHRAC(O)NHCHRBC(O)- (SEQ ID NO: 13), 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.

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.

[0336] 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 may 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.

[0337] "Chimeric" iRNA compounds, or "chimeras," in the context of the present invention, are iRNA compounds, *e.g.*, dsRNAs, that 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 may serve as a substrate for enzymes capable of 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 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.

[0338] 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 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., 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. Let., 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), 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 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 aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction may 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.

Delivery of iRNA

[0339] The delivery of an iRNA to a subject in need thereof can be achieved in a number of different ways. *In vivo* delivery can be performed directly by administering a composition comprising an iRNA, *e.g.* a dsRNA, to a subject. Alternatively, delivery can be performed indirectly by administering one or more vectors that encode and direct the expression of the iRNA. These alternatives are discussed further below.

Direct delivery

[0340] In general, any method of delivering a nucleic acid molecule can be adapted for use with an iRNA (see *e.g.*, Akhtar S. and Julian RL. (1992) Trends Cell. Biol. 2(5):139-144 and WO94/02595). However, there are three factors that are important to consider in order to successfully deliver an iRNA molecule *in vivo*: (a) biological stability of the delivered molecule, (2) preventing non-specific effects, and (3) 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 (as a non-limiting example, a tumor) or topically administering the preparation. Local admin-

istration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that may otherwise be harmed by the agent or that may 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*.

[0341] Modification of the 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 other groups, e.g., a lipid or carbohydrate group as described herein. Such conjugates can be used to target iRNA to particular cells, e.g., liver cells, e.g., hepatocytes. For example, GalNAc conjugates or lipid (e.g., LNP) formulations can be used to target iRNA to particular cells, e.g., liver cells, e.g., hepatocytes.

[0342] 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 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 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 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-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; Yoo, H., et al (1999) Pharm. Res. 16:1799-1804). An iRNA may form 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.

Vector encoded iRNAs

[0343] In another aspect, iRNA targeting the ALAS1 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., Proc. Natl. Acad. Sci. USA (1995) 92:1292).

[0344] 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. A dsRNA may be expressed

as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

[0345] An iRNA expression vector is typically a DNA plasmid or viral vector. An expression vector compatible with eukaryotic cells, *e.g.*, 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 contain 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.

[0346] An iRNA expression plasmid can be transfected into a target cell as a complex with a cationic lipid carrier (*e.g.*, Oligofectamine) or a non-cationic lipid-based carrier (*e.g.*, Transit-TKO™). Multiple lipid transfections for iRNA-mediated knockdowns targeting different regions of a target RNA over a period of a week or more are also contemplated by the invention. Successful introduction of vectors into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection of cells *ex vivo* can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (*e.g.*, antibiotics and drugs), such as hygromycin B resistance.

[0347] 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) SV40 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 may 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 further described below.

[0348] Vectors useful for the delivery of an iRNA will include regulatory elements (promoter, enhancer, *etc.*) sufficient for expression of the iRNA in the desired target cell or tissue. The regulatory elements can be chosen to provide either constitutive or regulated/inducible expression.

[0349] Expression of the iRNA can be precisely regulated, for example, by using an inducible regulatory sequence that is sensitive to certain physiological regulators, *e.g.*, circulating glucose levels, or hormones (Docherty et al., 1994, FASEB J. 8:20-24). Such inducible expression systems, suitable for the control of dsRNA expression in cells or in mammals include, for example, regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-β-D1-thiogalactopyranoside (IPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the iRNA transgene.

[0350] Viral vectors that contain nucleic acid sequences encoding an iRNA can be used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding an iRNA are cloned into one or more vectors, which facilitates delivery of the nucleic acid into a patient. More detail about retroviral vectors can be found, for example, in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993). Lentiviral vectors contemplated for use include, for example, the HIV based vectors described in U.S. Patent Nos. 6,143,520; 5,665,557; and 5,981,276.

[0351] Adenoviruses are also contemplated for use in delivery of iRNAs. Adenoviruses are especially attractive vehicles, e.g., for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143-155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). A suitable AV vector for expressing an iRNA featured in the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H et al. (2002), Nat. Biotech. 20: 1006-1010.

[0352] Use of Adeno-associated virus (AAV) vectors is also contemplated (Walsh et al., Proc. Soc. Exp. Biol. Med.

204:289-300 (1993); U.S. Pat. No. 5,436,146). In one embodiment, the iRNA can be expressed as two separate, complementary single-stranded RNA molecules from a recombinant AAV vector having, for example, either the U6 or H1 RNA promoters, or the cytomegalovirus (CMV) promoter. Suitable AAV vectors for expressing the dsRNA featured in the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R et al. (1987), J. Virol. 61: 3096-3101; Fisher K J et al. (1996), J. Virol, 70: 520-532; Samulski R et al. (1989), J. Virol. 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641.

[0353] Another typical viral vector is a pox virus such as a vaccinia virus, for example an attenuated vaccinia such as Modified Virus Ankara (MVA) or NYVAC, an avipox such as fowl pox or canary pox.

[0354] The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate. For example, lentiviral vectors can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors can be made to target different cells by engineering the vectors to express different capsid protein serotypes; see, *e.g.,* Rabinowitz J E et al. (2002), J Virol 76:791-801.

[0355] The pharmaceutical preparation of a vector can include the vector in an acceptable diluent, or can include a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

III. Pharmaceutical compositions containing iRNA

[0356] In one embodiment, the invention provides pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical composition containing the iRNA is useful for treating a disease or disorder related to the expression or activity of an ALAS1 gene (e.g., a disorder involving the porphyrin pathway). Such pharmaceutical compositions are formulated based on the mode of delivery. For example, compositions can be formulated for systemic administration via parenteral delivery, *e.g.*, by intravenous (IV) delivery. A composition provided herein (e.g., an LNP formulation) may be formulated for intravenous delivery. A composition provided herein (e.g., a composition comprising a GalNAc conjugate) may be formulated for subcutaneous delivery.

[0357] The pharmaceutical compositions featured herein are administered in a dosage sufficient to inhibit expression of an ALAS1 gene. In general, a suitable dose of iRNA will be in the range of 0.01 to 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of 1 to 50 mg per kilogram body weight per day. For example, the dsRNA can be administered at 0.05 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 3 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg, or 50 mg/kg per single dose. The pharmaceutical composition may be administered once daily, or the iRNA may be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the iRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, *e.g.*, using a conventional sustained release formulation which provides sustained release of the iRNA over a several day period. Sustained release formulations are well known in the art and are particularly useful for delivery of agents at a particular site, such as can be used with the agents of the present invention. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

[0358] The effect of a single dose on ALAS1 levels can be long lasting, such that subsequent doses are administered at not more than 3, 4, or 5 day intervals, or at not more than 1, 2, 3, or 4 week intervals.

[0359] The skilled artisan will appreciate that certain factors may 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.

[0360] Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as pathological processes related to ALAS1 expression (*e.g.*, pathological processes involving porphyrins or defects in the porphyrin pathway, such as, for example, porphyrias). Such models can be used for *in vivo* testing of iRNA, as well as for determining a therapeutically effective dose and/or an effective dosing regimen.

[0361] A suitable mouse model is, for example, a mouse containing a transgene expressing human ALAS1. Mice that have knock-in mutations (e.g., mutations that are associated with acute hepatic porphyrias in humans) can be used to determine the therapeutically effective dosage and/or duration of administration of ALAS1 siRNA. The present invention also includes pharmaceutical compositions and formulations that include the iRNA compounds featured in the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be 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.

[0362] The iRNA can be delivered in a manner to target a particular tissue, such as a tissue that produces erythrocytes. For example, the iRNA can be delivered to bone marrow, liver (*e.g.*, hepatocyes of liver), lymph glands, spleen, lungs (*e.g.*, pleura of lungs) or spine. In one embodiment, the iRNA is delivered to bone marrow.

[0363] Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful. 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, distearolyphosphatidyl choline) negative (*e.g.*, dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.*, dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). iRNAs featured in the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, iRNAs may 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, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C_{1-20} alkyl ester (*e.g.*, isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in U.S. Patent No. 6,747,014.

Liposomal formulations

[0364] There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

[0365] Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages *in vivo.*

[0366] In order to traverse 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. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

[0367] 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 drugs in their internal compartments from metabolism and degradation (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., 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.

[0368] Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes and as the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

[0369] Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that 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 a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

[0370] Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into the skin. Compounds including analgesics, antibodies, hormones and high-molecular weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis

[0371] Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome,

the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al., Biochem. Biophys. Res. Commun., 1987, 147, 980-985).

[0372] Liposomes which are pH-sensitive or negatively-charged, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some DNA is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al., Journal of Controlled Release, 1992, 19, 269-274).

[0373] One major type of liposomal composition includes phospholipids other than 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.

[0374] Several studies have assessed the topical delivery of liposomal drug formulations to the skin. Application of liposomes containing interferon to guinea pig skin resulted in a reduction of skin herpes sores while delivery of interferon via other means (*e.g.*, as a solution or as an emulsion) were ineffective (Weiner et al., Journal of Drug Targeting, 1992, 2, 405-410). Further, an additional study tested the efficacy of interferon administered as part of a liposomal formulation to the administration of interferon using an aqueous system, and concluded that the liposomal formulation was superior to aqueous administration (du Plessis et al., Antiviral Research, 1992, 18, 259-265).

[0375] 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 cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporin-A into different layers of the skin (Hu et al. S.T.P.Pharma. Sci., 1994, 4, 6, 466).

[0376] Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. 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) 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., FEBS Letters, 1987, 223, 42; Wu et al., Cancer Research, 1993, 53, 3765).

[0377] 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 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).

[0378] Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto et al. (Bull. Chem. Soc. Jpn., 1980, 53, 2778) described liposomes comprising a nonionic detergent, $2C_{1215G}$, that contains a PEG moiety. Illum et al. (FEBS Lett., 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (*e.g.*, PEG) are described by Sears (U.S. Pat. Nos. 4,426,330 and 4,534,899). Klibanov et al. (FEBS Lett., 1990, 268, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume et al. (Biochimica et Biophysica Acta, 1990, 1029, 91) extended such observations to other PEG-derivatized phospholipids, *e.g.*, DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle et al. (U.S. Pat. Nos. 5,013,556 and 5,356,633) and Martin et al. (U.S. Pat. No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Pat. No. 5,225,212 (both to Martin *et al.*) and in WO 94/20073 (Zalipsky et al.) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi et al). U.S. Pat. No. 5,540,935 (Miyazaki et al.) and U.S. Pat. No. 5,556,948 (Tagawa et al.) describe PEG-containing liposomes that can be further derivatized with functional moieties on their

surfaces.

[0379] A number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry et al. discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Pat. No. 5,264,221 to Tagawa et al. discloses protein-bonded liposomes and asserts that the contents of such liposomes may include a dsRNA. U.S. Pat. No. 5,665,710 to Rahman et al. describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love et al. discloses liposomes comprising dsRNAs targeted to the raf gene.

[0380] Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes may 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.

[0381] 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).

[0382] 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.

[0383] 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. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

[0384] 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 are the most used members of this class.

[0385] 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.

[0386] 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).

Nucleic acid lipid particles

[0387] An ALAS1 dsRNA may be fully encapsulated in the lipid formulation, *e.g.*, to form a SPLP, pSPLP, SNALP, or other nucleic acid-lipid particle. As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle, including SPLP. As used herein, the term "SPLP" refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle. SNALPs and SPLPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate). SNALPs and SPLPs 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). SPLPs 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 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; and PCT Publication No. WO 96/40964.

[0388] The lipid to drug ratio (mass/mass ratio) (*e.g.*, lipid to dsRNA ratio) may be in the range of from about 1:1 to about 50:1, from about 1:1 to 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.

[0389] The cationic lipid may be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(l -(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(l -(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-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-Dilinoleyloxo-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, (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)ethylazanediyl)didodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid may comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

[0390] 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.

[0391] The lipid-siRNA particle may include 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.

[0392] The non-cationic lipid may 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), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl- 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- phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid may 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.

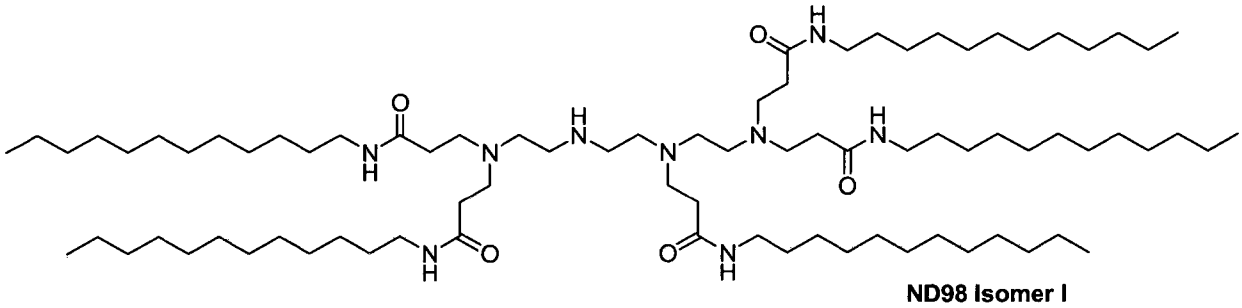
[0393] The conjugated lipid that inhibits aggregation of particles may be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate may be, for example, a PEG-dilauryloxypropyl (Ci_2), a PEG-dimyristyloxypropyl (Ci_4), a PEG-dipalmityloxypropyl (Ci_6), or a PEG- distearyloxypropyl ($C]_8$). The conjugated lipid that prevents aggregation of particles may be from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

[0394] The nucleic acid-lipid particle may further include cholesterol at, *e.g.*, about 10 mol % to about 60 mol % or about 48 mol % of the total lipid present in the particle.

[0395] The iRNA may be formulated in a lipid nanoparticle (LNP).

LNP01

[0396] In one embodiment, the lipidoid ND98·4HCl (MW 1487) (see U.S. Patent Application No. 12/056,230, filed 3/26/2008), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid-dsRNA nanoparticles (*e.g.*, LNP01 particles). Stock solutions of each in ethanol can be prepared as follows: ND98, 133 mg/ml; Cholesterol, 25 mg/ml, PEG-Ceramide 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

[0397] LNP01 formulations are described, *e.g.*, in International Application Publication No. WO 2008/042973.

[0398] Additional exemplary lipid-dsRNA formulations are provided in the following table.

Table 10: Examplary lipid formulations

	Cationic Lipid	**cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio**
SNALP	1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1
S-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
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/3 1.5/3.5 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 LipidaiRNA 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 LipidaiRNA 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 (C12-200)	C12-200/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1

(continued)

	Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio
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
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 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)		

[0399] SNALP (1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed April 15, 2009.

[0400] XTC comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/148,366, filed January 29, 2009; U.S. Provisional Serial No. 61/156,851, filed March 2, 2009; U.S. Provisional Serial No. filed June 10, 2009; U.S. Provisional Serial No. 61/228,373, filed July 24, 2009; U.S. Provisional Serial No. 61/239,686, filed September 3, 2009, and International Application No. PCT/US2010/022614, filed January 29, 2010.

[0401] MC3 comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/244,834, filed September 22, 2009, U.S. Provisional Serial No. 61/185,800, filed June 10, 2009, and International Application No. PCT/US10/28224,

filed June 10, 2010.

[0402] ALNY-100 comprising formulations are described, e.g., International patent application number PCT/US09/63933, filed on November 10, 2009.

[0403] C12-200 comprising formulations are described in U.S. Provisional Serial No. 61/175,770, filed May 5, 2009 and International Application No. PCT/US10/33777, filed May 5, 2010.

Synthesis of cationic lipids

[0404] Any of the compounds, e.g., cationic lipids and the like, used in the nucleic acid-lipid particles featured in the invention may be prepared by known organic synthesis techniques, including the methods described in more detail in the Examples. All substituents are as defined below unless indicated otherwise.

[0405] "Alkyl" means a straight chain or branched, noncyclic or cyclic, saturated aliphatic hydrocarbon containing from 1 to 24 carbon atoms. Representative saturated straight chain alkyls include methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, and the like; while saturated branched alkyls include isopropyl, *sec*-butyl, isobutyl, *tert*-butyl, isopentyl, and the like. Representative saturated cyclic alkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like; while unsaturated cyclic alkyls include cyclopentenyl and cyclohexenyl, and the like.

[0406] "Alkenyl" means an alkyl, as defined above, containing at least one double bond between adjacent carbon atoms. Alkenyls include both cis and trans isomers. Representative straight chain and branched alkenyls include ethylenyl, propylenyl, 1-butenyl, 2-butenyl, isobutylenyl, 1-pentenyl, 2-pentenyl, 3-methyl-1-butenyl, 2-methyl-2-butenyl, 2,3-dimethyl-2-butenyl, and the like.

[0407] "Alkynyl" means any alkyl or alkenyl, as defined above, which additionally contains at least one triple bond between adjacent carbons. Representative straight chain and branched alkynyls include acetylenyl, propynyl, 1-butynyl, 2-butynyl, 1-pentynyl, 2-pentynyl, 3-methyl-l butynyl, and the like.

[0408] "Acyl" means any alkyl, alkenyl, or alkynyl wherein the carbon at the point of attachment is substituted with an oxo group, as defined below. For example, -C(=O)alkyl, -C(=O)alkenyl, and -C(=O)alkynyl are acyl groups.

[0409] "Heterocycle" means a 5- to 7-membered monocyclic, or 7- to 10-membered bicyclic, heterocyclic ring which is either saturated, unsaturated, or aromatic, and which contains from 1 or 2 heteroatoms independently selected from nitrogen, oxygen and sulfur, and wherein the nitrogen and sulfur heteroatoms may be optionally oxidized, and the nitrogen heteroatom may be optionally quaternized, including bicyclic rings in which any of the above heterocycles are fused to a benzene ring. The heterocycle may be attached via any heteroatom or carbon atom. Heterocycles include heteroaryls as defined below. Heterocycles include morpholinyl, pyrrolidinonyl, pyrrolidinyl, piperidinyl, piperizynyl, hydantoinyl, valerolactamyl, oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydroprimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, and the like.

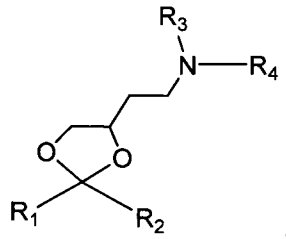
[0410] The terms "optionally substituted alkyl", "optionally substituted alkenyl", "optionally substituted alkynyl", "optionally substituted acyl", and "optionally substituted heterocycle" means that, when substituted, at least one hydrogen atom is replaced with a substituent. In the case of an oxo substituent (=O) two hydrogen atoms are replaced. In this regard, substituents include oxo, halogen, heterocycle, -CN, $-OR^x$, $-NR^xR^y$, $-NR^xC(=O)R^y$ $-NR^xSO_2R^y$, $-C(=O)R^x$, $-C(=O)OR^x$, $-C(=O)NR^xR^y$, $-SO_nR^x$ and $-SO_nNR^xR^y$, wherein n is 0, 1 or 2, R^x and R^y are the same or different and independently hydrogen, alkyl or heterocycle, and each of said alkyl and heterocycle substituents may be further substituted with one or more of oxo, halogen, -OH, -CN, alkyl, $-OR^x$, heterocycle, $-NR^xR^y$, $-NR^xC(=O)R^y$, $-NR^xSO_2R^y$, $-C(=O)R^x$, $-C(=O)OR^x$, $-C(=O)NR^xR^y$, $-SO_nR^x$ and $-SO_nNR^xR^y$.

[0411] "Halogen" means fluoro, chloro, bromo and iodo.

[0412] In some embodiments, the methods featured in the invention may require the use of protecting groups. Protecting group methodology is well known to those skilled in the art (*see, for example*, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, Green, T.W. et al., Wiley-Interscience, New York City, 1999). Briefly, protecting groups within the context of this invention are any group that reduces or eliminates unwanted reactivity of a functional group. A protecting group can be added to a functional group to mask its reactivity during certain reactions and then removed to reveal the original functional group. In some embodiments an "alcohol protecting group" is used. An "alcohol protecting group" is any group which decreases or eliminates unwanted reactivity of an alcohol functional group. Protecting groups can be added and removed using techniques well known in the art.

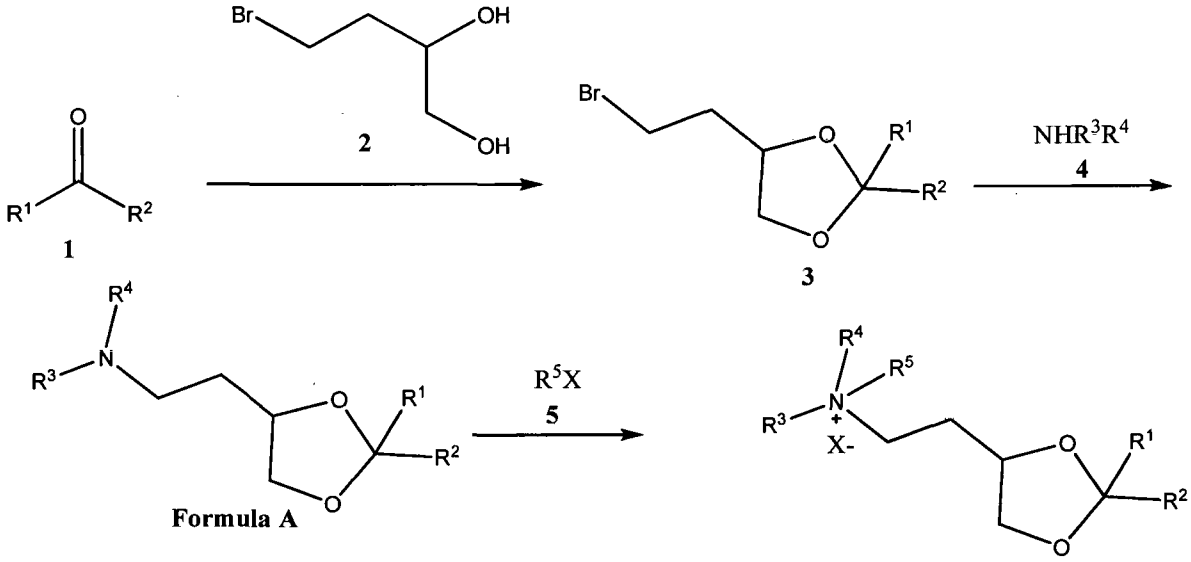
Synthesis of Formula A

[0413] In one embodiments, nucleic acid-lipid particles featured in the invention are formulated using a cationic lipid of formula A:



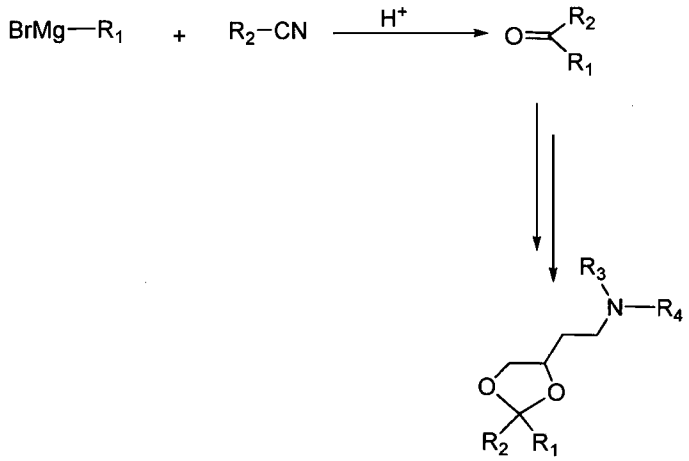
where R1 and R2 are independently alkyl, alkenyl or alkynyl, each can be optionally substituted, and R3 and R4 are independently lower alkyl or R3 and R4 can be taken together to form an optionally substituted heterocyclic ring. In some embodiments, the cationic lipid is XTC (2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane). In general, the lipid of formula A above may be made by the following Reaction Schemes 1 or 2, wherein all substituents are as defined above unless indicated otherwise.

Scheme 1



[0414] Lipid A, where R_1 and R_2 are independently alkyl, alkenyl or alkynyl, each can be optionally substituted, and R_3 and R_4 are independently lower alkyl or R_3 and R_4 can be taken together to form an optionally substituted heterocyclic ring, can be prepared according to Scheme 1. Ketone 1 and bromide 2 can be purchased or prepared according to methods known to those of ordinary skill in the art. Reaction of 1 and 2 yields ketal 3. Treatment of ketal 3 with amine 4 yields lipids of formula A. The lipids of formula A can be converted to the corresponding ammonium salt with an organic salt of formula 5, where X is anion counter ion selected from halogen, hydroxide, phosphate, sulfate, or the like.

Scheme 2



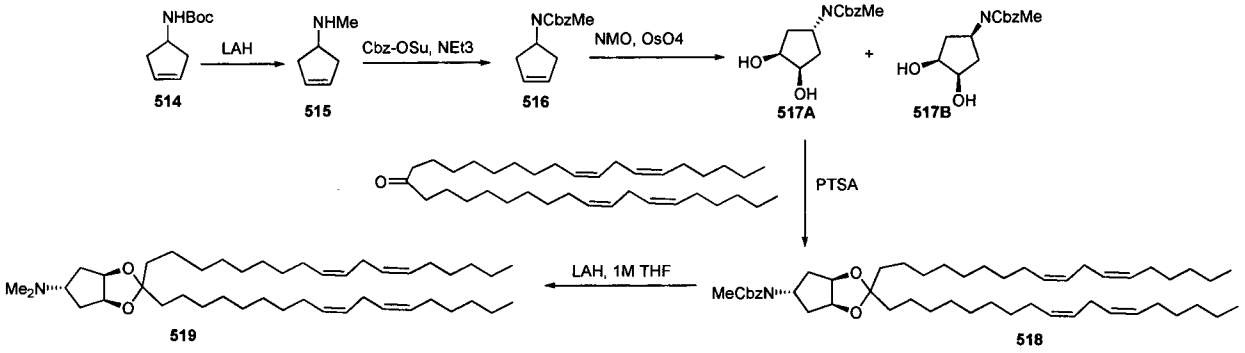
[0415] Alternatively, the ketone 1 starting material can be prepared according to Scheme 2. Grignard reagent 6 and cyanide 7 can be purchased or prepared according to methods known to those of ordinary skill in the art. Reaction of 6 and 7 yields ketone 1. Conversion of ketone 1 to the corresponding lipids of formula A is as described in Scheme 1.

Synthesis of MC3

[0416] Preparation of DLin-M-C3-DMA (*i.e.*, (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate) was as follows. A solution of (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol (0.53 g), 4-N,N-dimethylaminobutyric acid hydrochloride (0.51 g), 4-N,N-dimethylaminopyridine (0.61g) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.53 g) in dichloromethane (5 mL) was stirred at room temperature overnight. The solution was washed with dilute hydrochloric acid followed by dilute aqueous sodium bicarbonate. The organic fractions were dried over anhydrous magnesium sulphate, filtered and the solvent removed on a rotovap. The residue was passed down a silica gel column (20 g) using a 1-5% methanol/dichloromethane elution gradient. Fractions containing the purified product were combined and the solvent removed, yielding a colorless oil (0.54 g).

Synthesis of ALNY-100

[0417] Synthesis of ketal 519 [ALNY-100] was performed using the following scheme 3:



Synthesis of 515:

[0418] To a stirred suspension of LiAlH4 (3.74 g, 0.09852 mol) in 200 ml anhydrous THF in a two neck RBF (1L), was added a solution of 514 (10g, 0.04926mol) in 70 mL of THF slowly at 0 0C under nitrogen atmosphere. After complete addition, reaction mixture was warmed to room temperature and then heated to reflux for 4 h. Progress of the reaction was monitored by TLC. After completion of reaction (by TLC) the mixture was cooled to 0 0C and quenched with careful addition of saturated Na2SO4 solution. Reaction mixture was stirred for 4 h at room temperature and filtered off. Residue was washed well with THF. The filtrate and washings were mixed and diluted with 400 mL dioxane and 26 mL conc.

HCl and stirred for 20 minutes at room temperature. The volatilities were stripped off under vacuum to furnish the hydrochloride salt of 515 as a white solid. Yield: 7.12 g 1H-NMR (DMSO, 400MHz): δ= 9.34 (broad, 2H), 5.68 (s, 2H), 3.74 (m, 1H), 2.66-2.60 (m, 2H), 2.50-2.45 (m, 5H).

Synthesis of 516:

[0419] To a stirred solution of compound 515 in 100 mL dry DCM in a 250 mL two neck RBF, was added NEt3 (37.2 mL, 0.2669 mol) and cooled to 0 0C under nitrogen atmosphere. After a slow addition of N-(benzyloxy-carbonyloxy)-succinimide (20 g, 0.08007 mol) in 50 mL dry DCM, reaction mixture was allowed to warm to room temperature. After completion of the reaction (2-3 h by TLC) mixture was washed successively with IN HCl solution (1 x 100 mL) and saturated NaHCO3 solution (1 x 50 mL). The organic layer was then dried over anhyd. Na2SO4 and the solvent was evaporated to give crude material which was purified by silica gel column chromatography to get 516 as sticky mass. Yield: 11g (89%). 1H-NMR (CDCl3, 400MHz): δ = 7.36-7.27(m, 5H), 5.69 (s, 2H), 5.12 (s, 2H), 4.96 (br., 1H) 2.74 (s, 3H), 2.60(m, 2H), 2.30-2.25(m, 2H). LC-MS [M+H] -232.3 (96.94%).

Synthesis of 517A and 517B:

[0420] The cyclopentene 516 (5 g, 0.02164 mol) was dissolved in a solution of 220 mL acetone and water (10:1) in a single neck 500 mL RBF and to it was added N-methyl morpholine-N-oxide (7.6 g, 0.06492 mol) followed by 4.2 mL of 7.6% solution of OsO4 (0.275 g, 0.00108 mol) in tert-butanol at room temperature. After completion of the reaction (~ 3 h), the mixture was quenched with addition of solid Na2SO3 and resulting mixture was stirred for 1.5 h at room temperature. Reaction mixture was diluted with DCM (300 mL) and washed with water (2 x 100 mL) followed by saturated NaHCO3 (1 x 50 mL) solution, water (1 x 30 mL) and finally with brine (1x 50 mL). Organic phase was dried over an.Na2SO4 and solvent was removed in vacuum. Silica gel column chromatographic purification of the crude material was afforded a mixture of diastereomers, which were separated by prep HPLC. Yield: - 6 g crude
517A - Peak-1 (white solid), 5.13 g (96%). 1H-NMR (DMSO, 400MHz): δ= 7.39-7.31(m, 5H), 5.04(s, 2H), 4.78-4.73 (m, 1H), 4.48-4.47(d, 2H), 3.94-3.93(m, 2H), 2.71(s, 3H), 1.72- 1.67(m, 4H). LC-MS - [M+H]-266.3, [M+NH4 +]-283.5 present, HPLC-97.86%. Stereochemistry confirmed by X-ray.

Synthesis of 51.8:

[0421] Using a procedure analogous to that described for the synthesis of compound 505, compound 518 (1.2 g, 41%) was obtained as a colorless oil. 1H-NMR (CDCl3, 400MHz): δ= 7.35-7.33(m, 4H), 7.30-7.27(m, 1H), 5.37-5.27(m, 8H), 5.12(s, 2H), 4.75(m,1H), 4.58-4.57(m,2H), 2.78-2.74(m,7H), 2.06-2.00(m,8H), 1.96-1.91(m, 2H), 1.62(m, 4H), 1.48(m, 2H), 1.37-1.25(br m, 36H), 0.87(m, 6H). HPLC-98.65%.

General Procedure for the Synthesis of Compound 519:

[0422] A solution of compound 518 (1 eq) in hexane (15 mL) was added in a drop-wise fashion to an ice-cold solution of LAH in THF (1 M, 2 eq). After complete addition, the mixture was heated at 40°C over 0.5 h then cooled again on an ice bath. The mixture was carefully hydrolyzed with saturated aqueous Na2SO4 then filtered through celite and reduced to an oil. Column chromatography provided the pure 519 (1.3 g, 68%) which was obtained as a colorless oil. 13C NMR = 130.2, 130.1 (x2), 127.9 (x3), 112.3, 79.3, 64.4, 44.7, 38.3, 35.4, 31.5, 29.9 (x2), 29.7, 29.6 (x2), 29.5 (x3), 29.3 (x2), 27.2 (x3), 25.6, 24.5, 23.3, 226, 14.1; Electrospray MS (+ve): Molecular weight for C44H80NO2 (M + H)+ Calc. 654.6, Found 654.6.

[0423] Formulations prepared by either the standard or extrusion-free method can be characterized in similar manners. For example, formulations are typically characterized by visual inspection. They should be whitish translucent solutions free from aggregates or sediment. Particle size and particle size distribution of lipid-nanoparticles can be measured by light scattering using, for example, a Malvern Zetasizer Nano ZS (Malvern, USA). Particles should be about 20-300 nm, such as 40-100 nm in size. The particle size distribution should be unimodal. The total dsRNA concentration in the formulation, as well as the entrapped fraction, is estimated using a dye exclusion assay. A sample of the formulated dsRNA can be incubated with an RNA-binding dye, such as Ribogreen (Molecular Probes) in the presence or absence of a formulation disrupting surfactant, e.g., 0.5% Triton-X100. The total dsRNA in the formulation can be determined by the signal from the sample containing the surfactant, relative to a standard curve. The entrapped fraction is determined by subtracting the "free" dsRNA content (as measured by the signal in the absence of surfactant) from the total dsRNA content. Percent entrapped dsRNA is typically >85%. For SNALP formulation, the particle size is at least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm, at least 100 nm, at least 110 nm, and at least 120 nm. The suitable range is typically about at least 50 nm to about at least 110 nm, about at least 60 nm

to about at least 100 nm, or about at least 80 nm to about at least 90 nm.

[0424] 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 minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may 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 enhancers 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 ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic 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, 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 may 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, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (*e.g.*, p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, 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.

[0425] Compositions and formulations for parenteral, intraparenchymal (into the brain), intrathecal, intraventricular or intrahepatic administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[0426] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

[0427] The pharmaceutical formulations featured in the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0428] The compositions featured in the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

Additional Formulations

Emulsions

[0429] The compositions of the present invention may be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1μm 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, 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 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 may 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 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 may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants may also be present in emulsions as needed. Pharmaceutical emulsions may 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 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.

[0430] Emulsions are characterized by little or no thermodynamic stability. Often, the 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 may 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 may be incorporated into either phase of the emulsion. Emulsifiers may 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).

[0431] 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 may 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).

[0432] 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.

[0433] 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).

[0434] 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.

[0435] Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that may 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 may 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.

[0436] 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.

[0437] In one embodiment of the present invention, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion may 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).

[0438] 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.

[0439] Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 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, and 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 may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may 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 may 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.

[0440] 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 may form spontaneously when their components are brought together at ambient temperature. This may 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.

[0441] Microemulsions of the present invention may 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 may 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.

Penetration Enhancers

[0442] Various penetration enhancers may be employed 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 may cross cell membranes if the 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.

[0443] Penetration enhancers may be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating 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). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

[0444] *Surfactants:* In connection with the present invention, surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of iRNAs through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (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); and perfluorochemical emulsions, such as FC-43. Takahashi et al., J. Pharm. Pharmacol., 1988, 40, 252).

[0445] *Fatty acids:* Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C_{1-20} alkyl esters thereof (*e.g.*, methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (*i.e.*, oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, *etc.*) (see *e.g.,* Touitou, E., et al. Enhancement in Drug Delivery, CRC Press, Danvers, MA, 2006; Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri et al., J. Pharm. Pharmacol., 1992, 44, 651-654).

[0446] *Bile salts:* The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (see e.g., Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman et al. Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (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, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto et al., J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita et al., J. Pharm. Sci., 1990, 79, 579-583).

[0447] *Chelating Agents:* Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of iRNAs through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315-339). Suitable chelating agents include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric

acid, salicylates (*e.g.*, sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of β-diketones (enamines)(see *e.g.*, Katdare, A. et al., Excipient development for pharmaceutical, biotechnology, and drug delivery, CRC Press, Danvers, MA, 2006; Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur et al., J. Control Rel., 1990, 14,43-51).

[0448] *Non-chelating non-surfactants:* As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of iRNAs through the alimentary mucosa (see *e.g.*, Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., J. Pharm. Pharmacol., 1987, 39, 621-626).

[0449] Agents that enhance uptake of iRNAs at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi et al, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo et al., PCT Application WO 97/30731), are also known to enhance the cellular uptake of dsRNAs. Examples of commercially available transfection reagents include, for example Lipofectamine™ (Invitrogen; Carlsbad, CA), Lipofectamine 2000™ (Invitrogen; Carlsbad, CA), 293fectin™ (Invitrogen; Carlsbad, CA), Cellfectin™ (Invitrogen; Carlsbad, CA), DMRIE-C™ (Invitrogen; Carlsbad, CA), FreeStyle™ MAX (Invitrogen; Carlsbad, CA), Lipofectamine™ 2000 CD (Invitrogen; Carlsbad, CA), Lipofectamine™ (Invitrogen; Carlsbad, CA), RNAiMAX (Invitrogen; Carlsbad, CA), Oligofectamine™ (Invitrogen; Carlsbad, CA), Optifect™ (Invitrogen; Carlsbad, CA), X-tremeGENE Q2 Transfection Reagent (Roche; Grenzacherstrasse, Switzerland), DOTAP Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), DOSPER Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), or Fugene (Grenzacherstrasse, Switzerland), Transfectam® Reagent (Promega; Madison, WI), TransFast™ Transfection Reagent (Promega; Madison, WI), Tfx™-20 Reagent (Promega; Madison, WI), Tfx™-50 Reagent (Promega; Madison, WI), DreamFect™ (OZ Biosciences; Marseille, France), EcoTransfect (OZ Biosciences; Marseille, France), TransPass^{a} D1 Transfection Reagent (New England Biolabs; Ipswich, MA, USA), LyoVec™/LipoGen™ (Invivogen; San Diego, CA, USA), PerFectin Transfection Reagent (Genlantis; San Diego, CA, USA), NeuroPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), GenePORTER Transfection reagent (Genlantis; San Diego, CA, USA), GenePORTER 2 Transfection reagent (Genlantis; San Diego, CA, USA), Cytofectin Transfection Reagent (Genlantis; San Diego, CA, USA), BaculoPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), TroganPORTER™ transfection Reagent (Genlantis; San Diego, CA, USA), RiboFect (Bioline; Taunton, MA, USA), PlasFect (Bioline; Taunton, MA, USA), UniFECTOR (B-Bridge International; Mountain View, CA, USA), SureFECTOR (B-Bridge International; Mountain View, CA, USA), or HiFect™ (B-Bridge International, Mountain View, CA, USA), among others.

[0450] Other agents may be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

Carriers

[0451] Certain compositions of the present invention also incorporate carrier compounds 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 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 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.

Excipients

[0452] 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 may 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*).

[0453] 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.

[0454] Formulations for topical administration of nucleic acids may 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 may 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.

[0455] 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.

Other Components

[0456] The compositions may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

[0457] Aqueous suspensions may contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0458] In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA compounds and (b) one or more biologic agents which function by a non-RNAi mechanism. Examples of such biologic agents include agents that interfere with an interaction of ALAS1 and at least one ALAS1 binding partner.

[0459] 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 LD50/ED50. Compounds that exhibit high therapeutic indices are typical.

[0460] 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 in the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range 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 may 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.*, 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 may be measured, for example, by high performance liquid chromatography.

[0461] 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 diseases or disorders related to ALAS1 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.

Methods for treating diseases related to expression of an ALAS1 gene

[0462] The disclosure relates in particular to the use of an iRNA targeting ALAS1 to inhibit ALAS1 expression and/or to treat a disease, disorder, or pathological process that is related to ALAS1 expression.

[0463] As used herein, "a disorder related to ALAS1 expression," a "disease related to ALAS1 expression, a "pathological process related to ALAS1 expression," or the like includes any condition, disorder, or disease in which ALAS1 expression is altered (e.g., elevated), the level of one or more porphyrins is altered (e.g., elevated), the level or activity of one or more enzymes in the heme biosynthetic pathway (porphyrin pathway) is altered, or other mechisms that lead to pathological changes in the heme biosynthetic pathway. For example, an iRNA targeting an ALAS1 gene, or a combination thereof, may be used for treatment of conditions in which levels of a porphyrin or a porphyrin precursor (e.g., ALA or PBG) are elevated (*e.g.*, certain porphyrias), or conditions in which there are defects in the enzymes of the heme biosynthetic pathway (*e.g.*, certain porphyrias). Disorders related to ALAS1 expression include, for example, X-linked sideroblastic anemia (XLSA), ALA deyhdratase deficiency porphyria (Doss porphyria), acute intermittent porphyria (AIP), congenital erythropoietic porphyria, prophyria cutanea tarda, hereditary coproporphyria (coproporphyria), variegate porphyria, erythropoietic protoporphyria (EPP), and transient erythroporphyria of infancy.

[0464] As used herein, a "subject" to be treated according to the methods described herein, includes a human or non-human animal, *e.g.*, a mammal. The mammal may be, for example, a rodent (*e.g.*, a rat or mouse) or a primate (*e.g.*, a monkey). In some embodiments, the subject is a human.

[0465] The subject may be suffering from a disorder related to ALAS1 expression (*e.g.*, has been diagnosed with a porphyria or has suffered from one or more symptoms of porphyria and is a carrier of a mutation associated with porphyria) or is at risk of developing a disorder related to ALAS1 expression (*e.g.*, a subject with a family history of porphyria, or a subject who is a carrier of a genetic mutation associated with porphyria).

[0466] Classifications of porphyrias, including acute hepatic porphyrias, are described, e.g., in Balwani, M. & Desnick, R.J., Blood, 120(23), published online as Blood First Edition paper, July 12, 102; DOI 10.1182/blood-2012-05-423186. As described in Balwain & Desnick, acute intermittent porphyria (AIP) hereditary coproporphyria (HCP), variegate porphyria (VP) are autosomal dominant porphyrias and ALA deyhdratase deficiency porphyria (ADP) is autosomal recessive. In rare cases, AIP, HCP, and VP occur as homozygous dominant forms. In addition, there is a rare homozygous recessive form of porphyria cutanea tarda (PCT), which is the single hepatic cutaneous porphyria, and is also known as hepatoerythropoietic porphyria. The clinical and laboratory features of these porphyrias are described in Table 11 below.

Table 11: Human hepatic porphyrias: clinical and laboratory features

Porphyria	Deficient enzyme	Inheritance	Principal symptoms, NV or CP	Enzyme activity, % of normal	Increased porphyrin precursors and/or porphyrins*		
					Erythrocytes	Urine	Stool
Acute hepatic porphyrias							
ADP	ALA-dehydratase	AR	NV	~5	Zn-protoporphyrin	ALA, coproporphyrin III	-
AIP	HMB-synthase	AD	NV	~50	-	ALA, PBG, uroporphyrin	-
HCP	COPRO-oxidase	AD	NV and CP	~50	-	ALA, PBG, coproporphyrin III	coproporp hyrin III
VP	PROTO-oxidase	AD	NV and CP	~50	-	ALA, PBG coproporphyrin III	coproporp hyrin III, protoporp hyrin
Hepatic cutaneous porphyrias							
PCT	URO-decarboxylase	Sporadic or AD	CP	<20	-	uroporphyrin, 7-carboxylate porphyrin	uroporphy rin, 7-carboxylat e porphyrin
AR indicates autosomal recessive; AD, autosomal dominant; NV, neurovisceral; CP, cutaneous photosensitivity; and -, not applicable. *Increases that may be important for diagnosis.							

[0467] Described is that the subject has or is at risk for developing a porphyria, e.g., a hepatic porphyria, e.g., AIP, HCP, VP, ADP, or hepatoerythropoietic porphyria.

[0468] Described is that the porphyria is an acute hepatic porphyria, e.g., an acute hepatic porphyria iselected from acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP), and ALA deyhdratase deficiency porphyria (ADP).

[0469] Described is that the porphyria is a dual porphyria, e.g., at least two porphyrias. In some embodiments, the dual porphyria comprises two or more porphyrias selected from acute intermittent porphyria (AIP) hereditary coproporphyria (HCP), variegate porphyria (VP), and ALA deyhdratase deficiency porphyria (ADP).

[0470] Described is that the porphyria is a homozygous dominant hepatic porphyria (e.g., homozygous dominant AIP, HCP, or VP) or hepatoerythropoietic porphyria. Described is that the porphyria is AIP, HCP, VP, or hepatoerythropoietic porphyria, or a combination thereof (e.g., a dual porphyria). Described is that the AIP, HCP, or VP is either heterozygous dominant or homozygous dominant.

[0471] Described is that the subject has or is at risk for developing a porphyria, e.g., ADP, and shows an elevated level (e.g., an elevated urine level) of ALA and/or coproporphyrin III. Described is that the subject has or is at risk for developing a porphyria, e.g., ADP, and shows an elevated level of erythrocyte Zn-protoporphyrin.

[0472] Described is that the subject has or is at risk for developing a porphyria, e.g., AIP, and shows an elevated level (e.g., an elevated urine level) of ALA, PBG, and/or uroporphyrin.

[0473] Described is that the subject has or is at risk for developing a porphyria, e.g., HCP, and shows an elevated level (e.g., an elevated urine level) of ALA, PBG, and/or coproporphyrin III. Described is that the subject has or is at risk for developing a porphyria, e.g., HCP, and shows an elevated level (e.g., an elevated stool level) of coproporphyrin III.

[0474] Described is that the subject has or is at risk for developing a porphyria, e.g., VP, and shows an elevated level (e.g., an elevated urine level) of ALA, PBG, and/or coproporphyrin III.

[0475] Described is that the subject has or is at risk for developing a porphyria, e.g., HCP, and shows an elevated level (e.g., an elevated stool level) of coproporphyrin III and/or protoporphyrin.

[0476] Described is that the subject has or is at risk for developing a porphyria, e.g., PCT, (e.g.,hepatoerythropoietic porphyria) and shows an elevated level (e.g., an elevated urine level) of uroporphyrin and/or 7-carboxylate porphyrin. Described is that the subject has or is at risk for developing a porphyria, e.g., PCT, (e.g.,hepatoerythropoietic porphyria) and shows an elevated level (e.g., an elevated stool level) of uroporphyrin and/or 7-carboxylate porphyrin.

[0477] A mutation associated with porphyria includes any mutation in a gene encoding an enzyme in the heme biosynthetic pathway (porphyrin pathway) or a gene which alters the expression of a gene in the heme biosynthetic pathway. Described is that the subject carries one or more mutations in an enzyme of the porphyrin pathway (*e.g.*, a mutation in ALA deydratase or PBG deaminase). Described is that the subject is suffereing from an acute porphyria (e.g., AIP, ALA deydratase deficiency porphyria).

[0478] In some cases, patients with an acute hepatic porphyria (e.g., AIP), or patients who carry mutations associated with an acute hepatic porphyria (e.g., AIP) but who are asymptomatic, have elevated ALA and/or PBG levels compared with healthy individuals. See, e.g., Floderus, Y. et al, Clinical Chemistry, 52(4): 701-707, 2006; Sardh et al., Clinical Pharmacokinetics, 46(4): 335-349, 2007. In such cases, the level of ALA and/or PBG can be elevated even when the patient is not having, or has never had, an attack. In some such cases, the patient is otherwise completely asymptomatic. In some such cases, the patient suffers from pain, e.g., neuropathic pain, which can be chronic pain (e.g., chronic neuropathic pain). In some cases, the patient has a neuropathy. In some cases, the patient has a progressive neuropathy.

[0479] Described is that the subject to be treated according to the methods described herein has an elevated level of a porphyrin or a porphyrin precursor, e.g., ALA and/or PBG. Levels of a porphyrin or a porphyrin precursor can be assessed using methods known in the art or methods described herein. For example, methods of assessing uring and plasma ALA and PBG levels, as well as urine and plasma porphyrin levels, are disclosed in Floderus, Y. et al, Clinical Chemistry, 52(4): 701-707, 2006; and Sardh et al., Clinical Pharmacokinetics, 46(4): 335-349, 2007, the entire contents of which are hereby incorporated in their entirety.

[0480] Described is that the subject is an animal model of a porphyria, e.g., a mouse model of a porphyria (e.g., a mutant mouse as described in Lindberg et al. Nature Genetics, 12: 195-199, 1996). Described is that the subject is a human, e.g., a human who has or is at risk for developing a porphyria, as described herein. Described is that the subject is not having an acute attack of porphyria. Described is that the subject has never had an attack. Described is that the patient suffers from chronic pain. Described is that the patient has nerve damage. Described is that the subject has EMG changes and/or changes in nerve conduction velocity. In some embodiments, the subject is asymptomatic. Described is that the subject is at risk for developing a porphyria (e.g., carries a gene mutation associated with a porphyria) and is asymptomatic. Described is that the subject has previously had an acute attack but is asymptomatic at the time of treatment.

[0481] Described is that the subject is at risk for developing a porphyria and is treated prophylactically to prevent the development of a porphyria. Described is that the subject has an elevated level of a porphyrin or a porphyrin precursor, e.g., ALA and/or PBG. In some embodiments, the prophylactic treatment begins at puberty. Described is that the treatment

lowers the level (e.g., the plasma level or the urine level) of a porphyrin or a porphyrin precursor, e.g., ALA and/or PBG. Described is that the treatment prevents the development of an elevated level of a porphyrin or a porphyrin precursor, e.g., ALA and/or PBG. Described is that the treatment prevents the development of, or decreases the frequency or severity of, a symptom associated with a porphyria, e.g., pain or nerve damage.

[0482] Described is that the level of a porphyrin or a porphyrin precursor, e.g., ALA or PBG, is elevated, e.g., in a sample of plasma or urine from the subject. Described is that the level of a porphyrin or a porphyrin precursor, e.g., ALA or PBG, in the subject is assessed based on the absolute level of the porphyrin or the porphyrin precursor, e.g., ALA or PBG in a sample from the subject. Described is that the level of a porphyrin or a porphyrin precursor, e.g., ALA or PBG, in the subject is assessed based on the relative level of the porphyrin or porphyrin precursor, e.g., ALA or PBG, in a sample from the subject. Described is that the relative level is relative to the level of another protein or compound, e.g., the level of creatinine, in a sample from the subject. Described is that the sample is a urine sample. Described is that the sample is a plasma sample. Described is that the sample is a stool sample.

[0483] An elevated level of a porphyrin or a porphyrin precursor, e.g., ALA and/or PBG, can be established, e.g., by showing that the subject has a level of a porphyrin or a porphyrin precursor, e.g., ALA and/or PBG (e.g., a plasma or urine level of ALA and/or PBG) that is greater than, or greater than or equal to, a reference value. A physician with expertise in the treatment of porphyrias would be able to determine whether the level of a porphyrin or a porphyrin precursor, (e.g., ALA and/or PBG) is elevated, e.g., for the purpose of diagnosing a porphyria or for determining whether a subject is at risk for developing a porphyria, e.g., a subject may be predisposed to an acute attack or to pathology associated with a porphyria, such as, e.g., chronic pain (e.g., neuropathic pain) and neuropathy (e.g., progressive neuropathy).

[0484] As used herein, a "reference value" refers to a value from the subject when the subject is not in a disease state, or a value from a normal or healthy subject, or a value from a reference sample or population, e.g., a group of normal or healthy subjects (e.g., a group of subjects that does not carry a mutation associated with a porphyria and/or a group of subjects that does not suffer from symptoms associated with a porphyria).

[0485] Described is that the reference value is a pre-disease level in the same individual. In some embodiments, the reference value is a level in a reference sample or population. Described is that the reference value is the mean or median value in a reference sample or population. Described is that the reference value the value that is is two standard deviations above the mean in a reference sample or population. Described is that the reference value is the value that is 2.5, 3, 3.5, 4, 4.5, or 5 standard deviations above the mean in a reference sample or population.

[0486] Described is that wherein the subject has an elevated level of a porphyrin or a porphyrin precursor, e.g., ALA and/or PBG, the subject has a level of ALA and/or PBG that is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% higher than a reference value. Described is that the subject has a level of a porphyrin or a porphyrin precursor, e.g., ALA and/or PBG, that is at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold higher than a reference value.

[0487] Described is that the reference value is an upper reference limit. As used herein, an "upper reference limit" refers to a level that is the upper limit of the 95% confidence interval for a reference sample or population, e.g., a group of normal (e.g., wild type) or healthy individuals, e.g., individuals who do not carry a genetic mutation associated with a porphyria and/or individuals who do not suffer from a porphyria. Accordingly, a lower reference limit refers to a level that is the lower limit of the same 95% confidence interval.

[0488] When the subject has an elevated level, e.g., a plasma level or a urine level, of a porphyrin or a porphyrin precursor, e.g., ALA or PBG, the level may be greater than or equal to 2 times, 3 times, 4 times, or 5 times that of a reference value, e.g., an upper reference limit. Described is that the subject has a urine level of a porphyrin or a porphyrin precursor, e.g., ALA or PBG, that is greater than 4 times that of an upper reference limit.

[0489] Described is that the reference value is a value provided in Floderus, Y. et al, Clinical Chemistry, 52(4): 701-707, 2006 or Sardh et al., Clinical Pharmacokinetics, 46(4): 335-349, 2007. Described is that the reference value is a value provided in Table 1 of Sardh et al.

[0490] Described is that the subject is a human and has a urine level of PBG that is greater than or equal to 4.8 mmol/mol creatinine. Described is that the subject is a human and has a urine level of PBG that is greater than, or greater than or equal to, about 3, 4, 5, 6, 7, or 8 mmol/mol creatinine.

[0491] Described is that the reference value for plasma PBG is 0.12 μmol/L. Described is that the subject is a human and has a plasma PBG level that is greater than, or greater than or equal to, 0.10 μmol/L, 0.12 μmol/L, 0.24 μmol/L, 0.36 μmol/L, 0.48 μmol/L, or 0.60 μmol/L. Described is that the subject is a human and has a plasma level of PBG that is greater than, or greater than or equal to, 0.48 μmol/L.

[0492] Described is that the reference value for urine PBG is 1.2 mmol/mol creatinine. Described is that the subject is a human and has a urine PBG level that is greater than, or greater than or equal to, 1.0 mmol/mol creatinine, 1.2 mmol/mol creatinine, 2.4 mmol/mol creatinine, 3.6 mmol/mol creatinine, 4.8 mmol/mol creatinine, or 6.0 mmol/mol creatinine. Described is that the subject is a human and has a urine level of PBG that is greater than, or greater than or equal to, 4.8 mmol/mol creatinine.

[0493] Described is that the reference value for plasma ALA is 0.12 μmol/L. Described is that the subject is a human

and has a plasma ALA level that is greater than, or greater than or equal to, 0.10 μmol/L, 0.12 μmol/L, 0.24 μmol/L, 0.36 μmol/L, 0.48 μmol/L, or 0.60 μmol/L. Described is that the subject is a human and has a plasma ALA level that is greater than, or greater than or equal to 0.48 μmol/L.

[0494] Described is that the reference value for urine ALA is 3.1 mmol/mol creatinine. Described is that the subject is a human and has a urine ALA level that is greater than, or greater than or equal to, 2.5 mmol/mol creatinine, 3.1 mmol/mol creatinine, 6.2 mmol/mol creatinine, 9.3 mmol/mol creatinine, 12.4 mmol/mol creatinine, or 15.5 mmol/mol creatinine.

[0495] Described is that the reference value for plasma porphyrin is 10 nmol/L. Described is that the subject is a human and has a plasma porphyrin level that is greater than, or greater than or equal to, 10 nmol/L. Described is that the subject is a human and has a plasma porphyrin level that is greater than, or greater than or equal to, 8, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nmol/L. the subject is a human and has a plasma porphyrin level that is greater than, or greater than or equal to 40 nmol/L. Described is that the reference value for urine porphyrin is 25 μmol/mol creatinine. Described is that the subject is a human and has a urine porphyrin level that is greater than, or greater than or equal to, 25 μmol/mol creatinine. Described is that the subject is a human and has a urine porphyrin level that is greater than, or equal to, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80 μmol/mol creatinine.

[0496] Described is that the subject has a level, e.g., a plasma level or a urine level, of a porphyrin or a porphyrin precursor, e.g., ALA or PBG, that is greater than that of 99% of individuals in a sample of healthy individuals.

[0497] Described is that the subject has a level, e.g., a plasma level or a urine level, of ALA or PBG that is greater than two standard deviations above the mean level in a sample of healthy individuals.

[0498] Described is that the subject has a urine level of ALA that is 1.6 or more times that of the mean level in a normal subject (e.g., a subject that does not carry a mutation associated with a porphyria). Described is that the subject has a plasma level of ALA that is 2 or 3 times that of the mean level in a normal subject. Described is that the subject has a urine level of PBG that is four or more times that of the mean level in a normal subject. Described is that the subject has a plasma level of PBG that is four or more times that of the mean level in a normal subject.

[0499] Described is that the method is effective to decrease the level of a porphyrin or a porphyrin precursor, e.g., ALA and/or PBG. Described is that the method is effective to produce a predetermined reduction in the elevated level of the porphyrin or porphyrin precursor, e.g., ALA or PBG. Described is that the predetermined reduction is a decrease of at least 10%, 20%, 30%, 40%, or 50%. Described is that the predetermined reduction is a reduction that is effective to prevent or ameliorate symptoms, e.g., pain or recurring attacks.

[0500] Described is that the predetermined reduction is a reduction that is at least 1, 2, 3, or more standard deviations, wherein the standard deviation is determined based on the values from a reference sample, e.g., a reference sample as described herein.

[0501] Described is that the predetermined reduction is a reduction that brings the level of the porphyrin or porphyrin precursor to a level that is less than, or to a level that is less than or equal to, a reference value (e.g., a reference value as described herein).

[0502] Described is that the subject to be treated according to the methods described suffers from pain, e.g., chronic pain. Described is that the subject has or is at risk for developing a porphyria, e.g. an acute hepatic porphyria, e.g., AIP. Described is that the method is effective to treat the pain, e.g., by reducing the severity of the pain or curing the pain. In embodiments, the method is effective to decrease or prevent nerve damage.

[0503] Described is that the subject to be treated according to the methods described herein (a) has an elevated level of ALA and/or PBG and (b) suffers from pain, e.g., chronic pain. In embodiments, the method is effective to decrease an elevated level of ALA and/or PBG and/or to treat the pain, e.g., by reducing the severity of the pain or curing the pain.

[0504] Described is that the subject is an animal that serves as a model for a disorder related to ALAS 1 expression.

[0505] Described is that the subject is an animal that serves as a model for porphyria (*e.g.*, a genetically modified animal with one or more mutations). Described is that the porphyria is AIP and the subject is an animal model of AIP. Described is that the subject is a genetically modified mouse that is deficient in porphobilinogen deaminase, such as, for example, the mouse described in Lindberg et al., Nature Genetics, 12:195-199, 1996, or the homozygous R167Q mouse described in Yasuda, M., Yu, C. Zhang, J., Clavero, S., Edelmann, W., Gan, L., Phillips, J.D., & Desnick, R.J. Acute intermittent porphyria: A severely affected knock-in mouse that mimics the human homozygous dominant phenotype. (Abstract of Presentation on October 14, 2011 at the American Society of Human Genetics; Program No. 1308F; accessed online on April 4, 2012 at ichg2011.org/cgi-bin/showdetail.pl?absno=21167); both of these references are hereby incorporated herein in their entirety. Several knock-in models for mutations causing homozygous dominant AIP in humans have been generated. The mutations employed include, *e.g.*, R167Q, R173Q, and R173W in PBG deaminase. Viable homozygotes included the R167Q/R176Q and R167Q/R173Q, both of which exhibit constitutively elevated ALA and PBG levels analogous to the phenotype in human homozygous dominant AIP; such a viable homozygous AIP mouse model may be the subject.

[0506] Described is that a subject to be treated according to the methods described herein, (*e.g.*, a human subject or patient), is at risk of developing, or has been diagnosed, with a disorder related to ALAS1 expression, e.g. a porphyria. Described is that the subject is a subject who has suffered one or more acute attacks of one or more porphyric symptoms.

Described is that the subject is a subject who has suffered chronically from one or more symptoms of porphyria (e.g., pain, e.g., neuropathic pain and or neuropathy, e.g., progressive neuropathy). Described is that the subject carries a genetic alteration (e.g., a mutation) as described herein but is otherwise asymptomatic. Described is that the subject has previously been treated with a heme product (e.g., hemin, heme arginate, or heme albumin), as described herein.

[0507] Described is that a subject (*e.g.*, a subject with a porphyria, such as, *e.g.*, AIP) to be treated according to the methods described herein has recently experienced or is currently experiencing a prodrome. Described is that the subject is administered a combination treatment, e.g., an iRNA as described herein, and one or more additional treatments known to be effective against porphyria (*e.g.*, glucose and/or a heme product such as hemin, as described herein) or its associated symptoms.

[0508] Described is that an iRNA as described herein is administered in combination with glucose or dextrose. For example, 10-20% dextrose in normal saline may be provided intravenously. Typically, when glucose is administered, at least 300 g of 10% glucose is administered intravenously daily. The iRNA (e.g., an iRNA in an LNP formulation) may also be administered intravenously, as part of the same infusion that is used to administer the glucose or dextrose, or as a separate infusion that is administered before, concurrently, or after the administration of the glucose or dextrose. Described is that the iRNA is administered via a different route of administration (*e.g.*, subcutaneously). Described is that the iRNA is administered in combination with total parenteral nutrition. The iRNA may be administered before, concurrent with, or after the administration of total parenteral nutrition.

[0509] Described is that the iRNA is administered in combination with a heme product (*e.g.*, hemin, heme arginate, or heme albumin). Described is that the iRNA is administered in combination with a heme product and glucose, a heme product and dextrose, or a heme product and total parenteral nutrition.

[0510] A "prodrome," as used herein, includes any symptom that the individual subject has previously experienced immediately prior to developing an acute attack. Typical symptoms of a prodrome include, e.g., abdominal pain, nausea, headaches, psychological symptoms (e.g., anxiety), restlessness and/or insomnia. Described is that the subject experiences pain (e.g., abdominal pain and/or a headache) during the prodrome. Described is that the subject experiences nausea during the prodrome. Described is that the subject experiences psychological symptoms (e.g., anxiety) during the prodrome. Described is that the subject becomes restless and/or suffers from insomnia during the prodrome.

[0511] An acute "attack" of porphyria involves the onset of one or more symptoms of porphyria, typically in a patient who carries a mutation associated with porphyria (*e.g.*, a mutation in a gene that encodes an enzyme in the porphyrin pathway).

[0512] Described is that administration of an ALAS1 iRNA results in a decrease in the level of one or more porphyrins or porphyrin precursors, as described herein (*e.g.*, ALA and/or PBG). The decrease may be measured relative to any appropriate control or reference value. For example, the decrease in the level of one or more porphyrins or porphyrin precursors may be established in an individual subject, *e.g.*, as a decrease of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% or more compared with the level prior to treatment (*e.g.*, immediately prior to treatment). A decrease in the level of a porphyrin precursor, a porphyrin, or or a porphyrin metabolite may be measured using any method known in the art. For example, the level of PBG and/or ALA in urine or plasma may be assessed, using the Watson-Schwartz test, ion exchange chromatography, or high-performance liquid chromatography - mass spectrometry. See, *e.g.*, Thunell (1993).

[0513] Described is that administration of an ALAS1 siRNA is effective to reduce the level of ALA and/or PBG in the subject. The level of ALA or PBG in the subject can be assessed, e.g., based on the absolute level of ALA or PBG, or based on the relative level of ALA or PBG (e.g., relative to the level of another protein or compound, e.g., the level of creatinine) in a sample from the subject. In some embodiments, the sample is a urine sample. In some embodiments, the sample is a plasma sample.

[0514] Described is that an iRNA that targets ALAS1 is administered in combination one or more additional treatments, e.g., another treatment known to be effective in treating porphyria or symptoms of porphyria. For example, the other treatment may be glucose (*e.g.*, IV glucose) or a heme product (*e.g.*, hemin, heme arginate, or heme albumin). The additional treatment(s) may be administered before, after, or concurrent with the administration of iRNA.

[0515] The iRNA and an additional therapeutic agent can be administered in combination in the same composition, *e.g.*, intravenously, or the additional therapeutic agent can be administered as part of a separate composition or by another method described herein.

[0516] Described is that administration of iRNA, or administration of iRNA in combination one or more additional treatments (*e.g.*, glucose, dextrose or the like), decreases the frequency of acute attacks (e.g., by preventing acute attacks so that they no longer occur, or by reducing the number of attacks that occur in a certain time period, e.g., fewer attacks occur per year). In some such embodiments, the iRNA is administered according to a regular dosing regimen, *e.g.*, daily, weekly, biweekly, or monthly.

[0517] Described is that the iRNA is administered after an acute attack of porphyria. In some such embodiments, the iRNA is in a composition, e.g. a composition comprising a lipid formulation, e.g. an LNP formulation.

[0518] Described is that the iRNA is administered during an acute attack of porphyria. In some such embodiments,

the iRNA is in a composition, e.g. a composition comprising a lipid formulation (e.g., an LNP formulation) or a composition comprising a GalNAc conjugate.

[0519] Described is that administration of an ALAS 1 siRNA is effective to lessen the severity of the attack (e.g., by ameliorating one or more signs or symptoms associated with the attack). Described is that administration of an ALAS 1 siRNA is effective to shorten the duration of an attack. Described is that administration of an ALAS 1 siRNA is effective to stop an attack. Described is that the iRNA is administered prophylactically to prevent an acute attack of porphyria. Described is that the iRNA is in the form of a GalNAc conjugate, e.g., in a composition comprising a GalNAc conjugate. Described is that the prophylactic administration is before, during, or after exposure to or occurrence of a precipitating factor. Described is that the subject is at risk of developing porphyria.

[0520] Described is that the siRNA is administered during a prodrome. Described is that the prodrome is characterized by pain (e.g., headache and/or abdominal pain), nausea, psychological symptoms (e.g., anxiety), restlessness and/or insomnia.

[0521] Described is that the siRNA is administered during a particular phase of the menstrual cycle, e.g., during the luteal phase.

[0522] Described is that administration of an ALAS 1 siRNA is effective to prevent attacks (e.g., recurrent attacks that are associated with a prodrome and/or with a precipitating factor, e.g., with a particular phase of the menstrual cycle, e.g., the luteal phase). Described is that administration of an ALAS 1 siRNA is effective to reduce the frequency of attacks. In embodiments, administration of an ALAS 1 siRNA is effective to lessen the severity of the attack (e.g., by ameliorating one or more signs or symptoms associated with the attack). Described is that administration of an ALAS 1 siRNA is effective to shorten the duration of an attack. Described is that administration of an ALAS 1 siRNA is effective to stop an attack.

[0523] Described is that administration of an ALAS 1 siRNA is effective to prevent or decrease the frequency or severity of pain, e.g., neuropathic pain.

[0524] Described is that administration of an ALAS1 siRNA is effective to prevent or decrease the frequency or severity of neuropathy.

[0525] Effects of administration of an ALAS 1 siRNA can be established, for example, by comparison with an appropriate control. For example, a decrease in the frequency of acute attacks, as well as a decrease in the level of one or more porphyrins or porphyrin precursors, may be established, for example, in a group of patients with AIP, as a decreased frequency compared with an appropriate control group. A control group (e.g., a group of similar individuals or the same group of individuals in a crossover design) may include, for example, an untreated population, a population that has been treated with a conventional treatment for porphyria (*e.g.*, a conventional treatment for AIP may include glucose, hemin, or both); a population that has been treated with placebo, or a non-targeting iRNA, optionally in combination with one or more conventional treatments for porphyria (*e.g.*, glucose, *e.g.*, IV glucose), and the like.

[0526] A subject "at risk" of developing porphyria, as used herein, includes a subject with a family history of porphyria and/or a history of one or more recurring or chronic porphyric symptoms, and/or a subject who carries a genetic alteration (e.g., a mutation) in a gene encoding an enzyme of the heme biosynthetic pathway, and a subject who carries a genetic alteration, e.g., a mutation. known to be associated with porphyria.

[0527] Described is that the alteration, e.g., the mutation, makes an individual susceptible to an acute attack (e.g., upon exposure to a precipitating factor, e.g., a drug, dieting or other precipitating factor, e.g., a precipitating factor as disclosed herein). Described is that the alteration, e.g., the mutation, is associated with elevated levels of a porphyrin or a porphyrin precursor (e.g., ALA and/or PBG). Described is that the alteration, e.g., the mutation, is associated with chronic pain (e.g., chronic neuropathic pain) and/or neuropathy (e.g., progressive neuropathy). Described is that the alteration, e.g., the mutation, is associated with changes in EMG and/or nerve conduction velocities.

[0528] Described is that the alteration is a mutation in the ALAS1 gene. Described is that the alteration is a mutation in the ALAS1 gene promoter, or in regions upstream or downstream from the ALAS 1 gene. Described is that the alteration is a mutation in transcription factors or other genes that interact with ALAS1. Described is that the alteration is an alteration, e.g., a mutation, in a gene that encodes an enzyme in the heme biosynthetic pathway.

[0529] Described is that the subject has an genetic alteration as described herein (e.g., a genetic mutation known to be associated with a porphyria). Described is that the subject has an elevated level (e.g., urine or plasma level) of ALA and/or PBG. Described is that the subject does not have an elevated level of ALA and/or PBG. Described is that the subject has a genetic alteration as described herein and has other symptoms, e.g., chronic pain, EMG changes, changes in nerve conduction velocity, and/or other symptoms associated with a porphyria. Described is that the subject has a genetic alteration but does not suffer from acute attacks.

[0530] Described is that the subject has a mutation associated with AIP, HCP, VP, or ADP.

[0531] Described is that the porphyria is AIP. In some such embodiments, the subject has an alteration, e.g., at least one mutation, in the PBG deaminase gene. Many PBG deaminase mutations are known in the art, for example, as reported in Hrdinka, M. et al. Physiological Research, 55 (Suppl 2):S119-136 (2006). Described is that the subject is heterozygous for a PBG deaminase mutation. Described is that the subject is homozygous for a PBG deaminase

mutation. A homozygous subject may carry two identical mutations or two different mutations in the PBG deaminase gene.

[0532] Described is that the porphyria is HCP. Described is that the subject has an alteration, e.g., at least one mutation, in the gene that encodes the enzyme coproporphyrinogen III oxidase.

[0533] Described is that the porphyria is VP. Described is that the subject has an alteration, e.g., at least one mutation, in the gene that encodes protoporphrinogen oxidase.

[0534] Described is that the porphyria is ADP, e.g., autosomal recessive ADP. Described is that the subject has an alteration, e.g., at least one mutation, in the gene that encodes ALA dehydratase.

[0535] Methods of treatment provided herein may serve to ameliorate one or more symptoms associated with porphyria, to reduce the frequency of attacks associated with porphyria, to reduce the likelihood that an attack of one or more symptoms associated with porphyria will occur upon exposure to a precipitating factor, or to reduce the risk of developing conditions associated with porphyria (*e.g.*, neuropathy (e.g., progressive neuropathy), hepatocellular cancer). Additionally, the methods provided herein may serve to decrease the level of one or more porphyrin precursors, porphyrins and/or related porphyrin products or metabolites. The level of a porphyrin precursor or a porhyrin may be measured in any biological sample, such as, *e.g.*, urine, blood, feces, cerebrospinal fluid, or a tissue sample. The sample may be present within a subject or may be obtained or extracted from the subject. Described is that the porphyria is AIP, and the level of PBG and/or ALA is decreased. Described is that the porphyrin product or metabolite is porphobilin, porphobilinogen, or uroporphyrin. A decrease in the level of a porphyrin product or metabolite may be measured using any method known in the art. For example, the level of PBG and/or ALA in urine or plasma may be assessed, using the Watson-Schwartz test, ion exchange chromatography, or high-performance liquid chromatography - mass spectrometry. See, *e.g.*, Thunell (1993).

[0536] Methods described herein may also serve to reduce chronically elevated levels of porphyrin precursors (e.g., ALA and/or PBG) in subjects suffering from a porphyria (e.g., an acute hepatic porphyria, e.g., AIP) or at risk for developing a porphyria. Methods for assessing plasma and urine levels (e.g., chronically elevated levels) of porphyrin precursors include, e.g., HPLC-mass spectrometry and ion-exchange chromatography. The levels of porphyrin precursors may be expressed as the level relative to another protein or compound, e.g., creatinine. See, e.g., Floderus, Y. et al, Clinical Chemistry, 52(4): 701-707, 2006; Sardh et al., Clinical Pharmacokinetics, 46(4): 335-349, 2007.

[0537] A "precipitating factor" as used herein, refers to an endogenous or exogenous factor that may induce an acute attack of one or more symptoms associated with porphyria. Precipitating factors include fasting (or other forms of reduced or inadequate caloric intake, due to crash diets, long-distance athletics, etc.), metabolic stresses (*e.g.*, infections, surgery, international air travel, and psychological stress), endogenous hormones (e.g., progesterone), cigarette smoking, lipid-soluble foreign chemicals (including, *e.g.*, chemicals present in tobacco smoke, certain prescription drugs, organic solvents, biocides, components in alcoholic beverages), endocrine factors (e.g., reproductive hormones (women may experience exacerbations during the premenstrual period), synthetic estrogens, progesterones, ovulation stimulants, and hormone replacement therapy). See, for example, Thunell (1993). Common precipitating factors include cytochrome P450 inducing drugs and phenobarbital.

[0538] Symptoms associated with porphyria may include abdominal pain or cramping, headaches, effects caused by nervous system abnormalities, and light sensitivity, causing rashes, blistering, and scarring of the skin (photodermatitis). Described is that the porphyria is AIP. Symptoms of AIP include gastrointestinal symptoms (*e.g.*, severe and poorly localized abdominal pain, nausea/vomiting, constipation, diarrhea, ileus), urinary symptoms (dysuria, urinary retention/incontinence, or dark urine), neurologic symptoms (*e.g.*, sensory neuropathy, motor neuropathy (e.g., affecting the cranial nerves and/or leading to weakness in the arms or legs), seizures, neuropathic pain, progressive neuropathy, headaches, neuropsychiatric symptoms (*e.g.*, mental confusion, anxiety, agitation, hallucination, hysteria, delirium, apathy, depression, phobias, psychosis, insomnia, somnolence, coma), autonomic nervous system involvement (resulting *e.g.*, in cardiovascular sysmptoms such as tachycardia, hypertension, and/or arrhythmias, as well as other symptoms, such as, e.g., increased circulating catecholamine levels, sweating, restlessness, and/or tremor), dehydration, and electrolyte abnormalities.

[0539] Described is that an iRNA targeting ALAS 1 is administered together with (*e.g.*, before, after, or concurrent with) another treatment that may serve to alleviate one or more of the above symptoms. For example, abdominal pain may be treated, *e.g.*, with narcotic analgesics, seizures may be treated, *e.g.,* with anti-seizure medications, nausea/vomiting may be treated, *e.g.,* with phenothiazines, and tachycardia/hypertension may be treated, *e.g.*, with beta blockers,

[0540] The term "decrease" (or "increase") is intended to refer to a measurable change, e.g., a statistically significant change. The change may be, for example, at least 5%, 10%, 20%, 30%, 40%, 50% or more change (*e.g.*, decrease (or increase) relative to a reference value, *e.g.,* a reference where no iRNA is provided).

[0541] The disclosure further relates to the use of an iRNA or a pharmaceutical composition thereof, *e.g.*, for treating a disorder related to ALAS1 expression, 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 the disorder. Described is that the iRNA or pharmaceutical composition thereof can be administered in conjunction with a heme product (*e.g.*, hemin, heme arginate, or heme albumin, as described herein) and/or in

conjunction with intravenous glucose infusions. In some embodiments, the iRNA or pharmaceutical composition thereof is used prophylactically, *e.g.*, to prevent or ameliorate symptoms of an anticipated attack of acute porphyria. The prophylactic use may be timed according to the exposure or anticipated exposure of the subject to a precipitating factor. As described herein, a precipitating factor may be any endogenous or exogenous factor known to precipitate an acute attack. For example, the premenstrual phase is an endogenous precipitating factor, and a cytochrome P450 inducing drug is an exogenous precipitating factor.

[0542] The effective amount for the treatment of a disorder related to ALAS 1 expression (e.g., a porphyria such as AIP) depends on the type of disorder to be treated, the severity of the symptoms, the subject being treated, the sex, age and general condition of the subject, the mode of administration and so forth. For any given case, an appropriate "effective amount" can be determined by one of ordinary skill in the art using routine experimentation. 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 ALAS1 or pharmaceutical composition thereof, "effective against" a disorder related to ALAS 1 expression indicates that administration in a clinically appropriate manner results in a beneficial effect, e.g., for an individual patient or for at least a fraction of patients, e.g., a statistically significant fraction of patients. Beneficial effects include, e.g., prevention of or reduction of symptoms or other effects. For example, beneficial effects include, e.g., an improvement (e.g., decrease in the severity or frequency) of symptoms, a reduction in the severity or frequency of attacks, a reduced risk of developing associated disease (e.g., neuropathy (e.g., progressive neuropathy), hepatocellular cancer), an improved ability to tolerate a precipitating factor, an improvement in quality of life, a reduction in the expression of ALAS 1, a reduction in a level (e.g., a plasma or urine level) of a porphyrin or a porphyrin precursor (e.g., ALA and/or PBG) or other effect generally recognized as positive by medical doctors familiar with treating the particular type of disorder.

[0543] A treatment or preventive effect is evident when there is an improvement, e.g., a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, e.g., 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 experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker (e.g., plasma or urinary ALA or PBG) or symptom is observed.

[0544] Patients can be administered a therapeutic amount of iRNA. The therapeutic amount can be, e.g., 0.05-50 mg/kg. For example, the therapeutic amount can be 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, or 2.5, 3.0, 3.5, 4.0, 4.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 mg/kg dsRNA.

[0545] Described is that the iRNA is formulated as a lipid formulation, e.g., an LNP formulation as described herein. Described is that the therapeutic amount is 0.05-5 mg/kg, e.g., 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, or 5.0 mg/kg dsRNA. Described is that the lipid formulation, e.g., LNP formulation, is administered intravenously.

[0546] Described is that the iRNA is administered by intravenous infusion over a period of time, such as over a 5 minute, 10 minute, 15 minute, 20 minute, or 25 minute period.

[0547] Described is that the iRNA is in the form of a GalNAc conjugate as described herein. Described is that the therapeutic amount is 0.5-50 mg, e.g., 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 mg/kg dsRNA. Described is that the GalNAc conjugate is administered subcutaneously.

[0548] Described is that the administration is repeated, for example, on a regular basis, such as, daily, biweekly (*i.e.*, every two weeks) for one month, two months, three months, four months or longer. After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration biweekly for three months, administration can be repeated once per month, for six months or a year or longer.

[0549] Described is that the iRNA agent is administered in two or more doses. Described is that the number or amount of subsequent doses is dependent on the achievement of a desired effect, e.g., suppression of a ALAS gene, reduction of a level of a porphyrin or porphyrin precursor (e.g., ALA and/or PBG), or the achievement of a therapeutic or prophylactic effect, *e.g.*, reduction or prevention of one or more symptoms associated with porphyria (e.g., pain, e.g., neuropathic pain), and/or prevention of attacks or reduction in the frequency and/or severity of attacks associated with porphyria.

[0550] Described is that the iRNA agent is administered according to a schedule. For example, the iRNA agent may be administered once per week, twice per week, three times per week, four times per week, or five times per week. Described is that the schedule involves regularly spaced administrations, *e.g.*, hourly, every four hours, every six hours, every eight hours, every twelve hours, daily, every 2 days, every 3 days, every 4 days, every 5 days, weekly, biweekly, or monthly. Described is that the iRNA agent is administered weekly or biweekly to achieve a desired effect, e.g., to decrease the level of ALA and/or PBG, to decrease pain, and/or to prevent acute attacks.

[0551] Described is that the schedule involves closely spaced administrations followed by a longer period of time during which the agent is not administered. For example, the schedule may involve an initial set of doses that are administered in a relatively short period of time (*e.g.*, about every 6 hours, about every 12 hours, about every 24 hours,

about every 48 hours, or about every 72 hours) followed by a longer time period (*e.g.*, about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, or about 8 weeks) during which the iRNA agent is not administered. Described is that the iRNA agent is initially administered hourly and is later administered at a longer interval (*e.g.*, daily, weekly, biweekly, or monthly). Described is that the iRNA agent is initially administered daily and is later administered at a longer interval (*e.g.*, weekly, biweekly, or monthly). Described is that the longer interval increases over time or is determined based on the achievement of a desired effect. Described is that the iRNA agent is administered once daily during an acute attack, followed by weekly dosing starting on the eighth day of administration. Described is that the iRNA agent is administered every other day during a first week followed by weekly dosing starting on the eighth day of administration.

[0552] Described is that the iRNA agent is administered to prevent or reduce the severity or frequency of recurring attacks, e.g., cyclical attacks associated with a precipitating factor. In some embodiments, the precipitating factor is the menstrual cycle. Described is that the iRNA is administered repeatedly, e.g., at regular intervals to prevent or reduce the severity or frequency of recurring attacks, e.g., cyclical attacks associated with a precipitating factor, e.g., the menstrual cycle, e.g., a particular phase of the menstrual cycle, e.g., the luteal phase. In some embodiments, the iRNA is administered during a particular phase of the menstrual cycle or based on hormone levels of the patient being treated (e.g., based on hormone levels that are associated with a particular phase of the menstrual cycle). Described is that the iRNA is administered on one or more particular days of the menstrual cycle, e.g., on day 1, 2, 3, 4, 5, 6, 7, 8. 9. 10. 11. 12. 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or on day 28 (or later day for subjects who have a longer menstrual cycle). Described is that the iRNA is administered during the luteal phase, e.g., on one or more days between days 14-28 of the menstrual cycle (or later, in subjects who have a menstrual cycle longer than 28 days). Described is that ovulation of the subject is assessed (e.g., using a blood or urine test that detects a hormone associated with ovulation, e.g., LH) and the iRNA is administered at a predetermined interval after ovulation. Described is that the iRNA is administered immediately after ovulation. In some embodiments, the iRNA is administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 days after ovulation. Any of these schedules may optionally be repeated for one or more iterations. The number of iterations may depend on the achievement of a desired effect, *e.g.,* the suppression of a ALAS1 gene and/or the achievement of a therapeutic or prophylactic effect, *e.g.*, reduce or prevent one or more symptoms associated with porphyria, to reduce the frequency of attacks associated with porphyria.

[0553] Described is that an initial dose of the iRNA agent is administered and the level of ALA or PBG is tested, e.g., 1-48 hours, e.g., 2, 4, 8, 12, or 24 hours following administration of the initial dose. Described is that if the level of ALA and/or PBG has decreased (e.g., to achieve a predetermined reduction, e.g., a normalization), and/or if the symptoms associated with porphyria (e.g., pain) have improved (e.g., such that the patient is asymptomatic), no further dose is administered, whereas if the level of ALA and/or PBG has not decreased (e.g., has not achieved a predetermined reduction, e.g., has not normalized), a further dose of ALA or PBG is administered. Described is that the further dose is administered 12, 24, 36, 48, 60, or 72 hours after the initial dose. Described is that if the initial dose is not effective to decrease the level of ALA and/or PBG, the further dose is modified, e.g., increased to achieve a desired decrease (e.g., a predetermined reduction, e.g., a normalization) in ALA or PBG levels.

[0554] Described is that the predetermined reduction is a decrease of at least 10%, 20%, 30%, 40%, or 50%. Described is that the predetermined reduction is a reduction that is effective to prevent or ameliorate symptoms, e.g., pain, prodromal symptoms, or recurring attacks.

[0555] Described is that the predetermined reduction is a reduction of at least 1, 2, 3, or more standard deviations, wherein the standard deviation is determined based on the values from a reference sample, e.g., a reference sample as described herein.

[0556] Described is that the predetermined reduction is a reduction that brings the level of the porphyrin or porphyrin precursor to a level that is less than, or to a level that is less than or equal to, a reference value (e.g., a reference value as described herein).

[0557] As used herein, a "normalization" in ALA or PBG levels (or a "normal" or "normalized" level) refers to a level (e.g., a urine and/or plasma level) of either ALA, or PBG, or both, that is within the expected range for a healthy individual, an individual who is asymptomatic (e.g., an individual who does not experience pain and/or suffer from neuropathy), or an individual who does not have a mutation associated with a porphyria. For example, described is that a normalized level is within two standard deviations of the normal mean. Described is that a normalized level is within normal reference limits, e.g., within the 95% confidence interval for an appropriate control sample, e.g., a sample of healthy individuals or individuals who do not carry a gene mutation associated with a porphyria. Described is that the ALA and/or PBG level of the subject (e.g., the urine and/or plasma ALA and/or PBG level) is monitored at intervals, a further dose of the iRNA agent is administered when the level increases above the reference value.

[0558] Administration of the iRNA may reduce ALAS1 mRNA or protein levels, e.g., in a cell, tissue, blood, urine or other compartment of the patient by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80 % or at least 90% or more. Administration of the iRNA may reduce levels of products associated with ALAS1 gene expression, e.g., levels of one or more porphyrins or porphyrin precursors

(e.g., the level of ALA and/or PBG). Administration of the iRNA agent may also inhibit or prevent the upregulation of ALAS1 mRNA or protein levels during an acute attack of AIP.

[0559] Before administration of a full dose of the iRNA, patients can be administered a smaller dose, such as a 5% infusion dose, and monitored for adverse effects, such as an allergic reaction, or for elevated lipid levels or blood pressure. In another example, the patient can be monitored for unwanted effects.

Methods for modulating expression of an ALAS1 gene

[0560] In yet another aspect, the disclosure provides a method for modulating (e.g., inhibiting or activating) the expression of an ALAS 1 gene, *e.g.*, in a cell or in a subject. Described is that the cell is *ex vivo*, *in vitro*, or *in vivo*. Described is that the cell is an erythroid cell or a hepatocyte. Described is that the cell is in a subject (e.g., a mammal, such as, for example, a human). Described is that the subject (*e.g.*, the human) is at risk, or is diagnosed with a disease related to ALAS 1 expression, as described above.

[0561] Described is that the method includes contacting the cell with an iRNA as described herein, in an amount effective to decrease the expression of an ALAS 1 gene in the cell. "Contacting," as used herein, includes directly contacting a cell, as well as indirectly contacting a cell. For example, a cell within a subject (*e.g.*, an erythroid cell or a liver cell, such as a hepatocyte) may be contacted when a composition comprising an iRNA is administered (*e.g.*, intravenously or subcutaneously) to the subject.

[0562] The expression of an ALAS 1 gene may be assessed based on the level of expression of an ALAS1 mRNA, an ALAS1 protein, or the level of a parameter functionally linked to the level of expression of an ALAS 1 gene (*e.g.*, the level of a porphyrin or the incidence or severity of a symptom related to a porphyria). Described is that the expression of ALAS 1 is inhibited by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%. Described is that the iRNA has an IC_{50} in the range of 0.001-0.01 nM, 0.001-0.10 nM, 0.001-1.0 nM, 0.001-10 nM, 0.01-0.05 nM, 0.01-0.50 nM, 0.02-0.60 nM, 0.01-1.0 nM, 0.01-1.5 nM, 0.01-10 nM. The IC_{50} value may be normalized relative to an appropriate control value, e.g., the IC_{50} of a non-targeting iRNA.

[0563] Described is that the method includes introducing into the cell an iRNA as described herein and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of an ALAS1 gene, thereby inhibiting the expression of the ALAS 1 gene in the cell.

[0564] Described is that the method includes administering a composition described herein, *e.g.*, a composition comprising an iRNA that targets ALAS 1, to the mammal such that expression of the target ALAS 1 gene is decreased, such as for an extended duration, *e.g.*, at least two, three, four days or more, *e.g.*, one week, two weeks, three weeks, or four weeks or longer. In some embodiments, the decrease in expression of ALAS 1 is detectable within 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, or 24 hours of the first administration.

[0565] Described is that the method includes administering a composition as described herein to a mammal such that expression of the target ALAS 1 gene is increased by *e.g.*, at least 10% compared to an untreated animal. Described is that the activation of ALAS1 occurs over an extended duration, *e.g.*, at least two, three, four days or more, *e.g.*, one week, two weeks, three weeks, four weeks, or more. Without wishing to be bound by theory, an iRNA can activate ALAS 1 expression by stabilizing the ALAS1 mRNA transcript, interacting with a promoter in the genome, and/or inhibiting an inhibitor of ALAS 1 expression.

[0566] The iRNAs useful for the methods and compositions featured in the disclosure specifically target RNAs (primary or processed) of an ALAS1 gene. Compositions and methods for inhibiting the expression of an ALAS 1 gene using iRNAs can be prepared and performed as described elsewhere herein.

[0567] Described is that the method includes administering a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the ALAS 1 gene of the mammal to be treated. When the organism to be treated is a mammal such as a human, the composition may 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.

[0568] Described is that the compositions are administered by intravenous infusion or injection. In some such embodiments, the compositions comprise a lipid formulated siRNA (*e.g.*, an LNP formulation, such as an LNP11 formulation) for intravenous infusion. In particular embodiments, such compositions may be used to treat acute attacks of porphyria and/or for prophylaxis (*e.g.*, to decrease the severity or frequency of attacks).

[0569] Described is that the compositions are administered subcutaneously. In some such embodiments, the compositions comprise an iRNA conjugated to a GalNAc ligand. Described is that such compositions may be used to treat acute attacks of porphyria or for prophylaxis (*e.g.*, to decrease the severity or frequency of attacks).

Methods for decreasing a level of a porphyrin or porphyrin precursor

[0570] In another aspect, the disclosure provides a method for decreasing a level of a porphyrin or a porphyrin precursor, *e.g.*, in a cell or in a subject.

[0571] Described is that the cell is *ex vivo*, *in vitro*, or *in vivo*. Described is that the cell is an erythroid cell or a hepatocyte. Described is that the cell is a hepatocyte. In some embodiments, the cell is in a subject (*e.g.*, a mammal, such as, for example, a human).

[0572] Described is that the subject (*e.g.*, the human) is at risk, or is diagnosed with a porphyria, as described herein. Described is that the method is effective to treat a porphyria as described herein (*e.g.*, by ameliorating one or more symptoms associated with a porphyria, reducing the frequency of attacks associated with a porphyria, reducing the likelihood that an attack of one or more symptoms associated with porphyria will occur upon exposure to a precipitating factor, or reducing the risk of developing conditions associated with a porphyria (*e.g.*, neuropathy (e.g., progressive neuropathy), hepatocellular cancer). Described is that the method includes contacting the cell with an RNAi, as described herein, in an amount sufficient to decrease the level of the porphyrin or porphyrin precursor (e.g., ALA or PBG) in the cell, or in another related cell or group of cells, or in the subject. "Contacting," as used herein, includes directly contacting a cell, as well as indirectly contacting a cell. For example, a cell within a subject (*e.g.*, an erythroid cell or a liver cell, such as a hepatocyte) may be contacted when a composition comprising an RNAi is administered (*e.g.*, intravenously or subcutaneously) to the subject. "Another related cell or group of cells," as used herein, includes any cell or group of cells in which the level of the porphyrin or porphyrin precursor decreases as a result of the contacting. For example, the cell may be part of a tissue present within a subject (*e.g.*, a liver cell present within a subject), and contacting the cell within the subject (*e.g.*, contacting one or more liver cells present within a subject) with the RNAi may result in a decrease in the level of the porphyrin or porphyrin precursor in another related cell or group of cells (*e.g.*, nerve cells of the subject), or in a tissue or fluid of the subject (*e.g.*, in the urine, blood, plasma, or cerebrospinal fluid of the subject).

[0573] Described is that the porphyrin or porphyrin precursor is selected from the group consisting of δ-aminolevulinic acid (ALA), porphopilinogen (PBG), hydroxymethylbilane (HMB), uroporphyrinogen III, coproporphyrinogen III, protoporphrinogen IX, and protoporphyrin IX In some embodiments the porphyrin precursor is ALA. Described is that the porphyrin precursor is PBG. In some embodiments, the method decreases the level of ALA and PBG. The level of a porphyrin or a porphyrin precursor may be measured as described herein and as known in the art.

[0574] 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.

EXAMPLES

Example 1. siRNA synthesis

Source of reagents

[0575] Where the source of a reagent is not specifically given herein, such reagent may be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

Oligonucleotide Synthesis.

[0576] All oligonucleotides are synthesized on an AKTAoligopilot synthesizer. Commercially available controlled pore glass solid support (dT-CPG, 500Å, Prime Synthesis) and RNA phosphoramidites with standard protecting groups, 5'-*O*-dimethoxytrityl N6-benzoyl-2'-*t*-butyldimethylsilyl-adenosine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N4-acetyl-2'-*t*-butyldimethylsilyl-cytidine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N2--isobutryl-2'-*t*-butyldimethylsilyl-guanosine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, and 5'-*O*-dimethoxytrityl-2'-*t*-butyldimethylsilyl-uridine-3'-O-N,N'-diisopropyl-2-cyanoethylphosphoramidite (Pierce Nucleic Acids Technologies) were used for the oligonucleotide synthesis. The 2'-F phosphoramidites, 5'-*O*-dimethoxytrityl-N4-acetyl-2'-fluro-cytidine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite and 5'-*O*-dimethoxytrityl-2'-fluro-uridine-3'-O-N,N'-diisopropyl-2-cyanoethyl-phosphoramidite are purchased from (Promega). All phosphoramidites are used at a concentration of 0.2M in acetonitrile (CH_3CN) except for guanosine which is used at 0.2M concentration in 10% THF/ANC (v/v). Coupling/recycling time of 16 minutes is used. The activator is 5-ethyl thiotetrazole (0.75M, American International Chemicals); for the PO-oxidation iodine/water/pyridine is used and for the PS-oxidation PADS (2%) in 2,6-lutidine/ACN (1:1 v/v) is used.

[0577] 3'-ligand conjugated strands are synthesized using solid support containing the corresponding ligand. For example, the introduction of cholesterol unit in the sequence is performed from a hydroxyprolinol-cholesterol phosphoramidite. Cholesterol is tethered to *trans*-4-hydroxyprolinol via a 6-aminohexanoate linkage to obtain a hydroxyprolinol-

cholesterol moiety. 5'-end Cy-3 and Cy-5.5 (fluorophore) labeled iRNAs are synthesized from the corresponding Quasar-570 (Cy-3) phosphoramidite are purchased from Biosearch Technologies. Conjugation of ligands to 5'-end and or internal position is achieved by using appropriately protected ligand-phosphoramidite building block. An extended 15 min coupling of 0.1 M solution of phosphoramidite in anhydrous CH_3CN in the presence of 5-(ethylthio)-1*H*-tetrazole activator to a solid-support-bound oligonucleotide. Oxidation of the internucleotide phosphite to the phosphate is carried out using standard iodine-water as reported (1) or by treatment with *tert*-butyl hydroperoxide/acetonitrile/water (10: 87: 3) with 10 min oxidation wait time conjugated oligonucleotide. Phosphorothioate is introduced by the oxidation of phosphite to phosphorothioate by using a sulfur transfer reagent such as DDTT (purchased from AM Chemicals), PADS and or Beaucage reagent. The cholesterol phosphoramidite is synthesized in house and used at a concentration of 0.1 M in dichloromethane. Coupling time for the cholesterol phosphoramidite is 16 minutes.

Deprotection I (Nucleobase Deprotection)

[0578] After completion of synthesis, the support is transferred to a 100 mL glass bottle (VWR). The oligonucleotide is cleaved from the support with simultaneous deprotection of base and phosphate groups with 80 mL of a mixture of ethanolic ammonia [ammonia: ethanol (3:1)] for 6.5 h at 55°C. The bottle is cooled briefly on ice and then the ethanolic ammonia mixture is filtered into a new 250-mL bottle. The CPG is washed with 2 x 40 mL portions of ethanol/water (1:1 v/v). The volume of the mixture is then reduced to ~ 30 mL by roto-vap. The mixture is then frozen on dry ice and dried under vacuum on a speed vac.

Deprotection II (Removal of 2'-TBDMS group)

[0579] The dried residue is resuspended in 26 mL of triethylamine, triethylamine trihydrofluoride (TEA•3HF) or pyridine-HF and DMSO (3:4:6) and heated at 60°C for 90 minutes to remove the *tert*-butyldimethylsilyl (TBDMS) groups at the 2' position. The reaction is then quenched with 50 mL of 20 mM sodium acetate and the pH is adjusted to 6.5. Oligonucleotide is stored in a freezer until purification.

Analysis

[0580] The oligonucleotides are analyzed by high-performance liquid chromatography (HPLC) prior to purification and selection of buffer and column depends on nature of the sequence and or conjugated ligand.

HPLC Purification

[0581] The ligand-conjugated oligonucleotides are purified by reverse-phase preparative HPLC. The unconjugated oligonucleotides are purified by anion-exchange HPLC on a TSK gel column packed in house. The buffers are 20 mM sodium phosphate (pH 8.5) in 10% CH_3CN (buffer A) and 20 mM sodium phosphate (pH 8.5) in 10% CH_3CN, 1M NaBr (buffer B). Fractions containing full-length oligonucleotides are pooled, desalted, and lyophilized. Approximately 0.15 OD of desalted oligonucleotidess are diluted in water to 150 μL and then pipetted into special vials for CGE and LC/MS analysis. Compounds are then analyzed by LC-ESMS and CGE.

siRNA preparation

[0582] For the general preparation of siRNA, equimolar amounts of sense and antisense strand are heated in 1xPBS at 95°C for 5 min and slowly cooled to room temperature. Integrity of the duplex is confirmed by HPLC analysis. Nucleic acid sequences are represented below using standard nomenclature, and specifically the abbreviations of Table 1.

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
Ab	beta-L-adenosine-3'-phosphate
Abs	beta-L-adenosine-3'-phosphorothioate

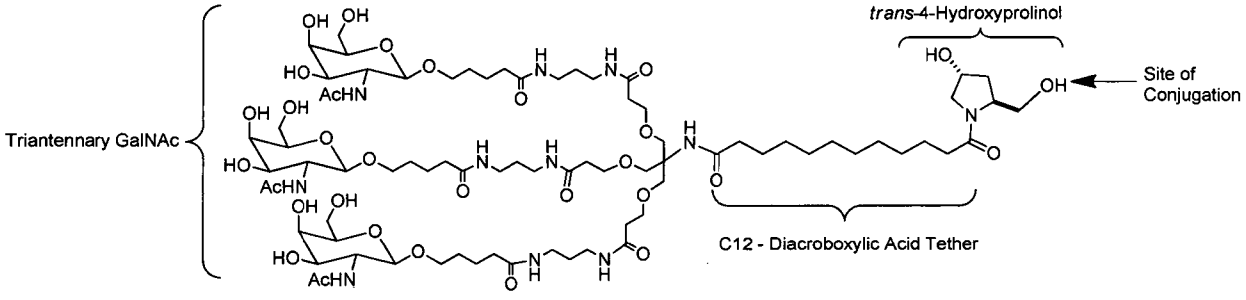
(continued)

Abbreviation	Nucleotide(s)
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cb	beta-L-cytidine-3'-phosphate
Cbs	beta-L-cytidine-3'-phosphorothioate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Chds)	2'-O-hexadecyl-cytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gb	beta-L-guanosine-3'-phosphate
Gbs	beta-L-guanosine-3'-phosphorothioate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tb	beta-L-thymidine-3'-phosphate
Tbs	beta-L-thymidine-3'-phosphorothioate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Ub	beta-L-uridine-3'-phosphate
Ubs	beta-L-uridine-3'-phosphorothioate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine-3'-phosphorothioate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate
(Uhds)	2'-O-hexadecyl-uridine-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

(continued)

Abbreviation	Nucleotide(s)
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
dA	2'-deoxyadenosine-3'-phosphate
dAs	2'-deoxyadenosine-3'-phosphorothioate
dC	2'-deoxycytidine-3'-phosphate
dCs	2'-deoxycytidine-3'-phosphorothioate
dG	2'-deoxyguanosine-3'-phosphate
dGs	2'-deoxyguanosine-3'-phosphorothioate
dT	2'-deoxythymidine
dTs	2'-deoxythymidine-3'-phosphorothioate
dU	2'-deoxyuridine
s	phosphorothioate linkage
L96[1]	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3
(Aeo)	2'-O-methoxyethyladenosine-3'-phosphate
(Aeos)	2'-O-methoxyethyladenosine-3'-phosphorothioate
(Geo)	2'-O-methoxyethylguanosine-3'-phosphate
(Geos)	2'-O-methoxyethylguanosine-3'- phosphorothioate
(Teo)	2'-O-methoxyethyl-5-methyluridine-3'-phosphate
(Teos)	2'-O-methoxyethyl-5-methyluridine-3'- phosphorothioate
(m5Ceo)	2'-O-methoxyethyl-5-methylcytidine-3'-phosphate
(m5Ceos)	2'-O-methoxyethyl-5-methylcytidine-3'-phosphorothioate

The chemical structure of L96 is as follows:



Example 2. ALAS1 siRNA Design and Synthesis

Experimental Methods

Bioinformatics

Transcripts

[0583] siRNA design was carried out to identify siRNAs targeting human, rhesus (*Macaca mulatta*), mouse, and rat ALAS1 transcripts annotated in the NCBI Gene database (http://www.ncbi.nlm.nih.gov/gene/). Design used the following transcripts from the NCBI RefSeq collection: Human -NM_000688.4 (see FIG.3), NM_199166.1; Rhesus - XM_001090440.2, XM_001090675.2; Mouse - NM_020559.2; Rat -NM_024484.2. Due to high primate/ rodent sequence divergence, siRNA duplexes were designed in several separate batches, including but not limited to batches containing duplexes matching human and rhesus transcripts only; human, rhesus, mouse, and rat transcripts only; and mouse and rat transcripts only. Most siRNA duplexes were designed that shared 100% identity the listed human transcript and other species transcripts considered in each design batch (above). In some instances, (see Table 8) mismatches between duplex and mRNA target were allowed at the first antisense (last sense) position when the antisense strand:target mRNA complementary basepair was a GC or CG pair. In these cases, duplexes were designed with UA or AU pairs at the first antisense:last sense pair. Thus the duplexes maintained complementarity but were mismatched with respect to target (U:C, U:G, A:C, or A:G). Eighteen of these "UA-swap" duplexes were designed as part of the human/rhesus/mouse/rat set (see duplexes in Table 8 with "C19U", "G19U", "C19A", or "G19A" labels in the Position column).

siRNA Design, Specificity, and Efficacy Prediction

[0584] The predicted specificity of all possible 19mers was predicted from each sequence. Candidate 19mers were then selected that lacked repeats longer than 7 nucleotides. These 1510 candidate human/rhesus, 114 human/rhesus/mouse/rat, and 717 mouse/rat siRNAs were used in comprehensive searches against the appropriate transcriptomes (defined as the set of NM_ and XM_ records within the human, rhesus, dog, mouse, or rat NCBI Refseq sets) using an exhaustive "brute-force" algorithm implemented in the python script 'BruteForce.py'. The script next parsed the transcript-oligo alignments to generate a score based on the position and number of mismatches between the siRNA and any potential 'off-target' transcript. The off-target score is weighted to emphasize differences in the 'seed' region of siRNAs, in positions 2-9 from the 5' end of the molecule. Each oligo-transcript pair from the brute-force search was given a mismatch score by summing the individual mismatch scores; mismatches in the position 2-9 were counted as 2.8, mismatches in the cleavage site positions 10-11 were counted as 1.2, and mismatches in region 12-19 counted as 1.0. An additional off-target prediction was carried out by comparing the frequency of heptamers and octomers derived from 3 distinct, seed-derived hexamers of each oligo. The hexamers from positions 2-7 relative to the 5' start is used to create 2 heptamers and one octomer. We create 'heptamer1' by adding a 3' A to the hexamer; we create heptamer2 by adding a 5' A to the hexamer; we create the octomer by adding an A to both 5' and 3' ends of the hexamer. The frequency of octomers and heptamers in the human, rhesus, mouse, or rat 3'UTRome (defined as the subsequence of the transcriptome from NCBI's Refseq database where the end of the coding region, the 'CDS', is clearly defined) was pre-calculated. The octomer frequency was normalized to the heptamer frequency using the median value from the range of octomer frequencies. A 'mirSeedScore' was then calculated by calculating the sum of ((3 X normalized octomer count) + (2 X heptamer2 count) + (1 X heptamer1 count)).

[0585] Both siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. We sorted by the specificity of the antisense strand. We then selected duplexes whose antisense oligos lacked GC at the first position, lacked G at both positions 13 and 14, and had 3 or more Us or As in the seed region (characteristics of duplexes with high predicted efficacy)

[0586] Candidate GalNac-conjugated duplexes, 21 and 23 nucleotides long on the sense and antisense strands respectively, were designed by extending antisense 19mers 4 additional nucleotides in the 3' direction (preserving perfect complementarity with the target transcript). The sense strand was specified as the reverse complement of the first 21 nucleotides of the antisense 23mer. Duplexes were selected that maintained perfect matches to all selected species transcripts across all 23 nucleotides.

siRNA sequence selection

[0587] A total of 90 sense and 90 antisense derived human/rhesus, 40 sense and 40 antisense derived human/rhesus/mouse/mouse/rat, and 40 sense and 40 antisense derived mouse/rat siRNA 19mer oligos were synthesized and

formed into duplexes. A total of 45 sense and 45 antisense derived human/rhesus 21/23mer oligos were synthesized to yield 45 GalNac-conjugated duplexes.

[0588] The sequences of the sense and antisense strands of the modified duplexes are shown in Table 2, and the sequences of the sense and antisense strands of the unmodified duplexes are shown in Table 3.

Synthesis of ALAS1 Sequences

[0589] ALAS1 sequences were synthesized on MerMade 192 synthesizer at either 1 or 0.2umol scale. Single strands were made with 2'O-methyl modifications for in vitro screening using transfection reagents. 3' GalNAc conjugates were made with sequences containing 2'F and 2'-O-methyl modifications on the sense strand in the 21-23 mer designs for free uptake in cells. For all the 21mer sequences in the list, 'endolight' chemistry was applied as detailed below.

- All pyrimidines (cytosine and uridine) in the sense strand contained 2'-O-Methyl bases (2' O-Methyl C and 2'-O-Methyl U)
- In the antisense strand, pyrimidines adjacent to(towards 5' position) ribo A nucleoside were replaced with their corresponding 2-O-Methyl nucleosides
- A two base dTsdT extension at 3' end of both sense and anti sense sequences was introduced
- The sequence file was converted to a text file to make it compatible for loading in the MerMade 192 synthesis software

[0590] For GalNAc conjugated sense strands and complementary antisense sequences, 2'F and other modified nucleosides were introduced in combination with ribo with 2'0-Methyl nucleosides. The synthesis was performed on a GalNAc modified CPG support for the sense strand and CPG modified with universal support on the antisense sequence.

Synthesis, Cleavage and deprotection:

[0591] The synthesis of ALAS1 sequences used solid supported oligonucleotide synthesis using phosphoramidite chemistry. For 21 mer endolight sequences, a deoxy thymidine CPG was used as the solid support while for the GalNAc conjugates, GalNAc solid support for sense strand and an universal CPG for the antisesense strand were used.

[0592] The synthesis of the above sequences was performed at either 1 or 0.2um scale in 96 well plates. The amidite solutions were prepared at 0.1 M concentration and ethyl thio tetrazole (0.6M in Acetonitrile) was used as activator.

[0593] The synthesized sequences were cleaved and deprotected in 96 well plates, using methylamine in the first step and fluoride reagent in the second step. For GalNAc and 2'F nucleoside containing sequences, deprotection conditions were modified. Sequences after cleavage and deprotection were precipitated using acetone: ethanol (80:20) mix and the pellet were re-suspended in 0.2M sodium acetate buffer. Samples from each sequence were analyzed by LC-MS to confirm the identity, UV for quantification and a selected set of samples by IEX chromatography to determine purity.

Purification and desalting:

[0594] ALAS1 sequences were precipitated and purified on AKTA Purifier system using Sephadex column. The ALAS1ess was run at ambient temperature. Sample injection and collection was performed in 96 well (1.8mL -deep well) plates. A single peak corresponding to the full length sequence was collected in the eluent. The desalted ALAS1 sequences were analyzed for concentration (by UV measurement at A260) and purity (by ion exchange HPLC). The complementary single strands were then combined in a 1:1 stoichiometric ratio to form siRNA duplexes.

Table 2: Human ALAS1 Modified Single Strands and Duplex Sequences

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2	3	522-540	AD-55078.2	cuccGGccAGuGAGAAAGAdTsdT	UCUUUCUcACUGGCCGGAGdTsdT
4	5	669-687	AD-55084.2	uGGcAGcAcAGAuGAAucAdTsdT	UGAUUcAUCUGUGCUGCcAdTsdT
6	7	790-808	AD-55090.2	cAGuGuGGuuAGuGuGAAAdTsdT	UUUcAcACuAACcAcACUGdTsdT
8	9	853-871	AD-55096.2	cAucAuGcAAAAGcAAAGAdTsdT	UCUUUGCUUUUGcAUGAUGdTsdT
10	11	876-894	AD-55102.2	AAAGAGuGucucAucuucudTsdT	AGAAGAUGAGAcACUCUUUdTsdT
12	13	877-895	AD-55106.2	AAGAGuGucucAucuucuudTsdT	AAGAAGAUGAGAcACUCUUdTsdT
14	15	914-932	AD-55111.2	ucuGuuuccAcuuuucAGudTsdT	ACUGAAAAGUGGAAAcAGAdTsdT
16	17	923-941	AD-55073.2	AcuuuucAGuAuGAucGuudTsdT	AACGAUcAuACUGAAAAG UdTsdT
18	19	926-944	AD-55079.2	uuucAGuAuGAucGuuucudTsdT	AGAAACGAUcAuACUGAAAdTsdT
20	21	927-945	AD-55085.2	uucAGuAuGAucGuuucuudTsdT	AAGAAACGAUcAuACUGAAdTsdT
22	23	928-946	AD-55091.2	ucAGuAuGAucGuuucuuudTsdT	AAAGAAACGAUcAuACUGAdTsdT
24	25	932-950	AD-55097.2	uAuGAucGuuucuuuGAGAdTsdT	UCUcAAAGAAACGAUcAuAdTsdT
26	27	973-991	AD-55103.2	uGAccAcAccuAucGAGuudTsdT	AACUCGAuAGGUGUGGUcAdTsdT
28	29	975-993	AD-55107.2	AccAcAccuAucGAGuuuudTsdT	AAAACUCGAuAGGUGUGGUdTsdT
30	31	1029-1047	AD-55112.2	uGGcAGAuGAcuAuucAGAdTsdT	UCUGAAuAGUcAUCUGCcAdTsdT
32	33	1077-1095	AD-55074.2	ucuGGuGcAGuAAuGAcuAdTsdT	uAGUcAUuACUGcACcAGAdTsdT
34	35	1124-1142	AD-55080.2	uGuGGGGcAGuuAuGGAcAdTsdT	UGUCcAuAACUGCCCcAcAdTsdT
36	37	1137-1155	AD-55086.2	uGGAcAcuuuGAAAcAAcAdTsdT	UGUUGUUUcAAAGUGUCcAdTsdT
38	39	1182-1200	AD-55098.2	AuAuuucuGGAAcuAGuAAdTsdT	UuACuAGUUCcAGAAAuAUdTsdT
40	41	1184-1202	AD-55104.2	AuuucuGGAAcuAGuAAAudTsdT	AUUuACuAGUUCcAGAAAUdTsdT
42	43	1185-1203	AD-55108.2	uuucuGGAAcuAGuAAAuudTsdT	AAUUuACuAGUUCcAGAAAdTsdT
44	45	1188-1206	AD-55113.2	cuGGAAcuAGuAAAuuccAdTsdT	UGGAAUUuACuAGUUCcAGdTsdT
46	47	1325-1343	AD-55075.2	uGuGAGAuuuAcucuGAuudTsdT	AAUcAGAGuAAAUCUcAcAdTsdT
48	49	1364-1382	AD-55081.2	AuccAAGGGAuucGAAAcAdTsdT	UGUUUCGAAUCCCUUGGAUdTsdT
50	51	1382-1400	AD-55087.2	AGccGAGuGccAAAGuAcAdTsdT	UGuACUUUGGcACUCGGCUdTsdT

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
52	53	1478-1496	AD-55093.2	uuuGAAAcuGuccAuucAAdTsdT	UUGAAUGGAcAGUUUcAAAdTsdT
54	55	1531-1549	AD-55099.2	uGAuGuGGcccAuGAGuuudTsdT	AAACUcAUGGGCcAcAUcAdTsdT
56	57	1631-1649	AD-53573.3	GucAuGccAAAAAuGGAcAdTsdT	UGUCcAUUUUUGGcAUGACdTsdT
58	59	1637-1655	AD-55109.2	ccAAAAAuGGAcAuCAuuudTsdT	AAAUGAUGUCcAUUUUUGGdTsdT
60	61	1706-1724	AD-55114.2	AcGAGuucucuGAuuGAcAdTsdT	UGUcAAUcAGAGAACUCGUdTsdT
62	63	1962-1980	AD-55076.2	AAGucuGuGAuGAAcuAAudTsdT	AUuAGUUcAUcAcAGACUUdTsdT
64	65	1967-1985	AD-55082.2	uGuGAuGAAcuAAuGAGcAdTsdT	UGCUcAUuAGUUcAUcAcAdTsdT
66	67	1977-1995	AD-55088.2	uAAuGAGcAGAcAuAAcAudTsdT	AUGUuAUGUCUGCUcAUuAdTsdT
68	69	2189-2207	AD-55094.2	uuuGAAGuGAuGAGuGAAAdTsdT	UUUcACUcAUcACUUcAAAdTsdT
70	71	2227-2245	AD-55100.2	AGGcuuGAGcAAGuuGGuAdTsdT	uACcAACUUGCUcAAGCCUdTsdT
72	73	2313-2331	AD-55105.2	ucuucAGAGuuGucuuuAudTsdT	AuAAAGAcAACUCUGAAGAdTsdT
74	75	2317-2335	AD-55110.2	cAGAGuuGucuuuAuAuGudTsdT	AcAuAuAAAGAcAACUCUGdTsdT
76	77	2319-2337	AD-55115.2	GAGuuGucuuuAuAuGuGAdTsdT	UcAcAuAuAAAGAcAACUCdTsdT
78	79	2320-2338	AD-55077.2	AGuuGucuuuAuAuGuGAAdTsdT	UUcAcAuAuAAAGAcAACUdTsdT
80	81	2344-2362	AD-55083.2	uuAuAuuAAAuuuuAAucudTsdT	AGAUuAAAAUUuAAuAuAAdTsdT
82	83	2352-2370	AD-55089.2	AAuuuuAAucuAuAGuAAAdTsdT	UUuACuAuAGAUuAAAAUUdTsdT
84	85	2353-2371	AD-55095.2	AuuuuAAucuAuAGuAAAAdTsdT	UUUuACuAuAGAUuAAAAUdTsdT
86	87	2376-2394	AD-55101.2	AGuccuGGAAAuAAAuucudTsdT	AGAAUUuAUUUCcAGGACUdTsdT
88	89	358-376	AD-53511.1	cuGcccAuucuuAucccGAdTsdT	UCGGGAuAAGAAUGGGcAGdTsdT
90	91	789-807	AD-53512.1	ccAGuGuGGuuAGuGuGAAdTsdT	UUcAcACuAACcAcACUGGdTsdT
92	93	1076-1094	AD-53513.1	GucuGGuGcAGuAAuGAcudTsdT	AGUcAUuACUGcACcAGACdTsdT
94	95	1253-1271	AD-53514.1	GcAcucuuGuuuuccucGudTsdT	ACGAGGAAAAcAAGAGUGCdTsdT
96	97	1544-1562	AD-53515.1	GAGuuuGGAGcAAucAccudTsdT	AGGUGAUUGCUCcAAACUCdTsdT
98	99	2228-2246	AD-53516.1	GGcuuGAGcAAGuuGGuAudTsdT	AuACcAACUUGCUcAAGCCdTsdT

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
100	101	404-422	AD-53517.1	GGcAAAucucuGuuGuucudTsdT	AGAAcAAcAGAGAUUUGCCdTsdT
102	103	404-422	AD-53517.1	GGcAAAucucuGuuGuucudTsdT	AGAAcAAcAGAGAUUUGCCdTsdT
104	105	866-884	AD-53518.1	cAAAGAccAGAAAGAGuGudTsdT	AcACUCUUUCUGGUCUUUGdTsdT
106	107	1080-1098	AD-53519.1	GGuGcAGuAAuGAcuAccudTsdT	AGGuAGUcAUuACUGcACCdTsdT
108	109	1258-1276	AD-53520.1	cuuGuuuuccucGuGcuuudTsdT	AAAGcACGAGGAAAAcAAGdTsdT
110	111	1616-1634	AD-53521.1	GGGGAucGGGAuGGAGucAdTsdT	UGACUCcAUCCCGAUCCCCdTsdT
112	113	2230-2248	AD-53522.1	cuuGAGcAAGuuGGuAucudTsdT	AGAuACcAACUUGCUcAAGdTsdT
114	115	436-454	AD-53523.1	ccccAAGAuGAuGGAAGuudTsdT	AACUUCcAUcAUCUUGGGGdTsdT
116	117	436-454	AD-53523.1	ccccAAGAuGAuGGAAGuudTsdT	AACUUCcAUcAUCUUGGGGdTsdT
118	119	885-903	AD-53524.1	cucAucuucuucAAGAuAAdTsdT	UuAUCUUGAAGAAGAUGAGdTsdT
120	121	1127-1145	AD-53525.1	GGGGcAGuuAuGGAcAcuudTsdT	AAGUGUCcAuAACUGCCCCdTsdT
122	123	1315-1333	AD-53526.1	GAuGccAGGcuGuGAGAuudTsdT	AAUCUcAcAGCCUGGcAUCdTsdT
124	125	1870-1888	AD-53527.1	GAGAcAGAuGcuAAuGGAudTsdT	AUCcAUuAGcAUCUGUCUCdTsdT
126	127	2286-2304	AD-53528.1	ccccAGGccAuuAucAuAudTsdT	AuAUGAuAAUGGCCUGGGGdTsdT
128	129	489-507	AD-53529.1	cAGcAGuAcAcuAccAAcAdTsdT	UGUUGGuAGUGuACUGCUGdTsdT
130	131	489-507	AD-53529.1	cAGcAGuAcAcuAccAAcAdTsdT	UGUUGGuAGUGuACUGCUGdTsdT
132	133	915-933	AD-53530.1	cuGuuuccAcuuuucAGuAdTsdT	uACUGAAAAGUGGAAAcAGdTsdT
134	135	1138-1156	AD-53531.1	GGAcAcuuuGAAAcAAcAudTsdT	AUGUUGUUUcAAAGUGUCCdTsdT
136	137	1324-1342	AD-53532.1	cuGuGAGAuuuAcucuGAudTsdT	AUcAGAGuAAAUCUcAcAGdTsdT
138	139	1927-1945	AD-53533.1	cccuGuGcGGGuuGcAGAudTsdT	AUCUGcAACCCGcAcAGGGdTsdT
140	141	2312-2330	AD-53534.1	GucuucAGAGuuGucuuuAdTsdT	uAAAGAcAACUCUGAAGACdTsdT
142	143	646-664	AD-53535.1	cAcuGcAAGcAAAuGcccudTsdT	AGGGcAUUUGCUUGcAGUGdTsdT
144	145	922-940	AD-53536.1	cAcuuuucAGuAuGAucGudTsdT	ACGAUcAuACUGAAAAGUGdTsdT
146	147	1163-1181	AD-53537.1	GGGGcAGGuGGuAcuAGAAdTsdT	UUCuAGuACcACCUGCCCCdTsdT

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
148	149	1347-1365	AD-53538.1	GGAAccAuGccuccAuGAudTsdT	AUcAUGGAGGcAUGGUUCCdTsdT
150	151	1964-1982	AD-53539.1	GucuG uGAuGAAcuAAuGAdTsdT	UcAUuAGUUcAUcAcAGACdTsdT
152	153	2321-2339	AD-53540.1	GuuGucuuuAuAuGuGAAudTsdT	AUUcAcAuAuAAAGAcAACdTsdT
154	155	671-689	AD-53541.1	GcAGcAcAGAuGAAucAGAdTsdT	UCUGAUUcAUCUGUGCUGCdTsdT
156	157	924-942	AD-53542.1	cuuuucAGuAuGAucGuuudTsdT	AAACGAUcAuACUGAAAAGdTsdT
158	159	1164-1182	AD-53543.1	GGGcAGGuGGuAcuAGAAAdTsdT	UUUCuAGuACcACCUGCCCdTsdT
160	161	1460-1478	AD-53544.1	GuccccAAGAuuGuGGcAudTsdT	AUGCcAcAAUCUUGGGGACdTsdT
162	163	1976-1994	AD-53545.1	cuAAuGAGcAGAcAuAAcAdTsdT	UGUuAUGUCUGCUcAUuAGdTsdT
164	165	786-804	AD-53546.1	GccccAGuGuGGuuAGuGudTsdT	AcACuAACcAcACUGGGGCdTsdT
166	167	935-953	AD-53547.1	GAucGuuucuuuGAGAAAAdTsdT	UUUUCUcAAAGAAACGAUCdTsdT
168	169	1165-1183	AD-53548.1	GGcAGGuGGuAcuAGAAAudTsdT	AUUUCuAGuACcACCUGCCdTsdT
170	171	1530-1548	AD-53549.1	GuGAuGuGGcccAuGAGuudTsdT	AACUcAUGGGCcAcAUcACdTsdT
172	173	2003-2021	AD-53550.1	cAAGcAAucAAuuAcccuAdTsdT	uAGGGuAAUUGAUUGCUUGdTsdT
174	175	788-806	AD-53551.1	cccAGuGuGGuuAGuGuGAdTsdT	UcAcACuAACcAcACUGGGdTsdT
176	177	974-992	AD-53552.1	GAccAcAccuAucGAGuuudTsdT	AAACUCGAuAGGUGUGGUCdTsdT
178	179	1191-1209	AD-53553.1	GAAcuAGuAAAuuccAuGudTsdT	AcAUGGAAUUuACuAGUUCdTsdT
180	181	1541-1559	AD-53554.1	cAuGAGuuuGGAGcAAucAdTsdT	UGAUUGCUCcAAACUcAUGdTsdT
182	183	2075-2093	AD-53555.1	ccccAGAuGAuGAAcuAcudTsdT	AGuAGUUcAUcAUCUGGGGdTsdT
184	185	360-378	AD-53561.1	GcccAuucuuAucccGAGudTsdT	ACUCGGGAuAAGAAUGGGCdTsdT
186	187	1356-1374	AD-53567.1	ccuccAuGAuccAAGGGAudTsdT	AUCCCUUGGAUcAUGGAGGdTsdT
188	189	1631-1649	AD-53573.1	GucAuGccAAAAAuGGAcAdTsdT	UGUCcAUUUUUGGcAUGACdTsdT
190	191	1634-1652	AD-53579.1	AuGccAAAAAuGGAcAucAdTsdT	UGAUGUCcAUUUUUGGcAUdTsdT

Table 3: Human ALAS1 Unmodified Single Strands and Duplex Sequences

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_000688.4	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
192	193	522-540	AD-55078.2	CUCCGGCCAGUGAGAAAGA	UCUUUCUCACUGGCCGGAG
194	195	669-687	AD-55084.2	UGGCAGCACAGAUGAAUCA	UGAUUCAUCUGUGCUGCCA
196	197	790-808	AD-55090.2	CAGUGUGGUUAGUGUGAAA	UUUCACACUAACCACACUG
198	199	853-871	AD-55096.2	CAUCAUGCAAAAGCAAAGA	UCUUUGCUUUUGCAUGAUG
200	201	876-894	AD-55102.2	AAAGAGUGUCUCAUCUUCU	AGAAGAUGAGACACUCUUU
202	203	877-895	AD-55106.2	AAGAGUGUCUCAUCUUCUU	AAGAAGAUGAGACACUCUU
204	205	914-932	AD-55111.2	UCUGUUUCCACUUUUCAGU	ACUGAAAAGUGGAAACAGA
206	207	923-941	AD-55073.2	ACUUUUCAGUAUGAUCGUU	AACGAUCAUACUGAAAAGU
208	209	926-944	AD-55079.2	UUUCAGUAUGAUCGUUUCU	AGAAACGAUCAUACUGAAA
210	211	927-945	AD-55085.2	UUCAGUAUGAUCGUUUCUU	AAGAAACGAUCAUACUGAA
212	213	928-946	AD-55091.2	UCAGUAUGAUCGUUUCUUU	AAAGAAACGAUCAUACUGA
214	215	932-950	AD-55097.2	UAUGAUCGUUUCUUUGAGA	UCUCAAAGAAACGAUCAUA
216	217	973-991	AD-55103.2	UGACCACACCUAUCGAGUU	AACUCGAUAGGUGUGGUCA
218	219	975-993	AD-55107.2	ACCACACCUAUCGAGUUUU	AAAACUCGAUAGGUGUGGU
220	221	1029-1047	AD-55112.2	UGGCAGAUGACUAUUCAGA	UCUGAAUAGUCAUCUGCCA
222	223	1077-1095	AD-55074.2	UCUGGUGCAGUAAUGACUA	UAGUCAUUACUGCACCAGA
224	225	1124-1142	AD-55080.2	UGUGGGGCAGUUAUGGACA	UGUCCAUAACUGCCCCACA
226	227	1137-1155	AD-55086.2	UGGACACUUUGAAACAACA	UGUUGUUUCAAAGUGUCCA
228	229	1182-1200	AD-55098.2	AUAUUUCUGGAACUAGUAA	UUACUAGUUCCAGAAAUAU
230	231	1184-1202	AD-55104.2	AUUUCUGGAACUAGUAAAU	AUUUACUAGUUCCAGAAAU
232	233	1185-1203	AD-55108.2	UUUCUGGAACUAGUAAAUU	AAUUUACUAGUUCCAGAAA
234	235	1188-1206	AD-55113.2	CUGGAACUAGUAAAUUCCA	UGGAAUUUACUAGUUCCAG
236	237	1325-1343	AD-55075.2	UGUGAGAUUUACUCUGAUU	AAUCAGAGUAAAUCUCACA
238	239	1364-1382	AD-55081.2	AUCCAAGGGAUUCGAAACA	UGUUUCGAAUCCCUUGGAU
240	241	1382-1400	AD-55087.2	AGCCGAGUGCCAAAGUACA	UGUACUUUGGCACUCGGCU

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_000688.4	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
242	243	1478-1496	AD-55093.2	UUUGAAACUGUCCAUUCAA	UUGAAUGGACAGUUUCAAA
244	245	1531-1549	AD-55099.2	UGAUGUGGCCCAUGAGUUU	AAACUCAUGGGCCACAUCA
246	247	1631-1649	AD-53573.3	GUCAUGCCAAAAAUGGACA	UGUCCAUUUUUGGCAUGAC
248	249	1637-1655	AD-55109.2	CCAAAAAUGGACAUCAUUU	AAAUGAUGUCCAUUUUUGG
250	251	1706-1724	AD-55114.2	ACGAGUUCUCUGAUUGACA	UGUCAAUCAGAGAACUCGU
252	253	1962-1980	AD-55076.2	AAGUCUGUGAUGAACUAAU	AUUAGUUCAUCACAGACUU
254	255	1967-1985	AD-55082.2	UGUGAUGAACUAAUGAGCA	UGCUCAUUAGUUCAUCACA
256	257	1977-1995	AD-55088.2	UAAUGAGCAGACAUAACAU	AUGUUAUGUCUGCUCAUUA
258	259	2189-2207	AD-55094.2	UUUGAAGUGAUGAGUGAAA	UUUCACUCAUCACUUCAAA
260	261	2227-2245	AD-55100.2	AGGCUUGAGCAAGUUGGUA	UACCAACUUGCUCAAGCCU
262	263	2313-2331	AD-55105.2	UCUUCAGAGUUGUCUUUAU	AUAAAGACAACUCUGAAGA
264	265	2317-2335	AD-55110.2	CAGAGUUGUCUUUAUAUGU	ACAUAUAAAGACAACUCUG
266	267	2319-2337	AD-55115.2	GAGUUGUCUUUAUAUGUGA	UCACAUAUAAAGACAACUC
268	269	2320-2338	AD-55077.2	AGUUGUCUUUAUAUGUGAA	UUCACAUAUAAAGACAACU
270	271	2344-2362	AD-55083.2	UUAUAUUAAAUUUUAAUCU	AGAUUAAAAUUUAAUAUAA
272	273	2352-2370	AD-55089.2	AAUUUUAAUCUAUAGUAAA	UUUACUAUAGAUUAAAAUU
274	275	2353-2371	AD-55095.2	AUUUUAAUCUAUAGUAAAA	UUUUACUAUAGAUUAAAAU
276	277	2376-2394	AD-55101.2	AGUCCUGGAAAUAAAUUCU	AGAAUUUAUUUCCAGGACU
278	279	358-376	AD-53511.1	CUGCCCAUUCUUAUCCCGA	UCGGGAUAAGAAUGGGCAG
280	281	789-807	AD-53512.1	CCAGUGUGGUUAGUGUGAA	UUCACACUAACCACACUGG
282	283	1076-1094	AD-53513.1	GUCUGGUGCAGUAAUGACU	AGUCAUUACUGCACCAGAC
284	285	1253-1271	AD-53514.1	GCACUCUUGUUUUCCUCGU	ACGAGGAAAACAAGAGUGC
286	287	1544-1562	AD-53515.1	GAGUUUGGAGCAAUCACCU	AGGUGAUUGCUCCAAACUC
288	289	2228-2246	AD-53516.1	GGCUUGAGCAAGUUGGUAU	AUACCAACUUGCUCAAGCC

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_000688.4	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
290	291	404-422	AD-53517.1	GGCAAAUCUCUGUUGUUCU	AGAACAACAGAGAUUUGCC
292	293	404-422	AD-53517.1	GGCAAAUCUCUGUUGUUCU	AGAACAACAGAGAUUUGCC
294	295	866-884	AD-53518.1	CAAAGACCAGAAAGAGUGU	ACACUCUUUCUGGUCUUUG
296	297	1080-1098	AD-53519.1	GGUGCAGUAAUGACUACCU	AGGUAGUCAUUACUGCACC
298	299	1258-1276	AD-53520.1	CUUGUUUUCCUCGUGCUUU	AAAGCACGAGGAAAACAAG
300	301	1616-1634	AD-53521.1	GGGGAUCGGGAUGGAGUCA	UGACUCCAUCCCGAUCCCC
302	303	2230-2248	AD-53522.1	CUUGAGCAAGUUGGUAUCU	AGAUACCAACUUGCUCAAG
304	305	436-454	AD-53523.1	CCCCAAGAUGAUGGAAGUU	AACUUCCAUCAUCUUGGGG
306	307	436-454	AD-53523.1	CCCCAAGAUGAUGGAAGUU	AACUUCCAUCAUCUUGGGG
308	309	885-903	AD-53524.1	CUCAUCUUCUUCAAGAUAA	UUAUCUUGAAGAAGAUGAG
310	311	1127-1145	AD-53525.1	GGGGCAGUUAUGGACACUU	AAGUGUCCAUAACUGCCCC
312	313	1315-1333	AD-53526.1	GAUGCCAGGCUGUGAGAUU	AAUCUCACAGCCUGGCAUC
314	315	1870-1888	AD-53527.1	GAGACAGAUGCUAAUGGAU	AUCCAUUAGCAUCUGUCUC
316	317	2286-2304	AD-53528.1	CCCCAGGCCAUUAUCAUAU	AUAUGAUAAUGGCCUGGGG
318	319	489-507	AD-53529.1	CAGCAGUACACUACCAACA	UGUUGGUAGUGUACUGCUG
320	321	489-507	AD-53529.1	CAGCAGUACACUACCAACA	UGUUGGUAGUGUACUGCUG
322	323	915-933	AD-53530.1	CUGUUUCCACUUUUCAGUA	UACUGAAAAGUGGAAACAG
324	325	1138-1156	AD-53531.1	GGACACUUUGAAACAACAU	AUGUUGUUUCAAAGUGUCC
326	327	1324-1342	AD-53532.1	CUGUGAGAUUUACUCUGAU	AUCAGAGUAAAUCUCACAG
328	329	1927-1945	AD-53533.1	CCCUGUGCGGGUUGCAGAU	AUCUGCAACCCGCACAGGG
330	331	2312-2330	AD-53534.1	GUCUUCAGAGUUGUCUUUA	UAAAGACAACUCUGAAGAC
332	333	646-664	AD-53535.1	CACUGCAAGCAAAUGCCCU	AGGGCAUUUGCUUGCAGUG
334	335	922-940	AD-53536.1	CACUUUUCAGUAUGAUCGU	ACGAUCAUACUGAAAAGUG
336	337	1163-1181	AD-53537.1	GGGGCAGGUGGUACUAGAA	UUCUAGUACCACCUGCCCC

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_000688.4	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
338	339	1347-1365	AD-53538.1	GGAACCAUGCCUCCAUGAU	AUCAUGGAGGCAUGGUUCC
340	341	1964-1982	AD-53539.1	GUCUGUGAUGAACUAAUGA	UCAUUAGUUCAUCACAGAC
342	343	2321-2339	AD-53540.1	GUUGUCUUUAUAUGUGAAU	AUUCACAUAUAAAGACAAC
344	345	671-689	AD-53541.1	GCAGCACAGAUGAAUCAGA	UCUGAUUCAUCUGUGCUGC
346	347	924-942	AD-53542.1	CUUUUCAGUAUGAUCGUUU	AAACGAUCAUACUGAAAAG
348	349	1164-1182	AD-53543.1	GGGCAGGUGGUACUAGAAA	UUUCUAGUACCACCUGCCC
350	351	1460-1478	AD-53544.1	GUCCCCAAGAUUGUGGCAU	AUGCCACAAUCUUGGGGAC
352	353	1976-1994	AD-53545.1	CUAAUGAGCAGACAUAACA	UGUUAUGUCUGCUCAUUAG
354	355	786-804	AD-53546.1	GCCCCAGUGUGGUUAGUGU	ACACUAACCACACUGGGGC
356	357	935-953	AD-53547.1	GAUCGUUUCUUUGAGAAAA	UUUUCUCAAAGAAACGAUC
358	359	1165-1183	AD-53548.1	GGCAGGUGGUACUAGAAAU	AUUUCUAGUACCACCUGCC
360	361	1530-1548	AD-53549.1	GUGAUGUGGCCCAUGAGUU	AACUCAUGGGCCACAUCAC
362	363	2003-2021	AD-53550.1	CAAGCAAUCAAUUACCCUA	UAGGGUAAUUGAUUGCUUG
364	365	788-806	AD-53551.1	CCCAGUGUGGUUAGUGUGA	UCACACUAACCACACUGGG
366	367	974-992	AD-53552.1	GACCACACCUAUCGAGUUU	AAACUCGAUAGGUGUGGUC
368	369	1191-1209	AD-53553.1	GAACUAGUAAAUUCCAUGU	ACAUGGAAUUUACUAGUUC
370	371	1541-1559	AD-53554.1	CAUGAGUUUGGAGCAAUCA	UGAUUGCUCCAAACUCAUG
372	373	2075-2093	AD-53555.1	CCCCAGAUGAUGAACUACU	AGUAGUUCAUCAUCUGGGG
374	375	360-378	AD-53561.1	GCCCAUUCUUAUCCCGAGU	ACUCGGGAUAAGAAUGGGC
376	377	1356-1374	AD-53567.1	CCUCCAUGAUCCAAGGGAU	AUCCCUUGGAUCAUGGAGG
378	379	1631-1649	AD-53573.1	GUCAUGCCAAAAAUGGACA	UGUCCAUUUUUGGCAUGAC
380	381	1634-1652	AD-53579.1	AUGCCAAAAAUGGACAUCA	UGAUGUCCAUUUUUGGCAU

Example 3. *In vitro* screening of ALAS1 siRNA duplexes for ALAS1 knockdown activity.

[0595] ALAS 1 siRNA duplexes were screened for the ability to knockdown ALAS 1 expression *in vitro.*

***In vitro* screening**

Cell culture and transfections

[0596] Hep3B cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in MEM (ATCC) supplemented with 10% FBS, before being released from the plate by trypsinization. Transfection was carried out by adding 14.8μl of Opti-MEM plus 0.2μl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5μl of siRNA duplexes per well into a 96-well plate and incubated at room temperature for 15 minutes. 80μl of complete growth media containing ~2 x 10^4 Hep3B cells were then added to the siRNA mixture. Cells were incubated for either 24 or 120 hours prior to RNA purification. Single dose experiments were performed at 10nM and 0.1nM final duplex concentration and dose response experiments were done at 10, 1.67, 0.27, 0.046, 0.0077, 0.0013, 0.00021, 0.00004 nM final duplex concentration.

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

[0597] Cells were harvested and lysed in 150μl of Lysis/Binding Buffer then mixed for 5 minutes at 850rpm using an Eppendorf Thermomixer (the mixing speed was the same throughout the process). Ten microliters of magnetic beads and 80μl Lysis/Binding Buffer mixture were added to a round bottom plate and mixed for 1 minute. Magnetic beads were captured using magnetic stand and the supernatant was removed without disturbing the beads. After removing supernatant, the lysed cells were added to the remaining beads and mixed for 5 minutes. After removing supernatant, magnetic beads were washed 2 times with 150μl Wash Buffer A and mixed for 1 minute. Beads were captured again and supernatant removed. Beads were then washed with 150μl Wash Buffer B, captured and supernatant was removed. Beads were next washed with 150μl Elution Buffer, captured and supernatant removed. Beads were allowed to dry for 2 minutes. After drying, 50μl of Elution Buffer was added and mixed for 5 minutes at 70°C. Beads were captured on magnet for 5 minutes. 40μl of supernatant was removed and added to another 96 well plate.

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

[0598] A master mix of 2)μl 10X Buffer, 0.8μl 25X dNTPs, 2μl Random primers, 1μl Reverse Transcriptase, 1μl RNase inhibitor and 3.2μl of H_2O per reaction were added into 10μl total RNA. cDNA was generated using a Bio-Rad C-1000 or S-1000 thermal cycler (Hercules, CA) through the following steps: 25°C 10 min, 37°C 120 min, 85°C 5 sec, 4°C hold.

Real time PCR

[0599] 2μl of cDNA were added to a master mix containing 0.5μl GAPDH TaqMan Probe (Applied Biosystems Cat #4326317E), 0.5μl ALAS1 TaqMan probe (Applied Biosystems cat # Hs00167441_ml) and 5μl Lightcycler 480 probe master mix (Roche Cat #04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a Roche LC480 Real Time PCR system (Roche) using the ΔΔCt(RQ) assay. Each duplex was tested in two independent transfections with two biological replicates each, and each transfection was assayed in duplicate, unless otherwise noted in the summary tables.

[0600] To calculate relative fold change, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC50s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 or naïve cells over the same dose range, or to its own lowest dose.

In vitro knockdown of endogenous ALAS1 expression by ALAS1 siRNA duplexes

[0601] Table 4 illustrates the knockdown of ALAS1 in Hep3B cells by ALAS1 modified siRNA duplexes (See Table 2). Silencing is expressed as the fraction RNA message remaining relative to the negative (luciferase) control siRNA AD-1955. Data were generated as described above following transfection of 10 nM or 0.1 nM of each siRNA. qPCR was run using the ALAS1 TaqMan probe Hs00167441_ml.

Table 4: ALAS1 expression in Hep3B cells following transfection with ALAS1 siRNA

Duplex ID	10nM Avg	0.1nM Avg	10nM STDEV	0.1nM STDEV
AD-55078.2	0.7	0.87	0.001	0.089
AD-55084.2	0.08	0.3	0	0.04
AD-55090.2	0.06	0.08	0.002	0.003
AD-55096.2	0.61	0.92	0.171	0.34
AD-55102.2	0.63	0.62	0.005	0.069
AD-55106.2	0.07	0.08	0.004	0.027
AD-55111.2	0.06	0.23	0.013	0.062
AD-55073.2	0.21	0.4	0.018	0.061
AD-55079.2	0.17	0.43	0.033	0.089
AD-55085.2	0.13	0.21	0.011	0.019
AD-55091.2	0.27	0.55	0.033	0.009
AD-55097.2	0.31	0.38	0.051	0.059
AD-55103.2	0.05	0.11	0.017	0.006
AD-55107.2	0.12	0.24	0.007	0.008
AD-55112.2	0.15	0.2	0.036	0.025
AD-55074.2	0.16	0.45	0.008	0.002
AD-55080.2	0.79	0.99	0.095	0.304
AD-55086.2	0.09	0.22	0.005	0.035
AD-55098.2	0.25	0.51	0.03	0.07
AD-55104.2	0.06	0.1	0.017	0.001
AD-55108.2	0.47	0.65	0.03	0.015
AD-55113.2	0.38	0.62	0.068	0.039
AD-55075.2	0.12	0.28	0.007	0.051
AD-55081.2	0.21	0.51	0.036	0.066
AD-55087.2	0.1	0.19	0.017	0.02
AD-55093.2	0.24	0.56	0.029	0.053
AD-55099.2	0.05	0.18	0.001	0.038
AD-53573.3	0.67	1.07	0.16	0.153
AD-55109.2	0.07	0.23	0.006	0.052
AD-55114.2	0.08	0.16	0.004	0.017
AD-55076.2	0.05	0.14	0.007	0.035
AD-55082.2	0.08	0.3	0.019	0.016
AD-55088.2	0.06	0.12	0.008	0.02
AD-55094.2	0.06	0.18	0.005	0.023
AD-55100.2	0.45	0.83	0.02	0.05
AD-55105.2	0.02	0.05	0.005	0.004
AD-55110.2	0.15	0.19	0.031	0.016

(continued)

Duplex ID	10nM Avg	0.1nM Avg	10nM STDEV	0.1nM STDEV
AD-55115.2	0.35	0.58	0.045	0.052
AD-55077.2	0.14	0.14	0.006	0.019
AD-55083.2	0.56	0.98	0.24	0.188
AD-55089.2	0.62	0.79	0.036	0.094
AD-55095.2	0.59	0.92	0.12	0.079
AD-55101.2	0.71	0.97	0.074	0.097
AD-1955	1.00	1.01	0.03	0.04
AD-53511.1	0.84	1.08	0.028	0.0515
AD-53512.1	0.15	0.65	0.062	0.023
AD-53513.1	0.34	0.86	0.055	0.011
AD-53514.1	0.12	0.61	0.003	0.008
AD-53515.1	0.25	0.66	0.005	0.004
AD-53516.1	1.05	1.02	0.032	0.011
AD-53517.1	0.145	0.725	0.025	0.0155
AD-53518.1	0.72	0.85	0.045	0.028
AD-53519.1	0.18	0.66	0.061	0.004
AD-53520.1	0.18	0.9	0.041	0.001
AD-53521.1	0.97	1.07	0.01	0.003
AD-53522.1	0.87	1.1	0.065	0.112
AD-53523.1	0.48	0.96	0.0305	0.0255
AD-53524.1	0.11	0.66	0.02	0.006
AD-53525.1	0.71	1.03	0.016	0.01
AD-53526.1	0.23	0.85	0.075	0.01
AD-53527.1	0.25	0.83	0.015	0.017
AD-53528.1	0.44	0.93	0.037	0.006
AD-53529.1	0.185	0.73	0.015	0.014
AD-53530.1	0.1	0.62	0.02	0.003
AD-53531.1	0.48	0.93	0.019	0.045
AD-53532.1	0.06	0.17	0	0.003
AD-53533.1	0.36	0.93	0.025	0.034
AD-53534.1	0.1	0.36	0.014	0.012
AD-53535.1	0.58	1.05	0.036	0.071
AD-53536.1	0.12	0.45	0.009	0.026
AD-53537.1	0.73	0.96	0.101	0.015
AD-53538.1	0.74	1.07	0	0.046
AD-53539.1	0.52	0.97	0.057	0.032
AD-53540.1	0.1	0.47	0.017	0.012
AD-53541.1	0.11	0.29	0.026	0.015

(continued)

Duplex ID	10nM Avg	0.1nM Avg	10nM STDEV	0.1nM STDEV
AD-53542.1	0.08	0.23	0.008	0.006
AD-53543.1	0.62	1.01	0.027	0.014
AD-53544.1	0.8	1.04	0.002	0.001
AD-53545.1	0.17	0.73	0.007	0.007
AD-53546.1	0.27	0.93	0.058	0.019
AD-53547.1	0.12	0.28	0.008	0.01
AD-53548.1	0.1	0.34	0.022	0.002
AD-53549.1	0.8	1.04	0.011	0.026
AD-53550.1	0.05	0.54	0.02	0.003
AD-53551.1	0.96	1.16	0.029	0.044
AD-53552.1	0.13	0.5	0.002	0.009
AD-53553.1	0.92	1.1	0.027	0.02
AD-53554.1	0.76	0.67	0.005	0.004
AD-53555.1	0.11	0.53	0.009	0.007
AD-53561.1	0.72	0.94	0.014	0.001
AD-53567.1	0.16	0.66	0.019	0.003
AD-53573.1	1.06	1.10	0.019	0.037
AD-53579.1	0.19	0.76	0.036	0.019

IC_{50}s of select ALAS1 siRNA duplexes in *in vitro* screen

[0602] Table 5 illustrates the IC_{50}s of select ALAS1 siRNA duplexes determined from the knockdown of endogenously expressed ALAS 1 in the Hep3B cell line, by ALAS 1 modified siRNA duplexes (see Table 2). Data were generated as described above, at 24 or 120 hours following transfection of each siRNA duplex. Silencing of ALAS1 is expressed as the fraction mRNA message remaining relative to the siRNA AD-1955, a non-targeting siRNA that was used as a negative control. Data from replicate transfection experiments were used to fit a single line to determine the IC_{50}. Several of the duplexes (e.g., AD-53541.1, AD-53542.1, and AD-53547.1) had an IC_{50} as low as about 0.03 nM at 24 hours. Numerous duplexes had an IC_{50} of less than 0.1 nM (e.g., AD-53534.1, AD-53536.1, AD-53540.1, AD-53541.1, AD-53542.1, AD-53547.1, AD-53548.1, AD-53550.1, AD-53552.1) at 24 hours, and some of these also had an IC_{50} of less than 0.1 nM (e.g., AD-53534.1, AD-53540.1, AD-53541.1, AD-53542.1, AD-53547.1, AD-53552.1) at 120 hours.

Table 5: IC_{50}s of select ALAS1 siRNA duplexes normalized to AD-1955

	IC50 (nM)	
DUPLEX ID	24hrs	120hrs
AD-53534.1	0.045	0.076
AD-53536.1	0.049	0.105
AD-53540.1	0.054	0.077
AD-53541.1	0.032	0.062
AD-53542.1	0.028	0.093
AD-53547.1	0.03	0.062
AD-53548.1	0.044	0.101
AD-53550.1	0.085	0.152

(continued)

	IC50 (nM)	
DUPLEX ID	24hrs	120hrs
AD-53552.1	0.077	0.063
AD-53567.1	0.219	0.357
AD-53579.1	0.217	0.566

Example 4. *In Vivo* Silencing using a mouse/rat ALAS1 siRNA formulated as a LNP

[0603] The sequences of the modified duplex AD-53558 are shown in Table 6 below.

Table 6: Sequences of ALAS1 siRNA Duplex AD-53558.4

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Start Position on transcript of NM_020559.2	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
383	384	1184	AD-53558	cuGuGAAAuuuAcucuGAudTsdT	AUcAGAGuAAAUUUcAcAGdTsdT

[0604] This duplex was formulated as a LNP11 formulation (see Table 10 above). The LNP-formulated AD-53558 siRNA was tested in *in vivo* in mice (N=25 animals; 5 animals per group) and rats (N=20 animals; 4 animals per group) and was confirmed to silence ALAS1 mRNA *in vivo.* The results are shown in FIG. 5 and FIG. 6.

[0605] FIG. 5 shows that the siRNA demonstrated a dose-response effect in mice. The expression of mouse ALAS1 (mALAS1) mRNA was reduced by about 78% when the siRNA was administered at 1mg/kg; mouse ALAS 1 mRNA was reduced by about 60% when the siRNA was administered at 0.3 mg/kg; and mouse ALAS1 mRNA was reduced by about 49% when the siRNA was administered at 0.1 mg/kg. These reductions are expressed relative to a PBS control. An AD-1955 LUC control was also employed, as shown in FIG. 5.

[0606] Similarly, FIG. 6 shows that the siRNA demonstrated a dose-response effect in rats. The expression of ALAS1 RNA was reduced by about 70% when the when the siRNA was administered at 1mg/kg; ALAS1 mRNA was reduced by about 62% when the siRNA was administered at 0.3 mg/kg; and ALAS 1 mRNA was reduced by about 34% when the siRNA was administered at 0.1 mg/kg.

[0607] The durability of silencing was also tested in mice (N=15; 3 animals per timepoint. The results are shown in FIG. 7, which shows that AD-53558 suppressed mALAS1 mRNA by about 80% for at least 9 days. Suppression of at least about 50% persisted for at least 14 days.

Example 5. Efficacy of ALAS1 siRNA in an Animal Model of AIP

[0608] The effects of the AD-53558 LNP11 formulation (a mouse/rat **ALAS1 siRNA** described in the previous example) were investigated in a mouse model of AIP. The PBGD knockout is not viable (-/-, 0% activity). Heterozygous PBGD knockout mice (+/-, ~50% activity) are available but do not have the full biochemical phenotype and thus do not recapitulate the human disease phenotype. Thus, a mouse model of AIP has been developed that is a compound heterozygote with T1/T2 alleles, including T1 (+/-) promoter disruption and T2 (-/-) splice-site alteration. These mice have been shown to have hepatic residual PBGD activity that is about ~30% of the wild-type level and normal or slightly elevated baseline plasma ALA and PBG levels. The mice have been found to appear normal early in life and to become slightly slower and ataxic with age. By six months of age, the mice have been documented to develop impaired motor coordination and muscular performance and axonal degeneration on pathological examination. Investigation of the pathology of the mouse model has shown axonal degeneration, impaired motor coordination and muscular performance in older mice. Urinary and plasma ALA and PBG have been found to markedly increase with serial i.p. administration of phenobarbital (see Lindberg et al., (1996), Nature Genetics, 12:195-219 and Lindberg et al., (1999), Journal of Clinical Investigation, 103:1127-34). The mice were rescued by AAV-mediated expression of PBGD in the liver (Yasuda et al. (2010), Molecular Medicine, 1:17-22 and Unzu et al. (2011), Molecular Medicine, 2:243-50).

[0609] On day 1, the mice were administered 1 mg/kg ALAS1 siRNA (n=5) or LUC AD-1955 control (n=3) by i.v. injection. Three phenobarbital injections were given (1 injection per day on days 2, 3, and 4) to induce hepatic ALAS1 and the porphyrin precursors, ALA and PBG. Plasma and overnight urine specimens were collected on day 5 and metabolite levels were measured by LC-MS. Metabolite levels were measured in plasma by LC-MS and were also measured in urine. Baseline levels of metabolites were measured prior to the first treatment on day 1. The results are shown in FIGs. 8-12 and in Tables 12 and 13.

[0610] FIG. 8 and FIG. 9 show the plasma ALA levels in μM. Baseline ALA levels were low, (n=4), and phenobarbital treatment induced significant increases in plasma ALA levels in the control LUC siRNA treated animals (n=3). Treatment with ALAS1 siRNA inhibited the induction of plasma ALA (n=5), as shown in FIG. 8. The ALAS1 siRNA was consistently effective in blocking the induction of plasma ALA in each of the individual animals studied (see FIG. 9). These results indicate that ALAS1 siRNA treatment was effective in preventing the increases in plasma ALA associated with the phenobarbital-induced acute attacks in this AIP animal model.

[0611] FIG. 10 and FIG. 11 show the plasma PBG levels in μM. Baseline PBG levels were low (n=4), and phenobarbital treatment induced significant increases in plasma PBG levels in the control LUC siRNA treated animals (n=3) Treatment with ALAS1 siRNA inhibited the induction of plasma PBG (n=5), as shown in FIG. 10. The ALAS1 siRNA was consistently effective in blocking the induction of plasma PBG in each of the individual animals studied (see FIG. 11). These results indicate that ALAS1 siRNA treatment was effective in preventing the increases in plasma PBG associated with the phenobarbital-induced acute attacks in this AIP animal model.

[0612] Tables 12 and 13 shows urine ALA and PBG levels at baseline and after phenobarbital treatment in LUC siRNA (n=2) control (CTR, which refers to a PBS buffer treated animal, n=1) and ALAS1 siRNA (n=5) treated animals.

Table 12: Urine data from individual animals showing prevention of induced acute attack

Mouse ID	ALA (micro M/l)	PBG (micro M/L)	Creatinine (ma/dl)	ALA (microM/mg creatinine)	PBG (microM/mg creatinine)	siRNA	PB
Ha-17-4-6				29.7	7.9	Baseline	-
Ha-19-5-4/2				15.7	5.1	Baseline	-
Ha-20-39-4/3				28.6	6.7	Baseline	-
Ha-20-38-4				21.4	4.7	Baseline	-
Ha-21-33-4	934.92	483.71	0.4205	222.33	115.03	Luc	+
Ha-21-36-9	944.08	563.53	0.5055	186.76	111.48	Luc	+
Ha-21-18-8	32.88	8.69	0.133	24.72	6.53	ALAS1; 1mg/kg	+
Ha-21-33-7	83.07	23.28	0.426	19.50	5.46	ALAS1; 1 mg/kg	+
Ha-21-34-5	59.15	18.41	0.263	22.49	7.00	ALAS1; 1 mg/kg	+
PB stands for phenobarbital. A "+" indicates that phenobarbital was administered.							

Table 13: Average Urine Data

Mean ALA (microM/mg creatinine)	Mean PBG (microM/mg creatinine)
23.8	6.1 AIP Baseline
204.55	113.26 Luc-siRNA
22.24	6.33 ALAS1-siRNA

[0613] Phenobarbital treatment induced strong increases (~25-30 fold increases) in urine ALA (~9-fold over baseline levels) and PBG (~19-fold over baseline levels) in the LUC siRNA treated mice, control, whereas such increases were not observed in the ALAS1 siRNA treated animals. Thus, ALAS1 siRNA blocked phenobarbital-induced increases in urinary ALA and PBG. These results are consistent with the plasma measurements and show that ALAS1 siRNA treatment was effective in preventing increases in urinary metabolites (ALA and PBG) associated with the phenobarbital-induced acute attacks in this AIP animal model.

[0614] In further experiments (FIG. 12), it was found that phenobarbital treatment induced large increases (~25 fold) in ALAS1 mRNA expression in the liver of the mouse model. Administration of ALAS1 siRNA completely blocked this ALAS 1 mRNA induction. These results provide further evidence that ALAS1 siRNA is effective in an animal model of AIP.

[0615] Collectively, the results provided in this Example show that ALAS1 siRNA was effective in treating acute attacks in an animal model of the acute hepatic porphyria AIP. Multiple outcome measures support this conclusion, including plasma ALA levels, plasma PBG levels, urine ALA levels, urine PBG levels, and liver ALAS1 mRNA expression levels.

Example 6. *In Vivo* Silencing using GalNAc-Conjugated Mouse ALAS1 siRNA

[0616] The experiments described in this example investigated the *in vivo* efficacy of three GalNAc-conjugated siRNAs (see Table 7). These siRNAs were designed and produced with methods such as those described in Example 2.

Table 7: Sequences AD-57929

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position of sense seq. on transcript NM_020559.2	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Position of antisense seq. on transcript NM_020559.2
385	386	775-795	AD-56211	AfaGfuCfuGfuUfUfCfcAfcUfuUfuCfa AfL96	uUfgAfaAfaGfuGfgaaAfcAfgAfcUf usUfsg	773-795
387	388	2168-2188	AD-56173	AfcAfuAfgUfaGfCfCfaGfaAfuUfgUfc UfL96	aGfaCfaAfuUfcUfggcUfaCfuAfuGf usGfsg	2166-2188
389	390	775-795	AD-57929	AfsasGfuCfuGfuUfUfCfcAfcUfuUfuCf aAfL96	usUfsgAfaAfaGfuGfgaaAfcAfgAfc Ufusug	773-795

[0617] The mice (n=40; n=4 per experimental condition) were divided into groups that received PBS or doses of 3 mg/kg, 10 mg/kg, or 30 mg/kg of siRNA administered subcutaneously. The level of mALAS1/mGAPDH mRNA, relative to the PBS control, was determined in liver cells at 72 hours post-administration. The results are shown in FIG. 13. There was not a clear dose-response effect for the siRNAs AD-56211 and AD-56173. In contrast, the ALAS1 siRNA AD-57929 showed a dose-response effect in inhibiting mALAS1 expression. These results demonstrate that an ALAS1 GalNAc conjugate was effective in inhibiting expression of ALAS1 mRNA *in vivo* and showed a dose-response effect.

Example 7. Human siRNAs

[0618] Additional human siRNAs were designed and produced as described in Example 2. The top 45 siRNAs were selected based on their predicted efficacy. The sequences of these 45 siRNAs are provided in Table 8.

Table 8: Human ALAS1 siRNA Sense and Antisense Sequences

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
391	392	1635-1657	CAUGCCAAAAAUGGACAUCAU	AUGAUGUCCAUUUUUGGCAUGAC
393	394	2352-2374	UAAAUUUUAAUCUAUAGUAAA	UUUACUAUAGAUUAAAAUUUAAU
395	396	1324-1346	GGCUGUGAGAUUUACUCUGAU	AUCAGAGUAAAUCUCACAGCCUG
397	398	1637-1659	UGCCAAAAAUGGACAUCAUUU	AAAUGAUGUCCAUUUUUGGCAUG
399	400	1363-1385	AUGAUCCAAGGGAUUCGAAAC	GUUUCGAAUCCCUUGGAUCAUGG
401	402	925-947	ACUUUUCAGUAUGAUCGUUUC	GAAACGAUCAUACUGAAAAGUGG
403	404	790-812	CCCAGUGUGGUUAGUGUGAAA	UUUCACACUAACCACACUGGGGC
405	406	1531-1553	UGUGAUGUGGCCCAUGAGUUU	AAACUCAUGGGCCACAUCACACA
407	408	2189-2211	AUUUUGAAGUGAUGAGUGAAA	UUUCACUCAUCACUUCAAAAUGC
409	410	929-951	UUCAGUAUGAUCGUUUCUUUG	CAAAGAAACGAUCAUACUGAAAA
411	412	872-894	GACCAGAAAGAGUGUCUCAUC	GAUGAGACACUCUUUCUGGUCUU
413	414	706-728	UUCUGCAAAGCCAGUCUUGAG	CUCAAGACUGGCUUUGCAGAAGA
415	416	1362-1384	CAUGAUCCAAGGGAUUCGAAA	UUUCGAAUCCCUUGGAUCAUGGA
417	418	1634-1656	UCAUGCCAAAAAUGGACAUCA	UGAUGUCCAUUUUUGGCAUGACU
419	420	1325-1347	GCUGUGAGAUUUACUCUGAUU	AAUCAGAGUAAAUCUCACAGCCU
421	422	2208-2230	AAGAGAGAAGUCCUAUUUCUC	GAGAAAUAGGACUUCUCUCUUUC
423	424	2344-2366	AGUUAUAUUAAAUUUUAAUCU	AGAUUAAAAUUUAAUAUAACUUA
425	426	924-946	CACUUUUCAGUAUGAUCGUUU	AAACGAUCAUACUGAAAAGUGGA
427	428	873-895	ACCAGAAAGAGUGUCUCAUCU	AGAUGAGACACUCUUUCUGGUCU
429	430	759-781	GAGGAAAGAGGUUGCUGAAAC	GUUUCAGCAACCUCUUUCCUCAC
431	432	871-893	AGACCAGAAAGAGUGUCUCAU	AUGAGACACUCUUUCUGGUCUUU
433	434	1183-1205	AAUAUUUCUGGAACUAGUAAA	UUUACUAGUUCCAGAAAUAUUUC
435	436	2229-2251	AGGCUUGAGCAAGUUGGUAUC	GAUACCAACUUGCUCAAGCCUGA
437	438	671-693	UGGCAGCACAGAUGAAUCAGA	UCUGAUUCAUCUGUGCUGCCAGG
439	440	2187-2209	GCAUUUUGAAGUGAUGAGUGA	UCACUCAUCACUUCAAAAUGCAG

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
441	442	913-935	AAAUCUGUUUCCACUUUUCAG	CUGAAAAGUGGAAACAGAUUUUG
443	444	1977-1999	ACUAAUGAGCAGACAUAACAU	AUGUUAUGUCUGCUCAUUAGUUC
445	446	1174-1196	GGUACUAGAAAUAUUUCUGGA	UCCAGAAAUAUUUCUAGUACCAC
447	448	1810-1832	AUCCUGAAGAGCGCUGAGGGA	UCCCUCAGCGCUCUUCAGGAUCC
449	450	892-914	CUUCUUCAAGAUAACUUGCCA	UGGCAAGUUAUCUUGAAGAAGAU
451	452	877-899	GAAAGAGUGUCUCAUCUUCUU	AAGAAGAUGAGACACUCUUUCUG
453	454	935-957	AUGAUCGUUUCUUUGAGAAAA	UUUUCUCAAAGAAACGAUCAUAC
455	456	1975-1997	GAACUAAUGAGCAGACAUAAC	GUUAUGUCUGCUCAUUAGUUCAU
457	458	1478-1500	CAUUUGAAACUGUCCAUUCAA	UUGAAUGGACAGUUUCAAAUGCC
459	460	2366-2388	UAGUAAAAACAUAGUCCUGGA	UCCAGGACUAUGUUUUUACUAUA
461	462	853-875	GACAUCAUGCAAAAGCAAAGA	UCUUUGCUUUUGCAUGAUGUCCU
463	464	1966-1988	GUCUGUGAUGAACUAAUGAGC	GCUCAUUAGUUCAUCACAGACUU
465	466	928-950	UUUCAGUAUGAUCGUUUCUUU	AAAGAAACGAUCAUACUGAAAAG
467	468	1186-1208	AUUUCUGGAACUAGUAAAUUC	GAAUUUACUAGUUCCAGAAAUAU
469	470	1189-1211	UCUGGAACUAGUAAAUUCCAU	AUGGAAUUUACUAGUUCCAGAAA
471	472	973-995	AAUGACCACACCUAUCGAGUU	AACUCGAUAGGUGUGGUCAUUCU
473	474	983-1005	CCUAUCGAGUUUUUAAAACUG	CAGUUUUAAAAACUCGAUAGGUG
475	476	1185-1207	UAUUUCUGGAACUAGUAAAUU	AAUUUACUAGUUCCAGAAAUAUU
477	478	2353-2375	AAAUUUUAAUCUAUAGUAAAA	UUUUACUAUAGAUUAAAAUUUAA
479	480	875-897	CAGAAAGAGUGUCUCAUCUUC	GAAGAUGAGACACUCUUUCUGGU
481	482	360-378	GCCCAUUCUUAUCCCGAGU	ACUCGGGAUAAGAAUGGGC
483	484	428-446	CAAAACUGCCCCAAGAUGA	UCAUCUUGGGGCAGUUUUG
485	486	873-891	CAGAAAGAGUGUCUCAUCU	AGAUGAGACACUCUUUCUG
487	488	874-892	AGAAAGAGUGUCUCAUCUU	AAGAUGAGACACUCUUUCU
489	490	877-895	AAGAGUGUCUCAUCUUCUU	AAGAAGAUGAGACACUCUU

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
491	492	1295-1313	CUCUUCACCCUGGCUAAGA	UCUUAGCCAGGGUGAAGAG
493	494	1296-1314	UCUUCACCCUGGCUAAGAU	AUCUUAGCCAGGGUGAAGA
495	496	1299-1317	UCACCCUGGCUAAGAUGAU	AUCAUCUUAGCCAGGGUGA
497	498	1347-1365	GGAACCAUGCCUCCAUGAU	AUCAUGGAGGCAUGGUUCC
499	500	1355-1373	GCCUCCAUGAUCCAAGGGA	UCCCUUGGAUCAUGGAGGC
501	502	1356-1374	CCUCCAUGAUCCAAGGGAU	AUCCCUUGGAUCAUGGAGG
503	504	1357-1375	CUCCAUGAUCCAAGGGAUU	AAUCCCUUGGAUCAUGGAG
505	506	1631-1649	GUCAUGCCAAAAAUGGACA	UGUCCAUUUUUGGCAUGAC
507	508	1634-1652	AUGCCAAAAAUGGACAUCA	UGAUGUCCAUUUUUGGCAU
509	510	1635-1653	UGCCAAAAAUGGACAUCAU	AUGAUGUCCAUUUUUGGCA
511	512	1791-1809	CCCUGGAGUCUGUGCGGAU	AUCCGCACAGACUCCAGGG
513	514	1794-1812	UGGAGUCUGUGCGGAUCCU	AGGAUCCGCACAGACUCCA
515	516	1921-1939	CAUCAUCCCUGUGCGGGUU	AACCCGCACAGGGAUGAUG
517	518	359-377	UGCCCAUUCUUAUCCCGAA	UUCGGGAUAAGAAUGGGCA
519	520	362-380	CCAUUCUUAUCCCGAGUCA	UGACUCGGGAUAAGAAUGG
521	522	363-381	CAUUCUUAUCCCGAGUCCA	UGGACUCGGGAUAAGAAUG
523	524	434-452	UGCCCCAAGAUGAUGGAAU	AUUCCAUCAUCUUGGGGCA
525	526	872-890	CCAGAAAGAGUGUCUCAUA	UAUGAGACACUCUUUCUGG
527	528	875-893	GAAAGAGUGUCUCAUCUUA	UAAGAUGAGACACUCUUUC
529	530	1112-1130	CACCCACGGGUGUGUGGGA	UCCCACACACCCGUGGGUG
531	532	1113-1131	ACCCACGGGUGUGUGGGGA	UCCCCACACACCCGUGGGU
533	534	1297-1315	CUUCACCCUGGCUAAGAUA	UAUCUUAGCCAGGGUGAAG
535	536	1300-1318	CACCCUGGCUAAGAUGAUA	UAUCAUCUUAGCCAGGGUG
537	538	1301-1319	ACCCUGGCUAAGAUGAUGA	UCAUCAUCUUAGCCAGGGU
539	540	1348-1366	GAACCAUGCCUCCAUGAUA	UAUCAUGGAGGCAUGGUUC

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
541	542	1481-1499	GAAACUGUCCAUUCAAUGA	UCAUUGAAUGGACAGUUUC
543	544	1786-1804	UGGAGCCCUGGAGUCUGUA	UACAGACUCCAGGGCUCCA
545	546	1795-1813	GGAGUCUGUGCGGAUCCUA	UAGGAUCCGCACAGACUCC
547	548	1919-1937	CACAUCAUCCCUGUGCGGA	UCCGCACAGGGAUGAUGUG
549	550	1922-1940	AUCAUCCCUGUGCGGGUUA	UAACCCGCACAGGGAUGAU
551	552	1923-1941	UCAUCCCUGUGCGGGUUGA	UCAACCCGCACAGGGAUGA

Example 8. Human siRNAs

[0619] Additional 19mer human siRNAs were generated. The sequences of these siRNAs are provided in Table 9. These siRNAs can be tested for efficacy using methods described herein and/or methods known in the art.

Table 9: Human ALAS1 siRNA Sense and Antisense Sequences

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
553	554	4-22	UAUAUUAAGGCGCCGGCGA	UCGCCGGCGCCUUAAUAUA
555	556	5-23	AUAUUAAGGCGCCGGCGAU	AUCGCCGGCGCCUUAAUAU
557	558	6-24	UAUUAAGGCGCCGGCGAUC	GAUCGCCGGCGCCUUAAUA
559	560	7-25	AUUAAGGCGCCGGCGAUCG	CGAUCGCCGGCGCCUUAAU
561	562	8-26	UUAAGGCGCCGGCGAUCGC	GCGAUCGCCGGCGCCUUAA
563	564	9-27	UAAGGCGCCGGCGAUCGCG	CGCGAUCGCCGGCGCCUUA
565	566	10-28	AAGGCGCCGGCGAUCGCGG	CCGCGAUCGCCGGCGCCUU
567	568	11-29	AGGCGCCGGCGAUCGCGGC	GCCGCGAUCGCCGGCGCCU
569	570	12-30	GGCGCCGGCGAUCGCGGCC	GGCCGCGAUCGCCGGCGCC
571	572	13-31	GCGCCGGCGAUCGCGGCCU	AGGCCGCGAUCGCCGGCGC
573	574	14-32	CGCCGGCGAUCGCGGCCUG	CAGGCCGCGAUCGCCGGCG
575	576	81-99	CUUGAGUGCCCGCCUCCUU	AAGGAGGCGGGCACUCAAG
577	578	82-100	UUGAGUGCCCGCCUCCUUC	GAAGGAGGCGGGCACUCAA
579	580	83-101	UGAGUGCCCGCCUCCUUCG	CGAAGGAGGCGGGCACUCA
581	582	84-102	GAGUGCCCGCCUCCUUCGC	GCGAAGGAGGCGGGCACUC
583	584	85-103	AGUGCCCGCCUCCUUCGCC	GGCGAAGGAGGCGGGCACU
585	586	86-104	GUGCCCGCCUCCUUCGCCG	CGGCGAAGGAGGCGGGCAC
587	588	87-105	UGCCCGCCUCCUUCGCCGC	GCGGCGAAGGAGGCGGGCA
589	590	88-106	GCCCGCCUCCUUCGCCGCC	GGCGGCGAAGGAGGCGGGC
591	592	89-107	CCCGCCUCCUUCGCCGCCG	CGGCGGCGAAGGAGGCGGG
593	594	90-108	CCGCCUCCUUCGCCGCCGC	GCGGCGGCGAAGGAGGCGG
595	596	91-109	CGCCUCCUUCGCCGCCGCC	GGCGGCGGCGAAGGAGGCG
597	598	92-110	GCCUCCUUCGCCGCCGCCU	AGGCGGCGGCGAAGGAGGC
599	600	93-111	CCUCCUUCGCCGCCGCCUC	GAGGCGGCGGCGAAGGAGG
601	602	356-374	CGCUGCCCAUUCUUAUCCC	GGGAUAAGAAUGGGCAGCG
603	604	357-375	GCUGCCCAUUCUUAUCCCG	CGGGAUAAGAAUGGGCAGC
605	606	359-377	UGCCCAUUCUUAUCCCGAG	CUCGGGAUAAGAAUGGGCA
607	608	361-379	CCCAUUCUUAUCCCGAGUC	GACUCGGGAUAAGAAUGGG
609	610	362-380	CCAUUCUUAUCCCGAGUCC	GGACUCGGGAUAAGAAUGG
611	612	363-381	CAUUCUUAUCCCGAGUCCC	GGGACUCGGGAUAAGAAUG
613	614	364-382	AUUCUUAUCCCGAGUCCCC	GGGGACUCGGGAUAAGAAU
615	616	365-383	UUCUUAUCCCGAGUCCCCC	GGGGGACUCGGGAUAAGAA
617	618	366-384	UCUUAUCCCGAGUCCCCCA	UGGGGGACUCGGGAUAAGA

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
619	620	367-385	CUUAUCCCGAGUCCCCCAG	CUGGGGGACUCGGGAUAAG
621	622	368-386	UUAUCCCGAGUCCCCCAGG	CCUGGGGGACUCGGGAUAA
623	624	369-387	UAUCCCGAGUCCCCCAGGC	GCCUGGGGGACUCGGGAUA
625	626	370-388	AUCCCGAGUCCCCCAGGCC	GGCCUGGGGGACUCGGGAU
627	628	371-389	UCCCGAGUCCCCCAGGCCU	AGGCCUGGGGGACUCGGGA
629	630	372-390	CCCGAGUCCCCCAGGCCUU	AAGGCCUGGGGGACUCGGG
631	632	373-391	CCGAGUCCCCCAGGCCUUU	AAAGGCCUGGGGGACUCGG
633	634	374-392	CGAGUCCCCCAGGCCUUUC	GAAAGGCCUGGGGGACUCG
635	636	375-393	GAGUCCCCCAGGCCUUUCU	AGAAAGGCCUGGGGGACUC
637	638	376-394	AGUCCCCCAGGCCUUUCUG	CAGAAAGGCCUGGGGGACU
639	640	377-395	GUCCCCCAGGCCUUUCUGC	GCAGAAAGGCCUGGGGGAC
641	642	378-396	UCCCCCAGGCCUUUCUGCA	UGCAGAAAGGCCUGGGGGA
643	644	379-397	CCCCCAGGCCUUUCUGCAG	CUGCAGAAAGGCCUGGGGG
645	646	380-398	CCCCAGGCCUUUCUGCAGA	UCUGCAGAAAGGCCUGGGG
647	648	381-399	CCCAGGCCUUUCUGCAGAA	UUCUGCAGAAAGGCCUGGG
649	650	382-400	CCAGGCCUUUCUGCAGAAA	UUUCUGCAGAAAGGCCUGG
651	652	383-401	CAGGCCUUUCUGCAGAAAG	CUUUCUGCAGAAAGGCCUG
653	654	384-402	AGGCCUUUCUGCAGAAAGC	GCUUUCUGCAGAAAGGCCU
655	656	385-403	GGCCUUUCUGCAGAAAGCA	UGCUUUCUGCAGAAAGGCC
657	658	386-404	GCCUUUCUGCAGAAAGCAG	CUGCUUUCUGCAGAAAGGC
659	660	387-405	CCUUUCUGCAGAAAGCAGG	CCUGCUUUCUGCAGAAAGG
661	662	388-406	CUUUCUGCAGAAAGCAGGC	GCCUGCUUUCUGCAGAAAG
663	664	389-407	UUUCUGCAGAAAGCAGGCA	UGCCUGCUUUCUGCAGAAA
665	666	390-408	UUCUGCAGAAAGCAGGCAA	UUGCCUGCUUUCUGCAGAA
667	668	391-409	UCUGCAGAAAGCAGGCAAA	UUUGCCUGCUUUCUGCAGA
669	670	392-410	CUGCAGAAAGCAGGCAAAU	AUUUGCCUGCUUUCUGCAG
671	672	393-411	UGCAGAAAGCAGGCAAAUC	GAUUUGCCUGCUUUCUGCA
673	674	394-412	GCAGAAAGCAGGCAAAUCU	AGAUUUGCCUGCUUUCUGC
675	676	395-413	CAGAAAGCAGGCAAAUCUC	GAGAUUUGCCUGCUUUCUG
677	678	396-414	AGAAAGCAGGCAAAUCUCU	AGAGAUUUGCCUGCUUUCU
679	680	397-415	GAAAGCAGGCAAAUCUCUG	CAGAGAUUUGCCUGCUUUC
681	682	398-416	AAAGCAGGCAAAUCUCUGU	ACAGAGAUUUGCCUGCUUU
683	684	399-417	AAGCAGGCAAAUCUCUGUU	AACAGAGAUUUGCCUGCUU
685	686	400-418	AGCAGGCAAAUCUCUGUUG	CAACAGAGAUUUGCCUGCU
687	688	401-419	GCAGGCAAAUCUCUGUUGU	ACAACAGAGAUUUGCCUGC
689	690	402-420	CAGGCAAAUCUCUGUUGUU	AACAACAGAGAUUUGCCUG

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
691	692	403-421	AGGCAAAUCUCUGUUGUUC	GAACAACAGAGAUUUGCCU
693	694	405-423	GCAAAUCUCUGUUGUUCUA	UAGAACAACAGAGAUUUGC
695	696	406-424	CAAAUCUCUGUUGUUCUAU	AUAGAACAACAGAGAUUUG
697	698	407-425	AAAUCUCUGUUGUUCUAUG	CAUAGAACAACAGAGAUUU
699	700	408-426	AAUCUCUGUUGUUCUAUGC	GCAUAGAACAACAGAGAUU
701	702	409-427	AUCUCUGUUGUUCUAUGCC	GGCAUAGAACAACAGAGAU
703	704	410-428	UCUCUGUUGUUCUAUGCCC	GGGCAUAGAACAACAGAGA
705	706	411-429	CUCUGUUGUUCUAUGCCCA	UGGGCAUAGAACAACAGAG
707	708	412-430	UCUGUUGUUCUAUGCCCAA	UUGGGCAUAGAACAACAGA
709	710	413-431	CUGUUGUUCUAUGCCCAAA	UUUGGGCAUAGAACAACAG
711	712	414-432	UGUUGUUCUAUGCCCAAAA	UUUUGGGCAUAGAACAACA
713	714	415-433	GUUGUUCUAUGCCCAAAAC	GUUUUGGGCAUAGAACAAC
715	716	416-434	UUGUUCUAUGCCCAAAACU	AGUUUUGGGCAUAGAACAA
717	718	417-435	UGUUCUAUGCCCAAAACUG	CAGUUUUGGGCAUAGAACA
719	720	418-436	GUUCUAUGCCCAAAACUGC	GCAGUUUUGGGCAUAGAAC
721	722	419-437	UUCUAUGCCCAAAACUGCC	GGCAGUUUUGGGCAUAGAA
723	724	420-438	UCUAUGCCCAAAACUGCCC	GGGCAGUUUUGGGCAUAGA
725	726	421-439	CUAUGCCCAAAACUGCCCC	GGGGCAGUUUUGGGCAUAG
727	728	422-440	UAUGCCCAAAACUGCCCCA	UGGGGCAGUUUUGGGCAUA
729	730	423-441	AUGCCCAAAACUGCCCCAA	UUGGGGCAGUUUUGGGCAU
731	732	424-442	UGCCCAAAACUGCCCCAAG	CUUGGGGCAGUUUUGGGCA
733	734	425-443	GCCCAAAACUGCCCCAAGA	UCUUGGGGCAGUUUUGGGC
735	736	426-444	CCCAAAACUGCCCCAAGAU	AUCUUGGGGCAGUUUUGGG
737	738	427-445	CCAAAACUGCCCCAAGAUG	CAUCUUGGGGCAGUUUUGG
739	740	429-447	AAAACUGCCCCAAGAUGAU	AUCAUCUUGGGGCAGUUUU
741	742	430-448	AAACUGCCCCAAGAUGAUG	CAUCAUCUUGGGGCAGUUU
743	744	431-449	AACUGCCCCAAGAUGAUGG	CCAUCAUCUUGGGGCAGUU
745	746	432-450	ACUGCCCCAAGAUGAUGGA	UCCAUCAUCUUGGGGCAGU
747	748	433-451	CUGCCCCAAGAUGAUGGAA	UUCCAUCAUCUUGGGGCAG
749	750	434-452	UGCCCCAAGAUGAUGGAAG	CUUCCAUCAUCUUGGGGCA
751	752	435-453	GCCCCAAGAUGAUGGAAGU	ACUUCCAUCAUCUUGGGGC
753	754	437-455	CCCAAGAUGAUGGAAGUUG	CAACUUCCAUCAUCUUGGG
755	756	438-456	CCAAGAUGAUGGAAGUUGG	CCAACUUCCAUCAUCUUGG
757	758	439-457	CAAGAUGAUGGAAGUUGGG	CCCAACUUCCAUCAUCUUG
759	760	440-458	AAGAUGAUGGAAGUUGGGG	CCCCAACUUCCAUCAUCUU
761	762	441-459	AGAUGAUGGAAGUUGGGGC	GCCCCAACUUCCAUCAUCU

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
763	764	442-460	GAUGAUGGAAGUUGGGGCC	GGCCCCAACUUCCAUCAUC
765	766	443-461	AUGAUGGAAGUUGGGGCCA	UGGCCCCAACUUCCAUCAU
767	768	444-462	UGAUGGAAGUUGGGGCCAA	UUGGCCCCAACUUCCAUCA
769	770	445-463	GAUGGAAGUUGGGGCCAAG	CUUGGCCCCAACUUCCAUC
771	772	446-464	AUGGAAGUUGGGGCCAAGC	GCUUGGCCCCAACUUCCAU
773	774	447-465	UGGAAGUUGGGGCCAAGCC	GGCUUGGCCCCAACUUCCA
775	776	448-466	GGAAGUUGGGGCCAAGCCA	UGGCUUGGCCCCAACUUCC
777	778	449-467	GAAGUUGGGGCCAAGCCAG	CUGGCUUGGCCCCAACUUC
779	780	450-468	AAGUUGGGGCCAAGCCAGC	GCUGGCUUGGCCCCAACUU
781	782	451-469	AGUUGGGGCCAAGCCAGCC	GGCUGGCUUGGCCCCAACU
783	784	452-470	GUUGGGGCCAAGCCAGCCC	GGGCUGGCUUGGCCCCAAC
785	786	453-471	UUGGGGCCAAGCCAGCCCC	GGGGCUGGCUUGGCCCCAA
787	788	454-472	UGGGGCCAAGCCAGCCCCU	AGGGGCUGGCUUGGCCCCA
789	790	455-473	GGGGCCAAGCCAGCCCCUC	GAGGGGCUGGCUUGGCCCC
791	792	456-474	GGGCCAAGCCAGCCCCUCG	CGAGGGGCUGGCUUGGCCC
793	794	457-475	GGCCAAGCCAGCCCCUCGG	CCGAGGGGCUGGCUUGGCC
795	796	458-476	GCCAAGCCAGCCCCUCGGG	CCCGAGGGGCUGGCUUGGC
797	798	459-477	CCAAGCCAGCCCCUCGGGC	GCCCGAGGGGCUGGCUUGG
799	800	460-478	CAAGCCAGCCCCUCGGGCA	UGCCCGAGGGGCUGGCUUG
801	802	461-479	AAGCCAGCCCCUCGGGCAU	AUGCCCGAGGGGCUGGCUU
803	804	462-480	AGCCAGCCCCUCGGGCAUU	AAUGCCCGAGGGGCUGGCU
805	806	463-481	GCCAGCCCCUCGGGCAUUG	CAAUGCCCGAGGGGCUGGC
807	808	464-482	CCAGCCCCUCGGGCAUUGU	ACAAUGCCCGAGGGGCUGG
809	810	465-483	CAGCCCCUCGGGCAUUGUC	GACAAUGCCCGAGGGGCUG
811	812	466-484	AGCCCCUCGGGCAUUGUCC	GGACAAUGCCCGAGGGGCU
813	814	467-485	GCCCCUCGGGCAUUGUCCA	UGGACAAUGCCCGAGGGGC
815	816	468-486	CCCCUCGGGCAUUGUCCAC	GUGGACAAUGCCCGAGGGG
817	818	469-487	CCCUCGGGCAUUGUCCACU	AGUGGACAAUGCCCGAGGG
819	820	470-488	CCUCGGGCAUUGUCCACUG	CAGUGGACAAUGCCCGAGG
821	822	471-489	CUCGGGCAUUGUCCACUGC	GCAGUGGACAAUGCCCGAG
823	824	472-490	UCGGGCAUUGUCCACUGCA	UGCAGUGGACAAUGCCCGA
825	826	473-491	CGGGCAUUGUCCACUGCAG	CUGCAGUGGACAAUGCCCG
827	828	474-492	GGGCAUUGUCCACUGCAGC	GCUGCAGUGGACAAUGCCC
829	830	475-493	GGCAUUGUCCACUGCAGCA	UGCUGCAGUGGACAAUGCC
831	832	476-494	GCAUUGUCCACUGCAGCAG	CUGCUGCAGUGGACAAUGC
833	834	477-495	CAUUGUCCACUGCAGCAGU	ACUGCUGCAGUGGACAAUG

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
835	836	478-496	AUUGUCCACUGCAGCAGUA	UACUGCUGCAGUGGACAAU
837	838	479-497	UUGUCCACUGCAGCAGUAC	GUACUGCUGCAGUGGACAA
839	840	480-498	UGUCCACUGCAGCAGUACA	UGUACUGCUGCAGUGGACA
841	842	481-499	GUCCACUGCAGCAGUACAC	GUGUACUGCUGCAGUGGAC
843	844	482-500	UCCACUGCAGCAGUACACU	AGUGUACUGCUGCAGUGGA
845	846	483-501	CCACUGCAGCAGUACACUA	UAGUGUACUGCUGCAGUGG
847	848	484-502	CACUGCAGCAGUACACUAC	GUAGUGUACUGCUGCAGUG
849	850	485-503	ACUGCAGCAGUACACUACC	GGUAGUGUACUGCUGCAGU
851	852	486-504	CUGCAGCAGUACACUACCA	UGGUAGUGUACUGCUGCAG
853	854	487-505	UGCAGCAGUACACUACCAA	UUGGUAGUGUACUGCUGCA
855	856	488-506	GCAGCAGUACACUACCAAC	GUUGGUAGUGUACUGCUGC
857	858	490-508	AGCAGUACACUACCAACAG	CUGUUGGUAGUGUACUGCU
859	860	491-509	GCAGUACACUACCAACAGA	UCUGUUGGUAGUGUACUGC
861	862	492-510	CAGUACACUACCAACAGAU	AUCUGUUGGUAGUGUACUG
863	864	493-511	AGUACACUACCAACAGAUC	GAUCUGUUGGUAGUGUACU
865	866	494-512	GUACACUACCAACAGAUCA	UGAUCUGUUGGUAGUGUAC
867	868	495-513	UACACUACCAACAGAUCAA	UUGAUCUGUUGGUAGUGUA
869	870	496-514	ACACUACCAACAGAUCAAA	UUUGAUCUGUUGGUAGUGU
871	872	497-515	CACUACCAACAGAUCAAAG	CUUUGAUCUGUUGGUAGUG
873	874	498-516	ACUACCAACAGAUCAAAGA	UCUUUGAUCUGUUGGUAGU
875	876	499-517	CUACCAACAGAUCAAAGAA	UUCUUUGAUCUGUUGGUAG
877	878	500-518	UACCAACAGAUCAAAGAAA	UUUCUUUGAUCUGUUGGUA
879	880	501-519	ACCAACAGAUCAAAGAAAC	GUUUCUUUGAUCUGUUGGU
881	882	502-520	CCAACAGAUCAAAGAAACC	GGUUUCUUUGAUCUGUUGG
883	884	523-541	UCCGGCCAGUGAGAAAGAC	GUCUUUCUCACUGGCCGGA
885	886	524-542	CCGGCCAGUGAGAAAGACA	UGUCUUUCUCACUGGCCGG
887	888	525-543	CGGCCAGUGAGAAAGACAA	UUGUCUUUCUCACUGGCCG
889	890	526-544	GGCCAGUGAGAAAGACAAA	UUUGUCUUUCUCACUGGCC
891	892	527-545	GCCAGUGAGAAAGACAAAA	UUUUGUCUUUCUCACUGGC
893	894	528-546	CCAGUGAGAAAGACAAAAC	GUUUUGUCUUUCUCACUGG
895	896	529-547	CAGUGAGAAAGACAAAACU	AGUUUUGUCUUUCUCACUG
897	898	530-548	AGUGAGAAAGACAAAACUG	CAGUUUUGUCUUUCUCACU
899	900	531-549	GUGAGAAAGACAAAACUGC	GCAGUUUUGUCUUUCUCAC
901	902	570-588	CUCCUGAUGGAUCCCAGCA	UGCUGGGAUCCAUCAGGAG
903	904	571-589	UCCUGAUGGAUCCCAGCAG	CUGCUGGGAUCCAUCAGGA
905	906	572-590	CCUGAUGGAUCCCAGCAGA	UCUGCUGGGAUCCAUCAGG

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
907	908	573-591	CUGAUGGAUCCCAGCAGAG	CUCUGCUGGGAUCCAUCAG
909	910	574-592	UGAUGGAUCCCAGCAGAGU	ACUCUGCUGGGAUCCAUCA
911	912	575-593	GAUGGAUCCCAGCAGAGUC	GACUCUGCUGGGAUCCAUC
913	914	576-594	AUGGAUCCCAGCAGAGUCC	GGACUCUGCUGGGAUCCAU
915	916	577-595	UGGAUCCCAGCAGAGUCCA	UGGACUCUGCUGGGAUCCA
917	918	578-596	GGAUCCCAGCAGAGUCCAG	CUGGACUCUGCUGGGAUCC
919	920	579-597	GAUCCCAGCAGAGUCCAGA	UCUGGACUCUGCUGGGAUC
921	922	580-598	AUCCCAGCAGAGUCCAGAU	AUCUGGACUCUGCUGGGAU
923	924	581-599	UCCCAGCAGAGUCCAGAUG	CAUCUGGACUCUGCUGGGA
925	926	582-600	CCCAGCAGAGUCCAGAUGG	CCAUCUGGACUCUGCUGGG
927	928	583-601	CCAGCAGAGUCCAGAUGGC	GCCAUCUGGACUCUGCUGG
929	930	584-602	CAGCAGAGUCCAGAUGGCA	UGCCAUCUGGACUCUGCUG
931	932	585-603	AGCAGAGUCCAGAUGGCAC	GUGCCAUCUGGACUCUGCU
933	934	586-604	GCAGAGUCCAGAUGGCACA	UGUGCCAUCUGGACUCUGC
935	936	587-605	CAGAGUCCAGAUGGCACAC	GUGUGCCAUCUGGACUCUG
937	938	588-606	AGAGUCCAGAUGGCACACA	UGUGUGCCAUCUGGACUCU
939	940	589-607	GAGUCCAGAUGGCACACAG	CUGUGUGCCAUCUGGACUC
941	942	590-608	AGUCCAGAUGGCACACAGC	GCUGUGUGCCAUCUGGACU
943	944	591-609	GUCCAGAUGGCACACAGCU	AGCUGUGUGCCAUCUGGAC
945	946	592-610	UCCAGAUGGCACACAGCUU	AAGCUGUGUGCCAUCUGGA
947	948	593-611	CCAGAUGGCACACAGCUUC	GAAGCUGUGUGCCAUCUGG
949	950	594-612	CAGAUGGCACACAGCUUCC	GGAAGCUGUGUGCCAUCUG
951	952	595-613	AGAUGGCACACAGCUUCCG	CGGAAGCUGUGUGCCAUCU
953	954	596-614	GAUGGCACACAGCUUCCGU	ACGGAAGCUGUGUGCCAUC
955	956	597-615	AUGGCACACAGCUUCCGUC	GACGGAAGCUGUGUGCCAU
957	958	598-616	UGGCACACAGCUUCCGUCU	AGACGGAAGCUGUGUGCCA
959	960	599-617	GGCACACAGCUUCCGUCUG	CAGACGGAAGCUGUGUGCC
961	962	600-618	GCACACAGCUUCCGUCUGG	CCAGACGGAAGCUGUGUGC
963	964	601-619	CACACAGCUUCCGUCUGGA	UCCAGACGGAAGCUGUGUG
965	966	602-620	ACACAGCUUCCGUCUGGAC	GUCCAGACGGAAGCUGUGU
967	968	603-621	CACAGCUUCCGUCUGGACA	UGUCCAGACGGAAGCUGUG
969	970	604-622	ACAGCUUCCGUCUGGACAC	GUGUCCAGACGGAAGCUGU
971	972	605-623	CAGCUUCCGUCUGGACACC	GGUGUCCAGACGGAAGCUG
973	974	606-624	AGCUUCCGUCUGGACACCC	GGGUGUCCAGACGGAAGCU
975	976	607-625	GCUUCCGUCUGGACACCCC	GGGGUGUCCAGACGGAAGC
977	978	608-626	CUUCCGUCUGGACACCCCU	AGGGGUGUCCAGACGGAAG

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
979	980	609-627	UUCCGUCUGGACACCCCUU	AAGGGGUGUCCAGACGGAA
981	982	610-628	UCCGUCUGGACACCCCUUG	CAAGGGGUGUCCAGACGGA
983	984	611-629	CCGUCUGGACACCCCUUGC	GCAAGGGGUGUCCAGACGG
985	986	612-630	CGUCUGGACACCCCUUGCC	GGCAAGGGGUGUCCAGACG
987	988	613-631	GUCUGGACACCCCUUGCCU	AGGCAAGGGGUGUCCAGAC
989	990	614-632	UCUGGACACCCCUUGCCUG	CAGGCAAGGGGUGUCCAGA
991	992	615-633	CUGGACACCCCUUGCCUGC	GCAGGCAAGGGGUGUCCAG
993	994	616-634	UGGACACCCCUUGCCUGCC	GGCAGGCAAGGGGUGUCCA
995	996	617-635	GGACACCCCUUGCCUGCCA	UGGCAGGCAAGGGGUGUCC
997	998	618-636	GACACCCCUUGCCUGCCAC	GUGGCAGGCAAGGGGUGUC
999	1000	619-637	ACACCCCUUGCCUGCCACA	UGUGGCAGGCAAGGGGUGU
1001	1002	620-638	CACCCCUUGCCUGCCACAA	UUGUGGCAGGCAAGGGGUG
1003	1004	621-639	ACCCCUUGCCUGCCACAAG	CUUGUGGCAGGCAAGGGGU
1005	1006	622-640	CCCCUUGCCUGCCACAAGC	GCUUGUGGCAGGCAAGGGG
1007	1008	623-641	CCCUUGCCUGCCACAAGCC	GGCUUGUGGCAGGCAAGGG
1009	1010	624-642	CCUUGCCUGCCACAAGCCA	UGGCUUGUGGCAGGCAAGG
1011	1012	625-643	CUUGCCUGCCACAAGCCAG	CUGGCUUGUGGCAGGCAAG
1013	1014	626-644	UUGCCUGCCACAAGCCAGG	CCUGGCUUGUGGCAGGCAA
1015	1016	627-645	UGCCUGCCACAAGCCAGGG	CCCUGGCUUGUGGCAGGCA
1017	1018	628-646	GCCUGCCACAAGCCAGGGC	GCCCUGGCUUGUGGCAGGC
1019	1020	629-647	CCUGCCACAAGCCAGGGCA	UGCCCUGGCUUGUGGCAGG
1021	1022	630-648	CUGCCACAAGCCAGGGCAC	GUGCCCUGGCUUGUGGCAG
1023	1024	631-649	UGCCACAAGCCAGGGCACU	AGUGCCCUGGCUUGUGGCA
1025	1026	632-650	GCCACAAGCCAGGGCACUG	CAGUGCCCUGGCUUGUGGC
1027	1028	633-651	CCACAAGCCAGGGCACUGC	GCAGUGCCCUGGCUUGUGG
1029	1030	634-652	CACAAGCCAGGGCACUGCA	UGCAGUGCCCUGGCUUGUG
1031	1032	635-653	ACAAGCCAGGGCACUGCAA	UUGCAGUGCCCUGGCUUGU
1033	1034	636-654	CAAGCCAGGGCACUGCAAG	CUUGCAGUGCCCUGGCUUG
1035	1036	637-655	AAGCCAGGGCACUGCAAGC	GCUUGCAGUGCCCUGGCUU
1037	1038	638-656	AGCCAGGGCACUGCAAGCA	UGCUUGCAGUGCCCUGGCU
1039	1040	639-657	GCCAGGGCACUGCAAGCAA	UUGCUUGCAGUGCCCUGGC
1041	1042	640-658	CCAGGGCACUGCAAGCAAA	UUUGCUUGCAGUGCCCUGG
1043	1044	641-659	CAGGGCACUGCAAGCAAAU	AUUUGCUUGCAGUGCCCUG
1045	1046	642-660	AGGGCACUGCAAGCAAAUG	CAUUUGCUUGCAGUGCCCU
1047	1048	643-661	GGGCACUGCAAGCAAAUGC	GCAUUUGCUUGCAGUGCCC
1049	1050	644-662	GGCACUGCAAGCAAAUGCC	GGCAUUUGCUUGCAGUGCC

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1051	1052	645-663	GCACUGCAAGCAAAUGCCC	GGGCAUUUGCUUGCAGUGC
1053	1054	647-665	ACUGCAAGCAAAUGCCCUU	AAGGGCAUUUGCUUGCAGU
1055	1056	648-666	CUGCAAGCAAAUGCCCUUU	AAAGGGCAUUUGCUUGCAG
1057	1058	649-667	UGCAAGCAAAUGCCCUUUC	GAAAGGGCAUUUGCUUGCA
1059	1060	650-668	GCAAGCAAAUGCCCUUUCC	GGAAAGGGCAUUUGCUUGC
1061	1062	651-669	CAAGCAAAUGCCCUUUCCU	AGGAAAGGGCAUUUGCUUG
1063	1064	652-670	AAGCAAAUGCCCUUUCCUG	CAGGAAAGGGCAUUUGCUU
1065	1066	653-671	AGCAAAUGCCCUUUCCUGG	CCAGGAAAGGGCAUUUGCU
1067	1068	654-672	GCAAAUGCCCUUUCCUGGC	GCCAGGAAAGGGCAUUUGC
1069	1070	655-673	CAAAUGCCCUUUCCUGGCA	UGCCAGGAAAGGGCAUUUG
1071	1072	656-674	AAAUGCCCUUUCCUGGCAG	CUGCCAGGAAAGGGCAUUU
1073	1074	657-675	AAUGCCCUUUCCUGGCAGC	GCUGCCAGGAAAGGGCAUU
1075	1076	658-676	AUGCCCUUUCCUGGCAGCA	UGCUGCCAGGAAAGGGCAU
1077	1078	659-677	UGCCCUUUCCUGGCAGCAC	GUGCUGCCAGGAAAGGGCA
1079	1080	660-678	GCCCUUUCCUGGCAGCACA	UGUGCUGCCAGGAAAGGGC
1081	1082	661-679	CCCUUUCCUGGCAGCACAG	CUGUGCUGCCAGGAAAGGG
1083	1084	662-680	CCUUUCCUGGCAGCACAGA	UCUGUGCUGCCAGGAAAGG
1085	1086	663-681	CUUUCCUGGCAGCACAGAU	AUCUGUGCUGCCAGGAAAG
1087	1088	664-682	UUUCCUGGCAGCACAGAUG	CAUCUGUGCUGCCAGGAAA
1089	1090	665-683	UUCCUGGCAGCACAGAUGA	UCAUCUGUGCUGCCAGGAA
1091	1092	666-684	UCCUGGCAGCACAGAUGAA	UUCAUCUGUGCUGCCAGGA
1093	1094	667-685	CCUGGCAGCACAGAUGAAU	AUUCAUCUGUGCUGCCAGG
1095	1096	668-686	CUGGCAGCACAGAUGAAUC	GAUUCAUCUGUGCUGCCAG
1097	1098	670-688	GGCAGCACAGAUGAAUCAG	CUGAUUCAUCUGUGCUGCC
1099	1100	672-690	CAGCACAGAUGAAUCAGAG	CUCUGAUUCAUCUGUGCUG
1101	1102	692-710	GGCAGCAGUGUCUUCUGCA	UGCAGAAGACACUGCUGCC
1103	1104	693-711	GCAGCAGUGUCUUCUGCAA	UUGCAGAAGACACUGCUGC
1105	1106	694-712	CAGCAGUGUCUUCUGCAAA	UUUGCAGAAGACACUGCUG
1107	1108	695-713	AGCAGUGUCUUCUGCAAAG	CUUUGCAGAAGACACUGCU
1109	1110	696-714	GCAGUGUCUUCUGCAAAGC	GCUUUGCAGAAGACACUGC
1111	1112	697-715	CAGUGUCUUCUGCAAAGCC	GGCUUUGCAGAAGACACUG
1113	1114	698-716	AGUGUCUUCUGCAAAGCCA	UGGCUUUGCAGAAGACACU
1115	1116	699-717	GUGUCUUCUGCAAAGCCAG	CUGGCUUUGCAGAAGACAC
1117	1118	700-718	UGUCUUCUGCAAAGCCAGU	ACUGGCUUUGCAGAAGACA
1119	1120	701-719	GUCUUCUGCAAAGCCAGUC	GACUGGCUUUGCAGAAGAC
1121	1122	702-720	UCUUCUGCAAAGCCAGUCU	AGACUGGCUUUGCAGAAGA

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1123	1124	703-721	CUUCUGCAAAGCCAGUCUU	AAGACUGGCUUUGCAGAAG
1125	1126	704-722	UUCUGCAAAGCCAGUCUUG	CAAGACUGGCUUUGCAGAA
1127	1128	705-723	UCUGCAAAGCCAGUCUUGA	UCAAGACUGGCUUUGCAGA
1129	1130	706-724	CUGCAAAGCCAGUCUUGAG	CUCAAGACUGGCUUUGCAG
1131	1132	707-725	UGCAAAGCCAGUCUUGAGC	GCUCAAGACUGGCUUUGCA
1133	1134	708-726	GCAAAGCCAGUCUUGAGCU	AGCUCAAGACUGGCUUUGC
1135	1136	709-727	CAAAGCCAGUCUUGAGCUU	AAGCUCAAGACUGGCUUUG
1137	1138	710-728	AAAGCCAGUCUUGAGCUUC	GAAGCUCAAGACUGGCUUU
1139	1140	711-729	AAGCCAGUCUUGAGCUUCA	UGAAGCUCAAGACUGGCUU
1141	1142	712-730	AGCCAGUCUUGAGCUUCAG	CUGAAGCUCAAGACUGGCU
1143	1144	713-731	GCCAGUCUUGAGCUUCAGG	CCUGAAGCUCAAGACUGGC
1145	1146	714-732	CCAGUCUUGAGCUUCAGGA	UCCUGAAGCUCAAGACUGG
1147	1148	715-733	CAGUCUUGAGCUUCAGGAG	CUCCUGAAGCUCAAGACUG
1149	1150	716-734	AGUCUUGAGCUUCAGGAGG	CCUCCUGAAGCUCAAGACU
1151	1152	717-735	GUCUUGAGCUUCAGGAGGA	UCCUCCUGAAGCUCAAGAC
1153	1154	718-736	UCUUGAGCUUCAGGAGGAU	AUCCUCCUGAAGCUCAAGA
1155	1156	719-737	CUUGAGCUUCAGGAGGAUG	CAUCCUCCUGAAGCUCAAG
1157	1158	720-738	UUGAGCUUCAGGAGGAUGU	ACAUCCUCCUGAAGCUCAA
1159	1160	721-739	UGAGCUUCAGGAGGAUGUG	CACAUCCUCCUGAAGCUCA
1161	1162	722-740	GAGCUUCAGGAGGAUGUGC	GCACAUCCUCCUGAAGCUC
1163	1164	723-741	AGCUUCAGGAGGAUGUGCA	UGCACAUCCUCCUGAAGCU
1165	1166	724-742	GCUUCAGGAGGAUGUGCAG	CUGCACAUCCUCCUGAAGC
1167	1168	725-743	CUUCAGGAGGAUGUGCAGG	CCUGCACAUCCUCCUGAAG
1169	1170	726-744	UUCAGGAGGAUGUGCAGGA	UCCUGCACAUCCUCCUGAA
1171	1172	727-745	UCAGGAGGAUGUGCAGGAA	UUCCUGCACAUCCUCCUGA
1173	1174	728-746	CAGGAGGAUGUGCAGGAAA	UUUCCUGCACAUCCUCCUG
1175	1176	729-747	AGGAGGAUGUGCAGGAAAU	AUUUCCUGCACAUCCUCCU
1177	1178	730-748	GGAGGAUGUGCAGGAAAUG	CAUUUCCUGCACAUCCUCC
1179	1180	731-749	GAGGAUGUGCAGGAAAUGA	UCAUUUCCUGCACAUCCUC
1181	1182	732-750	AGGAUGUGCAGGAAAUGAA	UUCAUUUCCUGCACAUCCU
1183	1184	733-751	GGAUGUGCAGGAAAUGAAU	AUUCAUUUCCUGCACAUCC
1185	1186	734-752	GAUGUGCAGGAAAUGAAUG	CAUUCAUUUCCUGCACAUC
1187	1188	735-753	AUGUGCAGGAAAUGAAUGC	GCAUUCAUUUCCUGCACAU
1189	1190	755-773	GUGAGGAAAGAGGUUGCUG	CAGCAACCUCUUUCCUCAC
1191	1192	756-774	UGAGGAAAGAGGUUGCUGA	UCAGCAACCUCUUUCCUCA
1193	1194	757-775	GAGGAAAGAGGUUGCUGAA	UUCAGCAACCUCUUUCCUC

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1195	1196	758-776	AGGAAAGAGGUUGCUGAAA	UUUCAGCAACCUCUUUCCU
1197	1198	759-777	GGAAAGAGGUUGCUGAAAC	GUUUCAGCAACCUCUUUCC
1199	1200	760-778	GAAAGAGGUUGCUGAAACC	GGUUUCAGCAACCUCUUUC
1201	1202	761-779	AAAGAGGUUGCUGAAACCU	AGGUUUCAGCAACCUCUUU
1203	1204	762-780	AAGAGGUUGCUGAAACCUC	GAGGUUUCAGCAACCUCUU
1205	1206	763-781	AGAGGUUGCUGAAACCUCA	UGAGGUUUCAGCAACCUCU
1207	1208	764-782	GAGGUUGCUGAAACCUCAG	CUGAGGUUUCAGCAACCUC
1209	1210	765-783	AGGUUGCUGAAACCUCAGC	GCUGAGGUUUCAGCAACCU
1211	1212	766-784	GGUUGCUGAAACCUCAGCA	UGCUGAGGUUUCAGCAACC
1213	1214	787-805	CCCCAGUGUGGUUAGUGUG	CACACUAACCACACUGGGG
1215	1216	791-809	AGUGUGGUUAGUGUGAAAA	UUUUCACACUAACCACACU
1217	1218	792-810	GUGUGGUUAGUGUGAAAAC	GUUUUCACACUAACCACAC
1219	1220	812-830	GAUGGAGGGGAUCCCAGUG	CACUGGGAUCCCCUCCAUC
1221	1222	813-831	AUGGAGGGGAUCCCAGUGG	CCACUGGGAUCCCCUCCAU
1223	1224	833-851	CUGCUGAAGAACUUCCAGG	CCUGGAAGUUCUUCAGCAG
1225	1226	834-852	UGCUGAAGAACUUCCAGGA	UCCUGGAAGUUCUUCAGCA
1227	1228	835-853	GCUGAAGAACUUCCAGGAC	GUCCUGGAAGUUCUUCAGC
1229	1230	836-854	CUGAAGAACUUCCAGGACA	UGUCCUGGAAGUUCUUCAG
1231	1232	837-855	UGAAGAACUUCCAGGACAU	AUGUCCUGGAAGUUCUUCA
1233	1234	838-856	GAAGAACUUCCAGGACAUC	GAUGUCCUGGAAGUUCUUC
1235	1236	839-857	AAGAACUUCCAGGACAUCA	UGAUGUCCUGGAAGUUCUU
1237	1238	840-858	AGAACUUCCAGGACAUCAU	AUGAUGUCCUGGAAGUUCU
1239	1240	841-859	GAACUUCCAGGACAUCAUG	CAUGAUGUCCUGGAAGUUC
1241	1242	842-860	AACUUCCAGGACAUCAUGC	GCAUGAUGUCCUGGAAGUU
1243	1244	843-861	ACUUCCAGGACAUCAUGCA	UGCAUGAUGUCCUGGAAGU
1245	1246	844-862	CUUCCAGGACAUCAUGCAA	UUGCAUGAUGUCCUGGAAG
1247	1248	845-863	UUCCAGGACAUCAUGCAAA	UUUGCAUGAUGUCCUGGAA
1249	1250	846-864	UCCAGGACAUCAUGCAAAA	UUUUGCAUGAUGUCCUGGA
1251	1252	847-865	CCAGGACAUCAUGCAAAAG	CUUUUGCAUGAUGUCCUGG
1253	1254	848-866	CAGGACAUCAUGCAAAAGC	GCUUUUGCAUGAUGUCCUG
1255	1256	849-867	AGGACAUCAUGCAAAAGCA	UGCUUUUGCAUGAUGUCCU
1257	1258	850-868	GGACAUCAUGCAAAAGCAA	UUGCUUUUGCAUGAUGUCC
1259	1260	851-869	GACAUCAUGCAAAAGCAAA	UUUGCUUUUGCAUGAUGUC
1261	1262	852-870	ACAUCAUGCAAAAGCAAAG	CUUUGCUUUUGCAUGAUGU
1263	1264	854-872	AUCAUGCAAAAGCAAAGAC	GUCUUUGCUUUUGCAUGAU
1265	1266	855-873	UCAUGCAAAAGCAAAGACC	GGUCUUUGCUUUUGCAUGA

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1267	1268	856-874	CAUGCAAAAGCAAAGACCA	UGGUCUUUGCUUUUGCAUG
1269	1270	857-875	AUGCAAAAGCAAAGACCAG	CUGGUCUUUGCUUUUGCAU
1271	1272	858-876	UGCAAAAGCAAAGACCAGA	UCUGGUCUUUGCUUUUGCA
1273	1274	859-877	GCAAAAGCAAAGACCAGAA	UUCUGGUCUUUGCUUUUGC
1275	1276	860-878	CAAAAGCAAAGACCAGAAA	UUUCUGGUCUUUGCUUUUG
1277	1278	861-879	AAAAGCAAAGACCAGAAAG	CUUUCUGGUCUUUGCUUUU
1279	1280	862-880	AAAGCAAAGACCAGAAAGA	UCUUUCUGGUCUUUGCUUU
1281	1282	863-881	AAGCAAAGACCAGAAAGAG	CUCUUUCUGGUCUUUGCUU
1283	1284	864-882	AGCAAAGACCAGAAAGAGU	ACUCUUUCUGGUCUUUGCU
1285	1286	865-883	GCAAAGACCAGAAAGAGUG	CACUCUUUCUGGUCUUUGC
1287	1288	867-885	AAAGACCAGAAAGAGUGUC	GACACUCUUUCUGGUCUUU
1289	1290	868-886	AAGACCAGAAAGAGUGUCU	AGACACUCUUUCUGGUCUU
1291	1292	869-887	AGACCAGAAAGAGUGUCUC	GAGACACUCUUUCUGGUCU
1293	1294	870-888	GACCAGAAAGAGUGUCUCA	UGAGACACUCUUUCUGGUC
1295	1296	871-889	ACCAGAAAGAGUGUCUCAU	AUGAGACACUCUUUCUGGU
1297	1298	872-890	CCAGAAAGAGUGUCUCAUC	GAUGAGACACUCUUUCUGG
1299	1300	875-893	GAAAGAGUGUCUCAUCUUC	GAAGAUGAGACACUCUUUC
1301	1302	878-896	AGAGUGUCUCAUCUUCUUC	GAAGAAGAUGAGACACUCU
1303	1304	879-897	GAGUGUCUCAUCUUCUUCA	UGAAGAAGAUGAGACACUC
1305	1306	880-898	AGUGUCUCAUCUUCUUCAA	UUGAAGAAGAUGAGACACU
1307	1308	881-899	GUGUCUCAUCUUCUUCAAG	CUUGAAGAAGAUGAGACAC
1309	1310	882-900	UGUCUCAUCUUCUUCAAGA	UCUUGAAGAAGAUGAGACA
1311	1312	883-901	GUCUCAUCUUCUUCAAGAU	AUCUUGAAGAAGAUGAGAC
1313	1314	884-902	UCUCAUCUUCUUCAAGAUA	UAUCUUGAAGAAGAUGAGA
1315	1316	886-904	UCAUCUUCUUCAAGAUAAC	GUUAUCUUGAAGAAGAUGA
1317	1318	887-905	CAUCUUCUUCAAGAUAACU	AGUUAUCUUGAAGAAGAUG
1319	1320	888-906	AUCUUCUUCAAGAUAACUU	AAGUUAUCUUGAAGAAGAU
1321	1322	889-907	UCUUCUUCAAGAUAACUUG	CAAGUUAUCUUGAAGAAGA
1323	1324	890-908	CUUCUUCAAGAUAACUUGC	GCAAGUUAUCUUGAAGAAG
1325	1326	891-909	UUCUUCAAGAUAACUUGCC	GGCAAGUUAUCUUGAAGAA
1327	1328	892-910	UCUUCAAGAUAACUUGCCA	UGGCAAGUUAUCUUGAAGA
1329	1330	893-911	CUUCAAGAUAACUUGCCAA	UUGGCAAGUUAUCUUGAAG
1331	1332	894-912	UUCAAGAUAACUUGCCAAA	UUUGGCAAGUUAUCUUGAA
1333	1334	895-913	UCAAGAUAACUUGCCAAAA	UUUUGGCAAGUUAUCUUGA
1335	1336	896-914	CAAGAUAACUUGCCAAAAU	AUUUUGGCAAGUUAUCUUG
1337	1338	897-915	AAGAUAACUUGCCAAAAUC	GAUUUUGGCAAGUUAUCUU

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1339	1340	898-916	AGAUAACUUGCCAAAAUCU	AGAUUUUGGCAAGUUAUCU
1341	1342	899-917	GAUAACUUGCCAAAAUCUG	CAGAUUUUGGCAAGUUAUC
1343	1344	900-918	AUAACUUGCCAAAAUCUGU	ACAGAUUUUGGCAAGUUAU
1345	1346	901-919	UAACUUGCCAAAAUCUGUU	AACAGAUUUUGGCAAGUUA
1347	1348	902-920	AACUUGCCAAAAUCUGUUU	AAACAGAUUUUGGCAAGUU
1349	1350	903-921	ACUUGCCAAAAUCUGUUUC	GAAACAGAUUUUGGCAAGU
1351	1352	904-922	CUUGCCAAAAUCUGUUUCC	GGAAACAGAUUUUGGCAAG
1353	1354	905-923	UUGCCAAAAUCUGUUUCCA	UGGAAACAGAUUUUGGCAA
1355	1356	906-924	UGCCAAAAUCUGUUUCCAC	GUGGAAACAGAUUUUGGCA
1357	1358	907-925	GCCAAAAUCUGUUUCCACU	AGUGGAAACAGAUUUUGGC
1359	1360	908-926	CCAAAAUCUGUUUCCACUU	AAGUGGAAACAGAUUUUGG
1361	1362	909-927	CAAAAUCUGUUUCCACUUU	AAAGUGGAAACAGAUUUUG
1363	1364	910-928	AAAAUCUGUUUCCACUUUU	AAAAGUGGAAACAGAUUUU
1365	1366	911-929	AAAUCUGUUUCCACUUUUC	GAAAAGUGGAAACAGAUUU
1367	1368	912-930	AAUCUGUUUCCACUUUUCA	UGAAAAGUGGAAACAGAUU
1369	1370	913-931	AUCUGUUUCCACUUUUCAG	CUGAAAAGUGGAAACAGAU
1371	1372	916-934	UGUUUCCACUUUUCAGUAU	AUACUGAAAAGUGGAAACA
1373	1374	917-935	GUUUCCACUUUUCAGUAUG	CAUACUGAAAAGUGGAAAC
1375	1376	918-936	UUUCCACUUUUCAGUAUGA	UCAUACUGAAAAGUGGAAA
1377	1378	919-937	UUCCACUUUUCAGUAUGAU	AUCAUACUGAAAAGUGGAA
1379	1380	920-938	UCCACUUUUCAGUAUGAUC	GAUCAUACUGAAAAGUGGA
1381	1382	921-939	CCACUUUUCAGUAUGAUCG	CGAUCAUACUGAAAAGUGG
1383	1384	925-943	UUUUCAGUAUGAUCGUUUC	GAAACGAUCAUACUGAAAA
1385	1386	929-947	CAGUAUGAUCGUUUCUUUG	CAAAGAAACGAUCAUACUG
1387	1388	930-948	AGUAUGAUCGUUUCUUUGA	UCAAAGAAACGAUCAUACU
1389	1390	931-949	GUAUGAUCGUUUCUUUGAG	CUCAAAGAAACGAUCAUAC
1391	1392	933-951	AUGAUCGUUUCUUUGAGAA	UUCUCAAAGAAACGAUCAU
1393	1394	934-952	UGAUCGUUUCUUUGAGAAA	UUUCUCAAAGAAACGAUCA
1395	1396	936-954	AUCGUUUCUUUGAGAAAAA	UUUUUCUCAAAGAAACGAU
1397	1398	937-955	UCGUUUCUUUGAGAAAAAA	UUUUUUCUCAAAGAAACGA
1399	1400	938-956	CGUUUCUUUGAGAAAAAAA	UUUUUUUCUCAAAGAAACG
1401	1402	939-957	GUUUCUUUGAGAAAAAAAU	AUUUUUUUCUCAAAGAAAC
1403	1404	940-958	UUUCUUUGAGAAAAAAAUU	AAUUUUUUUCUCAAAGAAA
1405	1406	941-959	UUCUUUGAGAAAAAAAUUG	CAAUUUUUUUCUCAAAGAA
1407	1408	942-960	UCUUUGAGAAAAAAAUUGA	UCAAUUUUUUUCUCAAAGA
1409	1410	943-961	CUUUGAGAAAAAAAUUGAU	AUCAAUUUUUUUCUCAAAG

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1411	1412	944-962	UUUGAGAAAAAAAUUGAUG	CAUCAAUUUUUUUCUCAAA
1413	1414	945-963	UUGAGAAAAAAAUUGAUGA	UCAUCAAUUUUUUUCUCAA
1415	1416	946-964	UGAGAAAAAAAUUGAUGAG	CUCAUCAAUUUUUUUCUCA
1417	1418	947-965	GAGAAAAAAAUUGAUGAGA	UCUCAUCAAUUUUUUUCUC
1419	1420	948-966	AGAAAAAAAUUGAUGAGAA	UUCUCAUCAAUUUUUUUCU
1421	1422	949-967	GAAAAAAAUUGAUGAGAAA	UUUCUCAUCAAUUUUUUUC
1423	1424	950-968	AAAAAAAUUGAUGAGAAAA	UUUUCUCAUCAAUUUUUUU
1425	1426	951-969	AAAAAAUUGAUGAGAAAAA	UUUUUCUCAUCAAUUUUUU
1427	1428	952-970	AAAAAUUGAUGAGAAAAAG	CUUUUUCUCAUCAAUUUUU
1429	1430	953-971	AAAAUUGAUGAGAAAAAGA	UCUUUUUCUCAUCAAUUUU
1431	1432	954-972	AAAUUGAUGAGAAAAAGAA	UUCUUUUUCUCAUCAAUUU
1433	1434	955-973	AAUUGAUGAGAAAAAGAAU	AUUCUUUUUCUCAUCAAUU
1435	1436	956-974	AUUGAUGAGAAAAAGAAUG	CAUUCUUUUUCUCAUCAAU
1437	1438	957-975	UUGAUGAGAAAAAGAAUGA	UCAUUCUUUUUCUCAUCAA
1439	1440	958-976	UGAUGAGAAAAAGAAUGAC	GUCAUUCUUUUUCUCAUCA
1441	1442	959-977	GAUGAGAAAAAGAAUGACC	GGUCAUUCUUUUUCUCAUC
1443	1444	960-978	AUGAGAAAAAGAAUGACCA	UGGUCAUUCUUUUUCUCAU
1445	1446	961-979	UGAGAAAAAGAAUGACCAC	GUGGUCAUUCUUUUUCUCA
1447	1448	962-980	GAGAAAAAGAAUGACCACA	UGUGGUCAUUCUUUUUCUC
1449	1450	963-981	AGAAAAAGAAUGACCACAC	GUGUGGUCAUUCUUUUUCU
1451	1452	964-982	GAAAAAGAAUGACCACACC	GGUGUGGUCAUUCUUUUUC
1453	1454	965-983	AAAAAGAAUGACCACACCU	AGGUGUGGUCAUUCUUUUU
1455	1456	966-984	AAAAGAAUGACCACACCUA	UAGGUGUGGUCAUUCUUUU
1457	1458	967-985	AAAGAAUGACCACACCUAU	AUAGGUGUGGUCAUUCUUU
1459	1460	968-986	AAGAAUGACCACACCUAUC	GAUAGGUGUGGUCAUUCUU
1461	1462	969-987	AGAAUGACCACACCUAUCG	CGAUAGGUGUGGUCAUUCU
1463	1464	970-988	GAAUGACCACACCUAUCGA	UCGAUAGGUGUGGUCAUUC
1465	1466	971-989	AAUGACCACACCUAUCGAG	CUCGAUAGGUGUGGUCAUU
1467	1468	972-990	AUGACCACACCUAUCGAGU	ACUCGAUAGGUGUGGUCAU
1469	1470	976-994	CCACACCUAUCGAGUUUUU	AAAAACUCGAUAGGUGUGG
1471	1472	977-995	CACACCUAUCGAGUUUUUA	UAAAAACUCGAUAGGUGUG
1473	1474	978-996	ACACCUAUCGAGUUUUUAA	UUAAAAACUCGAUAGGUGU
1475	1476	979-997	CACCUAUCGAGUUUUUAAA	UUUAAAAACUCGAUAGGUG
1477	1478	980-998	ACCUAUCGAGUUUUUAAAA	UUUUAAAAACUCGAUAGGU
1479	1480	981-999	CCUAUCGAGUUUUUAAAAC	GUUUUAAAAACUCGAUAGG
1481	1482	982-1000	CUAUCGAGUUUUUAAAACU	AGUUUUAAAAACUCGAUAG

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1483	1484	983-1001	UAUCGAGUUUUUAAAACUG	CAGUUUUAAAAACUCGAUA
1485	1486	984-1002	AUCGAGUUUUUAAAACUGU	ACAGUUUUAAAAACUCGAU
1487	1488	985-1003	UCGAGUUUUUAAAACUGUG	CACAGUUUUAAAAACUCGA
1489	1490	986-1004	CGAGUUUUUAAAACUGUGA	UCACAGUUUUAAAAACUCG
1491	1492	987-1005	GAGUUUUUAAAACUGUGAA	UUCACAGUUUUAAAAACUC
1493	1494	988-1006	AGUUUUUAAAACUGUGAAC	GUUCACAGUUUUAAAAACU
1495	1496	989-1007	GUUUUUAAAACUGUGAACC	GGUUCACAGUUUUAAAAAC
1497	1498	990-1008	UUUUUAAAACUGUGAACCG	CGGUUCACAGUUUUAAAAA
1499	1500	991-1009	UUUUAAAACUGUGAACCGG	CCGGUUCACAGUUUUAAAA
1501	1502	992-1010	UUUAAAACUGUGAACCGGC	GCCGGUUCACAGUUUUAAA
1503	1504	993-1011	UUAAAACUGUGAACCGGCG	CGCCGGUUCACAGUUUUAA
1505	1506	994-1012	UAAAACUGUGAACCGGCGA	UCGCCGGUUCACAGUUUUA
1507	1508	995-1013	AAAACUGUGAACCGGCGAG	CUCGCCGGUUCACAGUUUU
1509	1510	996-1014	AAACUGUGAACCGGCGAGC	GCUCGCCGGUUCACAGUUU
1511	1512	997-1015	AACUGUGAACCGGCGAGCA	UGCUCGCCGGUUCACAGUU
1513	1514	998-1016	ACUGUGAACCGGCGAGCAC	GUGCUCGCCGGUUCACAGU
1515	1516	999-1017	CUGUGAACCGGCGAGCACA	UGUGCUCGCCGGUUCACAG
1517	1518	1000-1018	UGUGAACCGGCGAGCACAC	GUGUGCUCGCCGGUUCACA
1519	1520	1001-1019	GUGAACCGGCGAGCACACA	UGUGUGCUCGCCGGUUCAC
1521	1522	1002-1020	UGAACCGGCGAGCACACAU	AUGUGUGCUCGCCGGUUCA
1523	1524	1003-1021	GAACCGGCGAGCACACAUC	GAUGUGUGCUCGCCGGUUC
1525	1526	1004-1022	AACCGGCGAGCACACAUCU	AGAUGUGUGCUCGCCGGUU
1527	1528	1005-1023	ACCGGCGAGCACACAUCUU	AAGAUGUGUGCUCGCCGGU
1529	1530	1006-1024	CCGGCGAGCACACAUCUUC	GAAGAUGUGUGCUCGCCGG
1531	1532	1007-1025	CGGCGAGCACACAUCUUCC	GGAAGAUGUGUGCUCGCCG
1533	1534	1008-1026	GGCGAGCACACAUCUUCCC	GGGAAGAUGUGUGCUCGCC
1535	1536	1028-1046	AUGGCAGAUGACUAUUCAG	CUGAAUAGUCAUCUGCCAU
1537	1538	1030-1048	GGCAGAUGACUAUUCAGAC	GUCUGAAUAGUCAUCUGCC
1539	1540	1031-1049	GCAGAUGACUAUUCAGACU	AGUCUGAAUAGUCAUCUGC
1541	1542	1032-1050	CAGAUGACUAUUCAGACUC	GAGUCUGAAUAGUCAUCUG
1543	1544	1033-1051	AGAUGACUAUUCAGACUCC	GGAGUCUGAAUAGUCAUCU
1545	1546	1034-1052	GAUGACUAUUCAGACUCCC	GGGAGUCUGAAUAGUCAUC
1547	1548	1035-1053	AUGACUAUUCAGACUCCCU	AGGGAGUCUGAAUAGUCAU
1549	1550	1036-1054	UGACUAUUCAGACUCCCUC	GAGGGAGUCUGAAUAGUCA
1551	1552	1037-1055	GACUAUUCAGACUCCCUCA	UGAGGGAGUCUGAAUAGUC
1553	1554	1038-1056	ACUAUUCAGACUCCCUCAU	AUGAGGGAGUCUGAAUAGU

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1555	1556	1039-1057	CUAUUCAGACUCCCUCAUC	GAUGAGGGAGUCUGAAUAG
1557	1558	1040-1058	UAUUCAGACUCCCUCAUCA	UGAUGAGGGAGUCUGAAUA
1559	1560	1041-1059	AUUCAGACUCCCUCAUCAC	GUGAUGAGGGAGUCUGAAU
1561	1562	1042-1060	UUCAGACUCCCUCAUCACC	GGUGAUGAGGGAGUCUGAA
1563	1564	1043-1061	UCAGACUCCCUCAUCACCA	UGGUGAUGAGGGAGUCUGA
1565	1566	1044-1062	CAGACUCCCUCAUCACCAA	UUGGUGAUGAGGGAGUCUG
1567	1568	1045-1063	AGACUCCCUCAUCACCAAA	UUUGGUGAUGAGGGAGUCU
1569	1570	1046-1064	GACUCCCUCAUCACCAAAA	UUUUGGUGAUGAGGGAGUC
1571	1572	1047-1065	ACUCCCUCAUCACCAAAAA	UUUUUGGUGAUGAGGGAGU
1573	1574	1048-1066	CUCCCUCAUCACCAAAAAG	CUUUUUGGUGAUGAGGGAG
1575	1576	1049-1067	UCCCUCAUCACCAAAAAGC	GCUUUUUGGUGAUGAGGGA
1577	1578	1050-1068	CCCUCAUCACCAAAAAGCA	UGCUUUUUGGUGAUGAGGG
1579	1580	1070-1088	GUGUCAGUCUGGUGCAGUA	UACUGCACCAGACUGACAC
1581	1582	1071-1089	UGUCAGUCUGGUGCAGUAA	UUACUGCACCAGACUGACA
1583	1584	1072-1090	GUCAGUCUGGUGCAGUAAU	AUUACUGCACCAGACUGAC
1585	1586	1073-1091	UCAGUCUGGUGCAGUAAUG	CAUUACUGCACCAGACUGA
1587	1588	1074-1092	CAGUCUGGUGCAGUAAUGA	UCAUUACUGCACCAGACUG
1589	1590	1075-1093	AGUCUGGUGCAGUAAUGAC	GUCAUUACUGCACCAGACU
1591	1592	1078-1096	CUGGUGCAGUAAUGACUAC	GUAGUCAUUACUGCACCAG
1593	1594	1079-1097	UGGUGCAGUAAUGACUACC	GGUAGUCAUUACUGCACCA
1595	1596	1081-1099	GUGCAGUAAUGACUACCUA	UAGGUAGUCAUUACUGCAC
1597	1598	1082-1100	UGCAGUAAUGACUACCUAG	CUAGGUAGUCAUUACUGCA
1599	1600	1083-1101	GCAGUAAUGACUACCUAGG	CCUAGGUAGUCAUUACUGC
1601	1602	1084-1102	CAGUAAUGACUACCUAGGA	UCCUAGGUAGUCAUUACUG
1603	1604	1085-1103	AGUAAUGACUACCUAGGAA	UUCCUAGGUAGUCAUUACU
1605	1606	1086-1104	GUAAUGACUACCUAGGAAU	AUUCCUAGGUAGUCAUUAC
1607	1608	1087-1105	UAAUGACUACCUAGGAAUG	CAUUCCUAGGUAGUCAUUA
1609	1610	1088-1106	AAUGACUACCUAGGAAUGA	UCAUUCCUAGGUAGUCAUU
1611	1612	1089-1107	AUGACUACCUAGGAAUGAG	CUCAUUCCUAGGUAGUCAU
1613	1614	1090-1108	UGACUACCUAGGAAUGAGU	ACUCAUUCCUAGGUAGUCA
1615	1616	1091-1109	GACUACCUAGGAAUGAGUC	GACUCAUUCCUAGGUAGUC
1617	1618	1092-1110	ACUACCUAGGAAUGAGUCG	CGACUCAUUCCUAGGUAGU
1619	1620	1093-1111	CUACCUAGGAAUGAGUCGC	GCGACUCAUUCCUAGGUAG
1621	1622	1094-1112	UACCUAGGAAUGAGUCGCC	GGCGACUCAUUCCUAGGUA
1623	1624	1095-1113	ACCUAGGAAUGAGUCGCCA	UGGCGACUCAUUCCUAGGU
1625	1626	1096-1114	CCUAGGAAUGAGUCGCCAC	GUGGCGACUCAUUCCUAGG

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1627	1628	1097-1115	CUAGGAAUGAGUCGCCACC	GGUGGCGACUCAUUCCUAG
1629	1630	1098-1116	UAGGAAUGAGUCGCCACCC	GGGUGGCGACUCAUUCCUA
1631	1632	1099-1117	AGGAAUGAGUCGCCACCCA	UGGGUGGCGACUCAUUCCU
1633	1634	1100-1118	GGAAUGAGUCGCCACCCAC	GUGGGUGGCGACUCAUUCC
1635	1636	1101-1119	GAAUGAGUCGCCACCCACG	CGUGGGUGGCGACUCAUUC
1637	1638	1102-1120	AAUGAGUCGCCACCCACGG	CCGUGGGUGGCGACUCAUU
1639	1640	1103-1121	AUGAGUCGCCACCCACGGG	CCCGUGGGUGGCGACUCAU
1641	1642	1104-1122	UGAGUCGCCACCCACGGGU	ACCCGUGGGUGGCGACUCA
1643	1644	1105-1123	GAGUCGCCACCCACGGGUG	CACCCGUGGGUGGCGACUC
1645	1646	1106-1124	AGUCGCCACCCACGGGUGU	ACACCCGUGGGUGGCGACU
1647	1648	1107-1125	GUCGCCACCCACGGGUGUG	CACACCCGUGGGUGGCGAC
1649	1650	1108-1126	UCGCCACCCACGGGUGUGU	ACACACCCGUGGGUGGCGA
1651	1652	1109-1127	CGCCACCCACGGGUGUGUG	CACACACCCGUGGGUGGCG
1653	1654	1110-1128	GCCACCCACGGGUGUGUGG	CCACACACCCGUGGGUGGC
1655	1656	1111-1129	CCACCCACGGGUGUGUGGG	CCCACACACCCGUGGGUGG
1657	1658	1112-1130	CACCCACGGGUGUGUGGGG	CCCCACACACCCGUGGGUG
1659	1660	1113-1131	ACCCACGGGUGUGUGGGGC	GCCCCACACACCCGUGGGU
1661	1662	1114-1132	CCCACGGGUGUGUGGGGCA	UGCCCCACACACCCGUGGG
1663	1664	1115-1133	CCACGGGUGUGUGGGGCAG	CUGCCCCACACACCCGUGG
1665	1666	1116-1134	CACGGGUGUGUGGGGCAGU	ACUGCCCCACACACCCGUG
1667	1668	1117-1135	ACGGGUGUGUGGGGCAGUU	AACUGCCCCACACACCCGU
1669	1670	1118-1136	CGGGUGUGUGGGGCAGUUA	UAACUGCCCCACACACCCG
1671	1672	1119-1137	GGGUGUGUGGGGCAGUUAU	AUAACUGCCCCACACACCC
1673	1674	1120-1138	GGUGUGUGGGGCAGUUAUG	CAUAACUGCCCCACACACC
1675	1676	1121-1139	GUGUGUGGGGCAGUUAUGG	CCAUAACUGCCCCACACAC
1677	1678	1122-1140	UGUGUGGGGCAGUUAUGGA	UCCAUAACUGCCCCACACA
1679	1680	1123-1141	GUGUGGGGCAGUUAUGGAC	GUCCAUAACUGCCCCACAC
1681	1682	1125-1143	GUGGGGCAGUUAUGGACAC	GUGUCCAUAACUGCCCCAC
1683	1684	1126-1144	UGGGGCAGUUAUGGACACU	AGUGUCCAUAACUGCCCCA
1685	1686	1128-1146	GGGCAGUUAUGGACACUUU	AAAGUGUCCAUAACUGCCC
1687	1688	1129-1147	GGCAGUUAUGGACACUUUG	CAAAGUGUCCAUAACUGCC
1689	1690	1130-1148	GCAGUUAUGGACACUUUGA	UCAAAGUGUCCAUAACUGC
1691	1692	1131-1149	CAGUUAUGGACACUUUGAA	UUCAAAGUGUCCAUAACUG
1693	1694	1132-1150	AGUUAUGGACACUUUGAAA	UUUCAAAGUGUCCAUAACU
1695	1696	1133-1151	GUUAUGGACACUUUGAAAC	GUUUCAAAGUGUCCAUAAC
1697	1698	1134-1152	UUAUGGACACUUUGAAACA	UGUUUCAAAGUGUCCAUAA

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1699	1700	1135-1153	UAUGGACACUUUGAAACAA	UUGUUUCAAAGUGUCCAUA
1701	1702	1136-1154	AUGGACACUUUGAAACAAC	GUUGUUUCAAAGUGUCCAU
1703	1704	1139-1157	GACACUUUGAAACAACAUG	CAUGUUGUUUCAAAGUGUC
1705	1706	1140-1158	ACACUUUGAAACAACAUGG	CCAUGUUGUUUCAAAGUGU
1707	1708	1141-1159	CACUUUGAAACAACAUGGU	ACCAUGUUGUUUCAAAGUG
1709	1710	1142-1160	ACUUUGAAACAACAUGGUG	CACCAUGUUGUUUCAAAGU
1711	1712	1143-1161	CUUUGAAACAACAUGGUGC	GCACCAUGUUGUUUCAAAG
1713	1714	1144-1162	UUUGAAACAACAUGGUGCU	AGCACCAUGUUGUUUCAAA
1715	1716	1145-1163	UUGAAACAACAUGGUGCUG	CAGCACCAUGUUGUUUCAA
1717	1718	1146-1164	UGAAACAACAUGGUGCUGG	CCAGCACCAUGUUGUUUCA
1719	1720	1147-1165	GAAACAACAUGGUGCUGGG	CCCAGCACCAUGUUGUUUC
1721	1722	1148-1166	AAACAACAUGGUGCUGGGG	CCCCAGCACCAUGUUGUUU
1723	1724	1149-1167	AACAACAUGGUGCUGGGGC	GCCCCAGCACCAUGUUGUU
1725	1726	1150-1168	ACAACAUGGUGCUGGGGCA	UGCCCCAGCACCAUGUUGU
1727	1728	1151-1169	CAACAUGGUGCUGGGGCAG	CUGCCCCAGCACCAUGUUG
1729	1730	1152-1170	AACAUGGUGCUGGGGCAGG	CCUGCCCCAGCACCAUGUU
1731	1732	1153-1171	ACAUGGUGCUGGGGCAGGU	ACCUGCCCCAGCACCAUGU
1733	1734	1154-1172	CAUGGUGCUGGGGCAGGUG	CACCUGCCCCAGCACCAUG
1735	1736	1155-1173	AUGGUGCUGGGGCAGGUGG	CCACCUGCCCCAGCACCAU
1737	1738	1156-1174	UGGUGCUGGGGCAGGUGGU	ACCACCUGCCCCAGCACCA
1739	1740	1157-1175	GGUGCUGGGGCAGGUGGUA	UACCACCUGCCCCAGCACC
1741	1742	1158-1176	GUGCUGGGGCAGGUGGUAC	GUACCACCUGCCCCAGCAC
1743	1744	1159-1177	UGCUGGGGCAGGUGGUACU	AGUACCACCUGCCCCAGCA
1745	1746	1160-1178	GCUGGGGCAGGUGGUACUA	UAGUACCACCUGCCCCAGC
1747	1748	1161-1179	CUGGGGCAGGUGGUACUAG	CUAGUACCACCUGCCCCAG
1749	1750	1162-1180	UGGGGCAGGUGGUACUAGA	UCUAGUACCACCUGCCCCA
1751	1752	1166-1184	GCAGGUGGUACUAGAAAUA	UAUUUCUAGUACCACCUGC
1753	1754	1167-1185	CAGGUGGUACUAGAAAUAU	AUAUUUCUAGUACCACCUG
1755	1756	1168-1186	AGGUGGUACUAGAAAUAUU	AAUAUUUCUAGUACCACCU
1757	1758	1169-1187	GGUGGUACUAGAAAUAUUU	AAAUAUUUCUAGUACCACC
1759	1760	1170-1188	GUGGUACUAGAAAUAUUUC	GAAAUAUUUCUAGUACCAC
1761	1762	1171-1189	UGGUACUAGAAAUAUUUCU	AGAAAUAUUUCUAGUACCA
1763	1764	1172-1190	GGUACUAGAAAUAUUUCUG	CAGAAAUAUUUCUAGUACC
1765	1766	1173-1191	GUACUAGAAAUAUUUCUGG	CCAGAAAUAUUUCUAGUAC
1767	1768	1174-1192	UACUAGAAAUAUUUCUGGA	UCCAGAAAUAUUUCUAGUA
1769	1770	1175-1193	ACUAGAAAUAUUUCUGGAA	UUCCAGAAAUAUUUCUAGU

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1771	1772	1176-1194	CUAGAAAUAUUUCUGGAAC	GUUCCAGAAAUAUUUCUAG
1773	1774	1177-1195	UAGAAAUAUUUCUGGAACU	AGUUCCAGAAAUAUUUCUA
1775	1776	1178-1196	AGAAAUAUUUCUGGAACUA	UAGUUCCAGAAAUAUUUCU
1777	1778	1179-1197	GAAAUAUUUCUGGAACUAG	CUAGUUCCAGAAAUAUUUC
1779	1780	1180-1198	AAAUAUUUCUGGAACUAGU	ACUAGUUCCAGAAAUAUUU
1781	1782	1181-1199	AAUAUUUCUGGAACUAGUA	UACUAGUUCCAGAAAUAUU
1783	1784	1183-1201	UAUUUCUGGAACUAGUAAA	UUUACUAGUUCCAGAAAUA
1785	1786	1186-1204	UUCUGGAACUAGUAAAUUC	GAAUUUACUAGUUCCAGAA
1787	1788	1187-1205	UCUGGAACUAGUAAAUUCC	GGAAUUUACUAGUUCCAGA
1789	1790	1189-1207	UGGAACUAGUAAAUUCCAU	AUGGAAUUUACUAGUUCCA
1791	1792	1190-1208	GGAACUAGUAAAUUCCAUG	CAUGGAAUUUACUAGUUCC
1793	1794	1192-1210	AACUAGUAAAUUCCAUGUG	CACAUGGAAUUUACUAGUU
1795	1796	1193-1211	ACUAGUAAAUUCCAUGUGG	CCACAUGGAAUUUACUAGU
1797	1798	1194-1212	CUAGUAAAUUCCAUGUGGA	UCCACAUGGAAUUUACUAG
1799	1800	1195-1213	UAGUAAAUUCCAUGUGGAC	GUCCACAUGGAAUUUACUA
1801	1802	1196-1214	AGUAAAUUCCAUGUGGACU	AGUCCACAUGGAAUUUACU
1803	1804	1197-1215	GUAAAUUCCAUGUGGACUU	AAGUCCACAUGGAAUUUAC
1805	1806	1198-1216	UAAAUUCCAUGUGGACUUA	UAAGUCCACAUGGAAUUUA
1807	1808	1199-1217	AAAUUCCAUGUGGACUUAG	CUAAGUCCACAUGGAAUUU
1809	1810	1200-1218	AAUUCCAUGUGGACUUAGA	UCUAAGUCCACAUGGAAUU
1811	1812	1201-1219	AUUCCAUGUGGACUUAGAG	CUCUAAGUCCACAUGGAAU
1813	1814	1202-1220	UUCCAUGUGGACUUAGAGC	GCUCUAAGUCCACAUGGAA
1815	1816	1222-1240	GGAGCUGGCAGACCUCCAU	AUGGAGGUCUGCCAGCUCC
1817	1818	1223-1241	GAGCUGGCAGACCUCCAUG	CAUGGAGGUCUGCCAGCUC
1819	1820	1224-1242	AGCUGGCAGACCUCCAUGG	CCAUGGAGGUCUGCCAGCU
1821	1822	1225-1243	GCUGGCAGACCUCCAUGGG	CCCAUGGAGGUCUGCCAGC
1823	1824	1226-1244	CUGGCAGACCUCCAUGGGA	UCCCAUGGAGGUCUGCCAG
1825	1826	1227-1245	UGGCAGACCUCCAUGGGAA	UUCCCAUGGAGGUCUGCCA
1827	1828	1228-1246	GGCAGACCUCCAUGGGAAA	UUUCCCAUGGAGGUCUGCC
1829	1830	1229-1247	GCAGACCUCCAUGGGAAAG	CUUUCCCAUGGAGGUCUGC
1831	1832	1230-1248	CAGACCUCCAUGGGAAAGA	UCUUUCCCAUGGAGGUCUG
1833	1834	1231-1249	AGACCUCCAUGGGAAAGAU	AUCUUUCCCAUGGAGGUCU
1835	1836	1232-1250	GACCUCCAUGGGAAAGAUG	CAUCUUUCCCAUGGAGGUC
1837	1838	1233-1251	ACCUCCAUGGGAAAGAUGC	GCAUCUUUCCCAUGGAGGU
1839	1840	1254-1272	CACUCUUGUUUUCCUCGUG	CACGAGGAAAACAAGAGUG
1841	1842	1255-1273	ACUCUUGUUUUCCUCGUGC	GCACGAGGAAAACAAGAGU

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1843	1844	1256-1274	CUCUUGUUUUCCUCGUGCU	AGCACGAGGAAAACAAGAG
1845	1846	1257-1275	UCUUGUUUUCCUCGUGCUU	AAGCACGAGGAAAACAAGA
1847	1848	1259-1277	UUGUUUUCCUCGUGCUUUG	CAAAGCACGAGGAAAACAA
1849	1850	1260-1278	UGUUUUCCUCGUGCUUUGU	ACAAAGCACGAGGAAAACA
1851	1852	1261-1279	GUUUUCCUCGUGCUUUGUG	CACAAAGCACGAGGAAAAC
1853	1854	1262-1280	UUUUCCUCGUGCUUUGUGG	CCACAAAGCACGAGGAAAA
1855	1856	1263-1281	UUUCCUCGUGCUUUGUGGC	GCCACAAAGCACGAGGAAA
1857	1858	1264-1282	UUCCUCGUGCUUUGUGGCC	GGCCACAAAGCACGAGGAA
1859	1860	1265-1283	UCCUCGUGCUUUGUGGCCA	UGGCCACAAAGCACGAGGA
1861	1862	1266-1284	CCUCGUGCUUUGUGGCCAA	UUGGCCACAAAGCACGAGG
1863	1864	1267-1285	CUCGUGCUUUGUGGCCAAU	AUUGGCCACAAAGCACGAG
1865	1866	1268-1286	UCGUGCUUUGUGGCCAAUG	CAUUGGCCACAAAGCACGA
1867	1868	1269-1287	CGUGCUUUGUGGCCAAUGA	UCAUUGGCCACAAAGCACG
1869	1870	1270-1288	GUGCUUUGUGGCCAAUGAC	GUCAUUGGCCACAAAGCAC
1871	1872	1271-1289	UGCUUUGUGGCCAAUGACU	AGUCAUUGGCCACAAAGCA
1873	1874	1272-1290	GCUUUGUGGCCAAUGACUC	GAGUCAUUGGCCACAAAGC
1875	1876	1273-1291	CUUUGUGGCCAAUGACUCA	UGAGUCAUUGGCCACAAAG
1877	1878	1274-1292	UUUGUGGCCAAUGACUCAA	UUGAGUCAUUGGCCACAAA
1879	1880	1275-1293	UUGUGGCCAAUGACUCAAC	GUUGAGUCAUUGGCCACAA
1881	1882	1276-1294	UGUGGCCAAUGACUCAACC	GGUUGAGUCAUUGGCCACA
1883	1884	1277-1295	GUGGCCAAUGACUCAACCC	GGGUUGAGUCAUUGGCCAC
1885	1886	1278-1296	UGGCCAAUGACUCAACCCU	AGGGUUGAGUCAUUGGCCA
1887	1888	1279-1297	GGCCAAUGACUCAACCCUC	GAGGGUUGAGUCAUUGGCC
1889	1890	1280-1298	GCCAAUGACUCAACCCUCU	AGAGGGUUGAGUCAUUGGC
1891	1892	1281-1299	CCAAUGACUCAACCCUCUU	AAGAGGGUUGAGUCAUUGG
1893	1894	1282-1300	CAAUGACUCAACCCUCUUC	GAAGAGGGUUGAGUCAUUG
1895	1896	1283-1301	AAUGACUCAACCCUCUUCA	UGAAGAGGGUUGAGUCAUU
1897	1898	1284-1302	AUGACUCAACCCUCUUCAC	GUGAAGAGGGUUGAGUCAU
1899	1900	1285-1303	UGACUCAACCCUCUUCACC	GGUGAAGAGGGUUGAGUCA
1901	1902	1286-1304	GACUCAACCCUCUUCACCC	GGGUGAAGAGGGUUGAGUC
1903	1904	1287-1305	ACUCAACCCUCUUCACCCU	AGGGUGAAGAGGGUUGAGU
1905	1906	1288-1306	CUCAACCCUCUUCACCCUG	CAGGGUGAAGAGGGUUGAG
1907	1908	1289-1307	UCAACCCUCUUCACCCUGG	CCAGGGUGAAGAGGGUUGA
1909	1910	1290-1308	CAACCCUCUUCACCCUGGC	GCCAGGGUGAAGAGGGUUG
1911	1912	1291-1309	AACCCUCUUCACCCUGGCU	AGCCAGGGUGAAGAGGGUU
1913	1914	1292-1310	ACCCUCUUCACCCUGGCUA	UAGCCAGGGUGAAGAGGGU

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1915	1916	1293-1311	CCCUCUUCACCCUGGCUAA	UUAGCCAGGGUGAAGAGGG
1917	1918	1294-1312	CCUCUUCACCCUGGCUAAG	CUUAGCCAGGGUGAAGAGG
1919	1920	1297-1315	CUUCACCCUGGCUAAGAUG	CAUCUUAGCCAGGGUGAAG
1921	1922	1298-1316	UUCACCCUGGCUAAGAUGA	UCAUCUUAGCCAGGGUGAA
1923	1924	1300-1318	CACCCUGGCUAAGAUGAUG	CAUCAUCUUAGCCAGGGUG
1925	1926	1301-1319	ACCCUGGCUAAGAUGAUGC	GCAUCAUCUUAGCCAGGGU
1927	1928	1302-1320	CCCUGGCUAAGAUGAUGCC	GGCAUCAUCUUAGCCAGGG
1929	1930	1303-1321	CCUGGCUAAGAUGAUGCCA	UGGCAUCAUCUUAGCCAGG
1931	1932	1304-1322	CUGGCUAAGAUGAUGCCAG	CUGGCAUCAUCUUAGCCAG
1933	1934	1305-1323	UGGCUAAGAUGAUGCCAGG	CCUGGCAUCAUCUUAGCCA
1935	1936	1306-1324	GGCUAAGAUGAUGCCAGGC	GCCUGGCAUCAUCUUAGCC
1937	1938	1307-1325	GCUAAGAUGAUGCCAGGCU	AGCCUGGCAUCAUCUUAGC
1939	1940	1308-1326	CUAAGAUGAUGCCAGGCUG	CAGCCUGGCAUCAUCUUAG
1941	1942	1309-1327	UAAGAUGAUGCCAGGCUGU	ACAGCCUGGCAUCAUCUUA
1943	1944	1310-1328	AAGAUGAUGCCAGGCUGUG	CACAGCCUGGCAUCAUCUU
1945	1946	1311-1329	AGAUGAUGCCAGGCUGUGA	UCACAGCCUGGCAUCAUCU
1947	1948	1312-1330	GAUGAUGCCAGGCUGUGAG	CUCACAGCCUGGCAUCAUC
1949	1950	1313-1331	AUGAUGCCAGGCUGUGAGA	UCUCACAGCCUGGCAUCAU
1951	1952	1314-1332	UGAUGCCAGGCUGUGAGAU	AUCUCACAGCCUGGCAUCA
1953	1954	1316-1334	AUGCCAGGCUGUGAGAUUU	AAAUCUCACAGCCUGGCAU
1955	1956	1317-1335	UGCCAGGCUGUGAGAUUUA	UAAAUCUCACAGCCUGGCA
1957	1958	1318-1336	GCCAGGCUGUGAGAUUUAC	GUAAAUCUCACAGCCUGGC
1959	1960	1319-1337	CCAGGCUGUGAGAUUUACU	AGUAAAUCUCACAGCCUGG
1961	1962	1320-1338	CAGGCUGUGAGAUUUACUC	GAGUAAAUCUCACAGCCUG
1963	1964	1321-1339	AGGCUGUGAGAUUUACUCU	AGAGUAAAUCUCACAGCCU
1965	1966	1322-1340	GGCUGUGAGAUUUACUCUG	CAGAGUAAAUCUCACAGCC
1967	1968	1323-1341	GCUGUGAGAUUUACUCUGA	UCAGAGUAAAUCUCACAGC
1969	1970	1326-1344	GUGAGAUUUACUCUGAUUC	GAAUCAGAGUAAAUCUCAC
1971	1972	1327-1345	UGAGAUUUACUCUGAUUCU	AGAAUCAGAGUAAAUCUCA
1973	1974	1328-1346	GAGAUUUACUCUGAUUCUG	CAGAAUCAGAGUAAAUCUC
1975	1976	1329-1347	AGAUUUACUCUGAUUCUGG	CCAGAAUCAGAGUAAAUCU
1977	1978	1330-1348	GAUUUACUCUGAUUCUGGG	CCCAGAAUCAGAGUAAAUC
1979	1980	1331-1349	AUUUACUCUGAUUCUGGGA	UCCCAGAAUCAGAGUAAAU
1981	1982	1332-1350	UUUACUCUGAUUCUGGGAA	UUCCCAGAAUCAGAGUAAA
1983	1984	1333-1351	UUACUCUGAUUCUGGGAAC	GUUCCCAGAAUCAGAGUAA
1985	1986	1334-1352	UACUCUGAUUCUGGGAACC	GGUUCCCAGAAUCAGAGUA

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
1987	1988	1335-1353	ACUCUGAUUCUGGGAACCA	UGGUUCCCAGAAUCAGAGU
1989	1990	1336-1354	CUCUGAUUCUGGGAACCAU	AUGGUUCCCAGAAUCAGAG
1991	1992	1337-1355	UCUGAUUCUGGGAACCAUG	CAUGGUUCCCAGAAUCAGA
1993	1994	1338-1356	CUGAUUCUGGGAACCAUGC	GCAUGGUUCCCAGAAUCAG
1995	1996	1339-1357	UGAUUCUGGGAACCAUGCC	GGCAUGGUUCCCAGAAUCA
1997	1998	1340-1358	GAUUCUGGGAACCAUGCCU	AGGCAUGGUUCCCAGAAUC
1999	2000	1341-1359	AUUCUGGGAACCAUGCCUC	GAGGCAUGGUUCCCAGAAU
2001	2002	1342-1360	UUCUGGGAACCAUGCCUCC	GGAGGCAUGGUUCCCAGAA
2003	2004	1343-1361	UCUGGGAACCAUGCCUCCA	UGGAGGCAUGGUUCCCAGA
2005	2006	1344-1362	CUGGGAACCAUGCCUCCAU	AUGGAGGCAUGGUUCCCAG
2007	2008	1345-1363	UGGGAACCAUGCCUCCAUG	CAUGGAGGCAUGGUUCCCA
2009	2010	1346-1364	GGGAACCAUGCCUCCAUGA	UCAUGGAGGCAUGGUUCCC
2011	2012	1348-1366	GAACCAUGCCUCCAUGAUC	GAUCAUGGAGGCAUGGUUC
2013	2014	1349-1367	AACCAUGCCUCCAUGAUCC	GGAUCAUGGAGGCAUGGUU
2015	2016	1350-1368	ACCAUGCCUCCAUGAUCCA	UGGAUCAUGGAGGCAUGGU
2017	2018	1351-1369	CCAUGCCUCCAUGAUCCAA	UUGGAUCAUGGAGGCAUGG
2019	2020	1352-1370	CAUGCCUCCAUGAUCCAAG	CUUGGAUCAUGGAGGCAUG
2021	2022	1353-1371	AUGCCUCCAUGAUCCAAGG	CCUUGGAUCAUGGAGGCAU
2023	2024	1354-1372	UGCCUCCAUGAUCCAAGGG	CCCUUGGAUCAUGGAGGCA
2025	2026	1358-1376	UCCAUGAUCCAAGGGAUUC	GAAUCCCUUGGAUCAUGGA
2027	2028	1359-1377	CCAUGAUCCAAGGGAUUCG	CGAAUCCCUUGGAUCAUGG
2029	2030	1360-1378	CAUGAUCCAAGGGAUUCGA	UCGAAUCCCUUGGAUCAUG
2031	2032	1361-1379	AUGAUCCAAGGGAUUCGAA	UUCGAAUCCCUUGGAUCAU
2033	2034	1362-1380	UGAUCCAAGGGAUUCGAAA	UUUCGAAUCCCUUGGAUCA
2035	2036	1363-1381	GAUCCAAGGGAUUCGAAAC	GUUUCGAAUCCCUUGGAUC
2037	2038	1365-1383	UCCAAGGGAUUCGAAACAG	CUGUUUCGAAUCCCUUGGA
2039	2040	1366-1384	CCAAGGGAUUCGAAACAGC	GCUGUUUCGAAUCCCUUGG
2041	2042	1367-1385	CAAGGGAUUCGAAACAGCC	GGCUGUUUCGAAUCCCUUG
2043	2044	1368-1386	AAGGGAUUCGAAACAGCCG	CGGCUGUUUCGAAUCCCUU
2045	2046	1369-1387	AGGGAUUCGAAACAGCCGA	UCGGCUGUUUCGAAUCCCU
2047	2048	1370-1388	GGGAUUCGAAACAGCCGAG	CUCGGCUGUUUCGAAUCCC
2049	2050	1371-1389	GGAUUCGAAACAGCCGAGU	ACUCGGCUGUUUCGAAUCC
2051	2052	1372-1390	GAUUCGAAACAGCCGAGUG	CACUCGGCUGUUUCGAAUC
2053	2054	1373-1391	AUUCGAAACAGCCGAGUGC	GCACUCGGCUGUUUCGAAU
2055	2056	1374-1392	UUCGAAACAGCCGAGUGCC	GGCACUCGGCUGUUUCGAA
2057	2058	1375-1393	UCGAAACAGCCGAGUGCCA	UGGCACUCGGCUGUUUCGA

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2059	2060	1376-1394	CGAAACAGCCGAGUGCCAA	UUGGCACUCGGCUGUUUCG
2061	2062	1377-1395	GAAACAGCCGAGUGCCAAA	UUUGGCACUCGGCUGUUUC
2063	2064	1378-1396	AAACAGCCGAGUGCCAAAG	CUUUGGCACUCGGCUGUUU
2065	2066	1379-1397	AACAGCCGAGUGCCAAAGU	ACUUUGGCACUCGGCUGUU
2067	2068	1380-1398	ACAGCCGAGUGCCAAAGUA	UACUUUGGCACUCGGCUGU
2069	2070	1381-1399	CAGCCGAGUGCCAAAGUAC	GUACUUUGGCACUCGGCUG
2071	2072	1383-1401	GCCGAGUGCCAAAGUACAU	AUGUACUUUGGCACUCGGC
2073	2074	1384-1402	CCGAGUGCCAAAGUACAUC	GAUGUACUUUGGCACUCGG
2075	2076	1385-1403	CGAGUGCCAAAGUACAUCU	AGAUGUACUUUGGCACUCG
2077	2078	1386-1404	GAGUGCCAAAGUACAUCUU	AAGAUGUACUUUGGCACUC
2079	2080	1387-1405	AGUGCCAAAGUACAUCUUC	GAAGAUGUACUUUGGCACU
2081	2082	1388-1406	GUGCCAAAGUACAUCUUCC	GGAAGAUGUACUUUGGCAC
2083	2084	1389-1407	UGCCAAAGUACAUCUUCCG	CGGAAGAUGUACUUUGGCA
2085	2086	1390-1408	GCCAAAGUACAUCUUCCGC	GCGGAAGAUGUACUUUGGC
2087	2088	1391-1409	CCAAAGUACAUCUUCCGCC	GGCGGAAGAUGUACUUUGG
2089	2090	1392-1410	CAAAGUACAUCUUCCGCCA	UGGCGGAAGAUGUACUUUG
2091	2092	1393-1411	AAAGUACAUCUUCCGCCAC	GUGGCGGAAGAUGUACUUU
2093	2094	1394-1412	AAGUACAUCUUCCGCCACA	UGUGGCGGAAGAUGUACUU
2095	2096	1395-1413	AGUACAUCUUCCGCCACAA	UUGUGGCGGAAGAUGUACU
2097	2098	1396-1414	GUACAUCUUCCGCCACAAU	AUUGUGGCGGAAGAUGUAC
2099	2100	1397-1415	UACAUCUUCCGCCACAAUG	CAUUGUGGCGGAAGAUGUA
2101	2102	1398-1416	ACAUCUUCCGCCACAAUGA	UCAUUGUGGCGGAAGAUGU
2103	2104	1399-1417	CAUCUUCCGCCACAAUGAU	AUCAUUGUGGCGGAAGAUG
2105	2106	1400-1418	AUCUUCCGCCACAAUGAUG	CAUCAUUGUGGCGGAAGAU
2107	2108	1401-1419	UCUUCCGCCACAAUGAUGU	ACAUCAUUGUGGCGGAAGA
2109	2110	1402-1420	CUUCCGCCACAAUGAUGUC	GACAUCAUUGUGGCGGAAG
2111	2112	1403-1421	UUCCGCCACAAUGAUGUCA	UGACAUCAUUGUGGCGGAA
2113	2114	1404-1422	UCCGCCACAAUGAUGUCAG	CUGACAUCAUUGUGGCGGA
2115	2116	1405-1423	CCGCCACAAUGAUGUCAGC	GCUGACAUCAUUGUGGCGG
2117	2118	1406-1424	CGCCACAAUGAUGUCAGCC	GGCUGACAUCAUUGUGGCG
2119	2120	1407-1425	GCCACAAUGAUGUCAGCCA	UGGCUGACAUCAUUGUGGC
2121	2122	1427-1445	CUCAGAGAACUGCUGCAAA	UUUGCAGCAGUUCUCUGAG
2123	2124	1428-1446	UCAGAGAACUGCUGCAAAG	CUUUGCAGCAGUUCUCUGA
2125	2126	1429-1447	CAGAGAACUGCUGCAAAGA	UCUUUGCAGCAGUUCUCUG
2127	2128	1430-1448	AGAGAACUGCUGCAAAGAU	AUCUUUGCAGCAGUUCUCU
2129	2130	1431-1449	GAGAACUGCUGCAAAGAUC	GAUCUUUGCAGCAGUUCUC

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2131	2132	1432-1450	AGAACUGCUGCAAAGAUCU	AGAUCUUUGCAGCAGUUCU
2133	2134	1433-1451	GAACUGCUGCAAAGAUCUG	CAGAUCUUUGCAGCAGUUC
2135	2136	1434-1452	AACUGCUGCAAAGAUCUGA	UCAGAUCUUUGCAGCAGUU
2137	2138	1435-1453	ACUGCUGCAAAGAUCUGAC	GUCAGAUCUUUGCAGCAGU
2139	2140	1436-1454	CUGCUGCAAAGAUCUGACC	GGUCAGAUCUUUGCAGCAG
2141	2142	1437-1455	UGCUGCAAAGAUCUGACCC	GGGUCAGAUCUUUGCAGCA
2143	2144	1457-1475	UCAGUCCCCAAGAUUGUGG	CCACAAUCUUGGGGACUGA
2145	2146	1458-1476	CAGUCCCCAAGAUUGUGGC	GCCACAAUCUUGGGGACUG
2147	2148	1459-1477	AGUCCCCAAGAUUGUGGCA	UGCCACAAUCUUGGGGACU
2149	2150	1461-1479	UCCCCAAGAUUGUGGCAUU	AAUGCCACAAUCUUGGGGA
2151	2152	1462-1480	CCCCAAGAUUGUGGCAUUU	AAAUGCCACAAUCUUGGGG
2153	2154	1463-1481	CCCAAGAUUGUGGCAUUUG	CAAAUGCCACAAUCUUGGG
2155	2156	1464-1482	CCAAGAUUGUGGCAUUUGA	UCAAAUGCCACAAUCUUGG
2157	2158	1465-1483	CAAGAUUGUGGCAUUUGAA	UUCAAAUGCCACAAUCUUG
2159	2160	1466-1484	AAGAUUGUGGCAUUUGAAA	UUUCAAAUGCCACAAUCUU
2161	2162	1467-1485	AGAUUGUGGCAUUUGAAAC	GUUUCAAAUGCCACAAUCU
2163	2164	1468-1486	GAUUGUGGCAUUUGAAACU	AGUUUCAAAUGCCACAAUC
2165	2166	1469-1487	AUUGUGGCAUUUGAAACUG	CAGUUUCAAAUGCCACAAU
2167	2168	1470-1488	UUGUGGCAUUUGAAACUGU	ACAGUUUCAAAUGCCACAA
2169	2170	1471-1489	UGUGGCAUUUGAAACUGUC	GACAGUUUCAAAUGCCACA
2171	2172	1472-1490	GUGGCAUUUGAAACUGUCC	GGACAGUUUCAAAUGCCAC
2173	2174	1473-1491	UGGCAUUUGAAACUGUCCA	UGGACAGUUUCAAAUGCCA
2175	2176	1474-1492	GGCAUUUGAAACUGUCCAU	AUGGACAGUUUCAAAUGCC
2177	2178	1475-1493	GCAUUUGAAACUGUCCAUU	AAUGGACAGUUUCAAAUGC
2179	2180	1476-1494	CAUUUGAAACUGUCCAUUC	GAAUGGACAGUUUCAAAUG
2181	2182	1477-1495	AUUUGAAACUGUCCAUUCA	UGAAUGGACAGUUUCAAAU
2183	2184	1479-1497	UUGAAACUGUCCAUUCAAU	AUUGAAUGGACAGUUUCAA
2185	2186	1480-1498	UGAAACUGUCCAUUCAAUG	CAUUGAAUGGACAGUUUCA
2187	2188	1481-1499	GAAACUGUCCAUUCAAUGG	CCAUUGAAUGGACAGUUUC
2189	2190	1482-1500	AAACUGUCCAUUCAAUGGA	UCCAUUGAAUGGACAGUUU
2191	2192	1483-1501	AACUGUCCAUUCAAUGGAU	AUCCAUUGAAUGGACAGUU
2193	2194	1484-1502	ACUGUCCAUUCAAUGGAUG	CAUCCAUUGAAUGGACAGU
2195	2196	1485-1503	CUGUCCAUUCAAUGGAUGG	CCAUCCAUUGAAUGGACAG
2197	2198	1486-1504	UGUCCAUUCAAUGGAUGGG	CCCAUCCAUUGAAUGGACA
2199	2200	1487-1505	GUCCAUUCAAUGGAUGGGG	CCCCAUCCAUUGAAUGGAC
2201	2202	1488-1506	UCCAUUCAAUGGAUGGGGC	GCCCCAUCCAUUGAAUGGA

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2203	2204	1508-1526	GUGUGCCCACUGGAAGAGC	GCUCUUCCAGUGGGCACAC
2205	2206	1509-1527	UGUGCCCACUGGAAGAGCU	AGCUCUUCCAGUGGGCACA
2207	2208	1510-1528	GUGCCCACUGGAAGAGCUG	CAGCUCUUCCAGUGGGCAC
2209	2210	1511-1529	UGCCCACUGGAAGAGCUGU	ACAGCUCUUCCAGUGGGCA
2211	2212	1512-1530	GCCCACUGGAAGAGCUGUG	CACAGCUCUUCCAGUGGGC
2213	2214	1513-1531	CCCACUGGAAGAGCUGUGU	ACACAGCUCUUCCAGUGGG
2215	2216	1514-1532	CCACUGGAAGAGCUGUGUG	CACACAGCUCUUCCAGUGG
2217	2218	1515-1533	CACUGGAAGAGCUGUGUGA	UCACACAGCUCUUCCAGUG
2219	2220	1516-1534	ACUGGAAGAGCUGUGUGAU	AUCACACAGCUCUUCCAGU
2221	2222	1517-1535	CUGGAAGAGCUGUGUGAUG	CAUCACACAGCUCUUCCAG
2223	2224	1518-1536	UGGAAGAGCUGUGUGAUGU	ACAUCACACAGCUCUUCCA
2225	2226	1519-1537	GGAAGAGCUGUGUGAUGUG	CACAUCACACAGCUCUUCC
2227	2228	1520-1538	GAAGAGCUGUGUGAUGUGG	CCACAUCACACAGCUCUUC
2229	2230	1521-1539	AAGAGCUGUGUGAUGUGGC	GCCACAUCACACAGCUCUU
2231	2232	1522-1540	AGAGCUGUGUGAUGUGGCC	GGCCACAUCACACAGCUCU
2233	2234	1523-1541	GAGCUGUGUGAUGUGGCCC	GGGCCACAUCACACAGCUC
2235	2236	1524-1542	AGCUGUGUGAUGUGGCCCA	UGGGCCACAUCACACAGCU
2237	2238	1525-1543	GCUGUGUGAUGUGGCCCAU	AUGGGCCACAUCACACAGC
2239	2240	1526-1544	CUGUGUGAUGUGGCCCAUG	CAUGGGCCACAUCACACAG
2241	2242	1527-1545	UGUGUGAUGUGGCCCAUGA	UCAUGGGCCACAUCACACA
2243	2244	1528-1546	GUGUGAUGUGGCCCAUGAG	CUCAUGGGCCACAUCACAC
2245	2246	1529-1547	UGUGAUGUGGCCCAUGAGU	ACUCAUGGGCCACAUCACA
2247	2248	1532-1550	GAUGUGGCCCAUGAGUUUG	CAAACUCAUGGGCCACAUC
2249	2250	1533-1551	AUGUGGCCCAUGAGUUUGG	CCAAACUCAUGGGCCACAU
2251	2252	1534-1552	UGUGGCCCAUGAGUUUGGA	UCCAAACUCAUGGGCCACA
2253	2254	1535-1553	GUGGCCCAUGAGUUUGGAG	CUCCAAACUCAUGGGCCAC
2255	2256	1536-1554	UGGCCCAUGAGUUUGGAGC	GCUCCAAACUCAUGGGCCA
2257	2258	1537-1555	GGCCCAUGAGUUUGGAGCA	UGCUCCAAACUCAUGGGCC
2259	2260	1538-1556	GCCCAUGAGUUUGGAGCAA	UUGCUCCAAACUCAUGGGC
2261	2262	1539-1557	CCCAUGAGUUUGGAGCAAU	AUUGCUCCAAACUCAUGGG
2263	2264	1540-1558	CCAUGAGUUUGGAGCAAUC	GAUUGCUCCAAACUCAUGG
2265	2266	1542-1560	AUGAGUUUGGAGCAAUCAC	GUGAUUGCUCCAAACUCAU
2267	2268	1543-1561	UGAGUUUGGAGCAAUCACC	GGUGAUUGCUCCAAACUCA
2269	2270	1545-1563	AGUUUGGAGCAAUCACCUU	AAGGUGAUUGCUCCAAACU
2271	2272	1546-1564	GUUUGGAGCAAUCACCUUC	GAAGGUGAUUGCUCCAAAC
2273	2274	1547-1565	UUUGGAGCAAUCACCUUCG	CGAAGGUGAUUGCUCCAAA

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2275	2276	1548-1566	UUGGAGCAAUCACCUUCGU	ACGAAGGUGAUUGCUCCAA
2277	2278	1549-1567	UGGAGCAAUCACCUUCGUG	CACGAAGGUGAUUGCUCCA
2279	2280	1550-1568	GGAGCAAUCACCUUCGUGG	CCACGAAGGUGAUUGCUCC
2281	2282	1551-1569	GAGCAAUCACCUUCGUGGA	UCCACGAAGGUGAUUGCUC
2283	2284	1552-1570	AGCAAUCACCUUCGUGGAU	AUCCACGAAGGUGAUUGCU
2285	2286	1553-1571	GCAAUCACCUUCGUGGAUG	CAUCCACGAAGGUGAUUGC
2287	2288	1554-1572	CAAUCACCUUCGUGGAUGA	UCAUCCACGAAGGUGAUUG
2289	2290	1555-1573	AAUCACCUUCGUGGAUGAG	CUCAUCCACGAAGGUGAUU
2291	2292	1556-1574	AUCACCUUCGUGGAUGAGG	CCUCAUCCACGAAGGUGAU
2293	2294	1557-1575	UCACCUUCGUGGAUGAGGU	ACCUCAUCCACGAAGGUGA
2295	2296	1558-1576	CACCUUCGUGGAUGAGGUC	GACCUCAUCCACGAAGGUG
2297	2298	1559-1577	ACCUUCGUGGAUGAGGUCC	GGACCUCAUCCACGAAGGU
2299	2300	1560-1578	CCUUCGUGGAUGAGGUCCA	UGGACCUCAUCCACGAAGG
2301	2302	1561-1579	CUUCGUGGAUGAGGUCCAC	GUGGACCUCAUCCACGAAG
2303	2304	1562-1580	UUCGUGGAUGAGGUCCACG	CGUGGACCUCAUCCACGAA
2305	2306	1563-1581	UCGUGGAUGAGGUCCACGC	GCGUGGACCUCAUCCACGA
2307	2308	1564-1582	CGUGGAUGAGGUCCACGCA	UGCGUGGACCUCAUCCACG
2309	2310	1565-1583	GUGGAUGAGGUCCACGCAG	CUGCGUGGACCUCAUCCAC
2311	2312	1566-1584	UGGAUGAGGUCCACGCAGU	ACUGCGUGGACCUCAUCCA
2313	2314	1567-1585	GGAUGAGGUCCACGCAGUG	CACUGCGUGGACCUCAUCC
2315	2316	1568-1586	GAUGAGGUCCACGCAGUGG	CCACUGCGUGGACCUCAUC
2317	2318	1569-1587	AUGAGGUCCACGCAGUGGG	CCCACUGCGUGGACCUCAU
2319	2320	1570-1588	UGAGGUCCACGCAGUGGGG	CCCCACUGCGUGGACCUCA
2321	2322	1571-1589	GAGGUCCACGCAGUGGGGC	GCCCCACUGCGUGGACCUC
2323	2324	1572-1590	AGGUCCACGCAGUGGGGCU	AGCCCCACUGCGUGGACCU
2325	2326	1595-1613	GGGGCUCGAGGCGGAGGGA	UCCCUCCGCCUCGAGCCCC
2327	2328	1596-1614	GGGCUCGAGGCGGAGGGAU	AUCCCUCCGCCUCGAGCCC
2329	2330	1597-1615	GGCUCGAGGCGGAGGGAUU	AAUCCCUCCGCCUCGAGCC
2331	2332	1598-1616	GCUCGAGGCGGAGGGAUUG	CAAUCCCUCCGCCUCGAGC
2333	2334	1599-1617	CUCGAGGCGGAGGGAUUGG	CCAAUCCCUCCGCCUCGAG
2335	2336	1600-1618	UCGAGGCGGAGGGAUUGGG	CCCAAUCCCUCCGCCUCGA
2337	2338	1601-1619	CGAGGCGGAGGGAUUGGGG	CCCCAAUCCCUCCGCCUCG
2339	2340	1602-1620	GAGGCGGAGGGAUUGGGGA	UCCCCAAUCCCUCCGCCUC
2341	2342	1603-1621	AGGCGGAGGGAUUGGGGAU	AUCCCCAAUCCCUCCGCCU
2343	2344	1604-1622	GGCGGAGGGAUUGGGGAUC	GAUCCCCAAUCCCUCCGCC
2345	2346	1605-1623	GCGGAGGGAUUGGGGAUCG	CGAUCCCCAAUCCCUCCGC

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2347	2348	1606-1624	CGGAGGGAUUGGGGAUCGG	CCGAUCCCCAAUCCCUCCG
2349	2350	1607-1625	GGAGGGAUUGGGGAUCGGG	CCCGAUCCCCAAUCCCUCC
2351	2352	1608-1626	GAGGGAUUGGGGAUCGGGA	UCCCGAUCCCCAAUCCCUC
2353	2354	1609-1627	AGGGAUUGGGGAUCGGGAU	AUCCCGAUCCCCAAUCCCU
2355	2356	1610-1628	GGGAUUGGGGAUCGGGAUG	CAUCCCGAUCCCCAAUCCC
2357	2358	1611-1629	GGAUUGGGGAUCGGGAUGG	CCAUCCCGAUCCCCAAUCC
2359	2360	1612-1630	GAUUGGGGAUCGGGAUGGA	UCCAUCCCGAUCCCCAAUC
2361	2362	1613-1631	AUUGGGGAUCGGGAUGGAG	CUCCAUCCCGAUCCCCAAU
2363	2364	1614-1632	UUGGGGAUCGGGAUGGAGU	ACUCCAUCCCGAUCCCCAA
2365	2366	1615-1633	UGGGGAUCGGGAUGGAGUC	GACUCCAUCCCGAUCCCCA
2367	2368	1617-1635	GGGAUCGGGAUGGAGUCAU	AUGACUCCAUCCCGAUCCC
2369	2370	1618-1636	GGAUCGGGAUGGAGUCAUG	CAUGACUCCAUCCCGAUCC
2371	2372	1619-1637	GAUCGGGAUGGAGUCAUGC	GCAUGACUCCAUCCCGAUC
2373	2374	1620-1638	AUCGGGAUGGAGUCAUGCC	GGCAUGACUCCAUCCCGAU
2375	2376	1621-1639	UCGGGAUGGAGUCAUGCCA	UGGCAUGACUCCAUCCCGA
2377	2378	1622-1640	CGGGAUGGAGUCAUGCCAA	UUGGCAUGACUCCAUCCCG
2379	2380	1623-1641	GGGAUGGAGUCAUGCCAAA	UUUGGCAUGACUCCAUCCC
2381	2382	1624-1642	GGAUGGAGUCAUGCCAAAA	UUUUGGCAUGACUCCAUCC
2383	2384	1625-1643	GAUGGAGUCAUGCCAAAAA	UUUUUGGCAUGACUCCAUC
2385	2386	1626-1644	AUGGAGUCAUGCCAAAAAU	AUUUUUGGCAUGACUCCAU
2387	2388	1627-1645	UGGAGUCAUGCCAAAAAUG	CAUUUUUGGCAUGACUCCA
2389	2390	1628-1646	GGAGUCAUGCCAAAAAUGG	CCAUUUUUGGCAUGACUCC
2391	2392	1629-1647	GAGUCAUGCCAAAAAUGGA	UCCAUUUUUGGCAUGACUC
2393	2394	1630-1648	AGUCAUGCCAAAAAUGGAC	GUCCAUUUUUGGCAUGACU
2395	2396	1632-1650	UCAUGCCAAAAAUGGACAU	AUGUCCAUUUUUGGCAUGA
2397	2398	1633-1651	CAUGCCAAAAAUGGACAUC	GAUGUCCAUUUUUGGCAUG
2399	2400	1636-1654	GCCAAAAAUGGACAUCAUU	AAUGAUGUCCAUUUUUGGC
2401	2402	1638-1656	CAAAAAUGGACAUCAUUUC	GAAAUGAUGUCCAUUUUUG
2403	2404	1639-1657	AAAAAUGGACAUCAUUUCU	AGAAAUGAUGUCCAUUUUU
2405	2406	1640-1658	AAAAUGGACAUCAUUUCUG	CAGAAAUGAUGUCCAUUUU
2407	2408	1641-1659	AAAUGGACAUCAUUUCUGG	CCAGAAAUGAUGUCCAUUU
2409	2410	1642-1660	AAUGGACAUCAUUUCUGGA	UCCAGAAAUGAUGUCCAUU
2411	2412	1643-1661	AUGGACAUCAUUUCUGGAA	UUCCAGAAAUGAUGUCCAU
2413	2414	1644-1662	UGGACAUCAUUUCUGGAAC	GUUCCAGAAAUGAUGUCCA
2415	2416	1645-1663	GGACAUCAUUUCUGGAACA	UGUUCCAGAAAUGAUGUCC
2417	2418	1646-1664	GACAUCAUUUCUGGAACAC	GUGUUCCAGAAAUGAUGUC

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2419	2420	1647-1665	ACAUCAUUUCUGGAACACU	AGUGUUCCAGAAAUGAUGU
2421	2422	1648-1666	CAUCAUUUCUGGAACACUU	AAGUGUUCCAGAAAUGAUG
2423	2424	1649-1667	AUCAUUUCUGGAACACUUG	CAAGUGUUCCAGAAAUGAU
2425	2426	1650-1668	UCAUUUCUGGAACACUUGG	CCAAGUGUUCCAGAAAUGA
2427	2428	1651-1669	CAUUUCUGGAACACUUGGC	GCCAAGUGUUCCAGAAAUG
2429	2430	1652-1670	AUUUCUGGAACACUUGGCA	UGCCAAGUGUUCCAGAAAU
2431	2432	1653-1671	UUUCUGGAACACUUGGCAA	UUGCCAAGUGUUCCAGAAA
2433	2434	1654-1672	UUCUGGAACACUUGGCAAA	UUUGCCAAGUGUUCCAGAA
2435	2436	1655-1673	UCUGGAACACUUGGCAAAG	CUUUGCCAAGUGUUCCAGA
2437	2438	1656-1674	CUGGAACACUUGGCAAAGC	GCUUUGCCAAGUGUUCCAG
2439	2440	1657-1675	UGGAACACUUGGCAAAGCC	GGCUUUGCCAAGUGUUCCA
2441	2442	1658-1676	GGAACACUUGGCAAAGCCU	AGGCUUUGCCAAGUGUUCC
2443	2444	1659-1677	GAACACUUGGCAAAGCCUU	AAGGCUUUGCCAAGUGUUC
2445	2446	1660-1678	AACACUUGGCAAAGCCUUU	AAAGGCUUUGCCAAGUGUU
2447	2448	1661-1679	ACACUUGGCAAAGCCUUUG	CAAAGGCUUUGCCAAGUGU
2449	2450	1662-1680	CACUUGGCAAAGCCUUUGG	CCAAAGGCUUUGCCAAGUG
2451	2452	1682-1700	UGUGUUGGAGGGUACAUCG	CGAUGUACCCUCCAACACA
2453	2454	1683-1701	GUGUUGGAGGGUACAUCGC	GCGAUGUACCCUCCAACAC
2455	2456	1684-1702	UGUUGGAGGGUACAUCGCC	GGCGAUGUACCCUCCAACA
2457	2458	1685-1703	GUUGGAGGGUACAUCGCCA	UGGCGAUGUACCCUCCAAC
2459	2460	1686-1704	UUGGAGGGUACAUCGCCAG	CUGGCGAUGUACCCUCCAA
2461	2462	1687-1705	UGGAGGGUACAUCGCCAGC	GCUGGCGAUGUACCCUCCA
2463	2464	1688-1706	GGAGGGUACAUCGCCAGCA	UGCUGGCGAUGUACCCUCC
2465	2466	1689-1707	GAGGGUACAUCGCCAGCAC	GUGCUGGCGAUGUACCCUC
2467	2468	1690-1708	AGGGUACAUCGCCAGCACG	CGUGCUGGCGAUGUACCCU
2469	2470	1691-1709	GGGUACAUCGCCAGCACGA	UCGUGCUGGCGAUGUACCC
2471	2472	1692-1710	GGUACAUCGCCAGCACGAG	CUCGUGCUGGCGAUGUACC
2473	2474	1693-1711	GUACAUCGCCAGCACGAGU	ACUCGUGCUGGCGAUGUAC
2475	2476	1694-1712	UACAUCGCCAGCACGAGUU	AACUCGUGCUGGCGAUGUA
2477	2478	1695-1713	ACAUCGCCAGCACGAGUUC	GAACUCGUGCUGGCGAUGU
2479	2480	1696-1714	CAUCGCCAGCACGAGUUCU	AGAACUCGUGCUGGCGAUG
2481	2482	1697-1715	AUCGCCAGCACGAGUUCUC	GAGAACUCGUGCUGGCGAU
2483	2484	1698-1716	UCGCCAGCACGAGUUCUCU	AGAGAACUCGUGCUGGCGA
2485	2486	1699-1717	CGCCAGCACGAGUUCUCUG	CAGAGAACUCGUGCUGGCG
2487	2488	1700-1718	GCCAGCACGAGUUCUCUGA	UCAGAGAACUCGUGCUGGC
2489	2490	1701-1719	CCAGCACGAGUUCUCUGAU	AUCAGAGAACUCGUGCUGG

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2491	2492	1702-1720	CAGCACGAGUUCUCUGAUU	AAUCAGAGAACUCGUGCUG
2493	2494	1703-1721	AGCACGAGUUCUCUGAUUG	CAAUCAGAGAACUCGUGCU
2495	2496	1704-1722	GCACGAGUUCUCUGAUUGA	UCAAUCAGAGAACUCGUGC
2497	2498	1705-1723	CACGAGUUCUCUGAUUGAC	GUCAAUCAGAGAACUCGUG
2499	2500	1707-1725	CGAGUUCUCUGAUUGACAC	GUGUCAAUCAGAGAACUCG
2501	2502	1727-1745	GUACGGUCCUAUGCUGCUG	CAGCAGCAUAGGACCGUAC
2503	2504	1728-1746	UACGGUCCUAUGCUGCUGG	CCAGCAGCAUAGGACCGUA
2505	2506	1729-1747	ACGGUCCUAUGCUGCUGGC	GCCAGCAGCAUAGGACCGU
2507	2508	1730-1748	CGGUCCUAUGCUGCUGGCU	AGCCAGCAGCAUAGGACCG
2509	2510	1731-1749	GGUCCUAUGCUGCUGGCUU	AAGCCAGCAGCAUAGGACC
2511	2512	1732-1750	GUCCUAUGCUGCUGGCUUC	GAAGCCAGCAGCAUAGGAC
2513	2514	1733-1751	UCCUAUGCUGCUGGCUUCA	UGAAGCCAGCAGCAUAGGA
2515	2516	1734-1752	CCUAUGCUGCUGGCUUCAU	AUGAAGCCAGCAGCAUAGG
2517	2518	1735-1753	CUAUGCUGCUGGCUUCAUC	GAUGAAGCCAGCAGCAUAG
2519	2520	1736-1754	UAUGCUGCUGGCUUCAUCU	AGAUGAAGCCAGCAGCAUA
2521	2522	1737-1755	AUGCUGCUGGCUUCAUCUU	AAGAUGAAGCCAGCAGCAU
2523	2524	1738-1756	UGCUGCUGGCUUCAUCUUC	GAAGAUGAAGCCAGCAGCA
2525	2526	1739-1757	GCUGCUGGCUUCAUCUUCA	UGAAGAUGAAGCCAGCAGC
2527	2528	1740-1758	CUGCUGGCUUCAUCUUCAC	GUGAAGAUGAAGCCAGCAG
2529	2530	1741-1759	UGCUGGCUUCAUCUUCACC	GGUGAAGAUGAAGCCAGCA
2531	2532	1742-1760	GCUGGCUUCAUCUUCACCA	UGGUGAAGAUGAAGCCAGC
2533	2534	1743-1761	CUGGCUUCAUCUUCACCAC	GUGGUGAAGAUGAAGCCAG
2535	2536	1744-1762	UGGCUUCAUCUUCACCACC	GGUGGUGAAGAUGAAGCCA
2537	2538	1745-1763	GGCUUCAUCUUCACCACCU	AGGUGGUGAAGAUGAAGCC
2539	2540	1746-1764	GCUUCAUCUUCACCACCUC	GAGGUGGUGAAGAUGAAGC
2541	2542	1747-1765	CUUCAUCUUCACCACCUCU	AGAGGUGGUGAAGAUGAAG
2543	2544	1748-1766	UUCAUCUUCACCACCUCUC	GAGAGGUGGUGAAGAUGAA
2545	2546	1749-1767	UCAUCUUCACCACCUCUCU	AGAGAGGUGGUGAAGAUGA
2547	2548	1750-1768	CAUCUUCACCACCUCUCUG	CAGAGAGGUGGUGAAGAUG
2549	2550	1751-1769	AUCUUCACCACCUCUCUGC	GCAGAGAGGUGGUGAAGAU
2551	2552	1752-1770	UCUUCACCACCUCUCUGCC	GGCAGAGAGGUGGUGAAGA
2553	2554	1753-1771	CUUCACCACCUCUCUGCCA	UGGCAGAGAGGUGGUGAAG
2555	2556	1754-1772	UUCACCACCUCUCUGCCAC	GUGGCAGAGAGGUGGUGAA
2557	2558	1755-1773	UCACCACCUCUCUGCCACC	GGUGGCAGAGAGGUGGUGA
2559	2560	1756-1774	CACCACCUCUCUGCCACCC	GGGUGGCAGAGAGGUGGUG
2561	2562	1757-1775	ACCACCUCUCUGCCACCCA	UGGGUGGCAGAGAGGUGGU

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2563	2564	1758-1776	CCACCUCUCUGCCACCCAU	AUGGGUGGCAGAGAGGUGG
2565	2566	1759-1777	CACCUCUCUGCCACCCAUG	CAUGGGUGGCAGAGAGGUG
2567	2568	1760-1778	ACCUCUCUGCCACCCAUGC	GCAUGGGUGGCAGAGAGGU
2569	2570	1761-1779	CCUCUCUGCCACCCAUGCU	AGCAUGGGUGGCAGAGAGG
2571	2572	1762-1780	CUCUCUGCCACCCAUGCUG	CAGCAUGGGUGGCAGAGAG
2573	2574	1763-1781	UCUCUGCCACCCAUGCUGC	GCAGCAUGGGUGGCAGAGA
2575	2576	1764-1782	CUCUGCCACCCAUGCUGCU	AGCAGCAUGGGUGGCAGAG
2577	2578	1765-1783	UCUGCCACCCAUGCUGCUG	CAGCAGCAUGGGUGGCAGA
2579	2580	1766-1784	CUGCCACCCAUGCUGCUGG	CCAGCAGCAUGGGUGGCAG
2581	2582	1767-1785	UGCCACCCAUGCUGCUGGC	GCCAGCAGCAUGGGUGGCA
2583	2584	1768-1786	GCCACCCAUGCUGCUGGCU	AGCCAGCAGCAUGGGUGGC
2585	2586	1769-1787	CCACCCAUGCUGCUGGCUG	CAGCCAGCAGCAUGGGUGG
2587	2588	1770-1788	CACCCAUGCUGCUGGCUGG	CCAGCCAGCAGCAUGGGUG
2589	2590	1771-1789	ACCCAUGCUGCUGGCUGGA	UCCAGCCAGCAGCAUGGGU
2591	2592	1772-1790	CCCAUGCUGCUGGCUGGAG	CUCCAGCCAGCAGCAUGGG
2593	2594	1773-1791	CCAUGCUGCUGGCUGGAGC	GCUCCAGCCAGCAGCAUGG
2595	2596	1774-1792	CAUGCUGCUGGCUGGAGCC	GGCUCCAGCCAGCAGCAUG
2597	2598	1775-1793	AUGCUGCUGGCUGGAGCCC	GGGCUCCAGCCAGCAGCAU
2599	2600	1776-1794	UGCUGCUGGCUGGAGCCCU	AGGGCUCCAGCCAGCAGCA
2601	2602	1777-1795	GCUGCUGGCUGGAGCCCUG	CAGGGCUCCAGCCAGCAGC
2603	2604	1778-1796	CUGCUGGCUGGAGCCCUGG	CCAGGGCUCCAGCCAGCAG
2605	2606	1779-1797	UGCUGGCUGGAGCCCUGGA	UCCAGGGCUCCAGCCAGCA
2607	2608	1780-1798	GCUGGCUGGAGCCCUGGAG	CUCCAGGGCUCCAGCCAGC
2609	2610	1781-1799	CUGGCUGGAGCCCUGGAGU	ACUCCAGGGCUCCAGCCAG
2611	2612	1782-1800	UGGCUGGAGCCCUGGAGUC	GACUCCAGGGCUCCAGCCA
2613	2614	1783-1801	GGCUGGAGCCCUGGAGUCU	AGACUCCAGGGCUCCAGCC
2615	2616	1784-1802	GCUGGAGCCCUGGAGUCUG	CAGACUCCAGGGCUCCAGC
2617	2618	1785-1803	CUGGAGCCCUGGAGUCUGU	ACAGACUCCAGGGCUCCAG
2619	2620	1786-1804	UGGAGCCCUGGAGUCUGUG	CACAGACUCCAGGGCUCCA
2621	2622	1787-1805	GGAGCCCUGGAGUCUGUGC	GCACAGACUCCAGGGCUCC
2623	2624	1788-1806	GAGCCCUGGAGUCUGUGCG	CGCACAGACUCCAGGGCUC
2625	2626	1789-1807	AGCCCUGGAGUCUGUGCGG	CCGCACAGACUCCAGGGCU
2627	2628	1790-1808	GCCCUGGAGUCUGUGCGGA	UCCGCACAGACUCCAGGGC
2629	2630	1792-1810	CCUGGAGUCUGUGCGGAUC	GAUCCGCACAGACUCCAGG
2631	2632	1793-1811	CUGGAGUCUGUGCGGAUCC	GGAUCCGCACAGACUCCAG
2633	2634	1795-1813	GGAGUCUGUGCGGAUCCUG	CAGGAUCCGCACAGACUCC

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2635	2636	1796-1814	GAGUCUGUGCGGAUCCUGA	UCAGGAUCCGCACAGACUC
2637	2638	1797-1815	AGUCUGUGCGGAUCCUGAA	UUCAGGAUCCGCACAGACU
2639	2640	1798-1816	GUCUGUGCGGAUCCUGAAG	CUUCAGGAUCCGCACAGAC
2641	2642	1799-1817	UCUGUGCGGAUCCUGAAGA	UCUUCAGGAUCCGCACAGA
2643	2644	1800-1818	CUGUGCGGAUCCUGAAGAG	CUCUUCAGGAUCCGCACAG
2645	2646	1801-1819	UGUGCGGAUCCUGAAGAGC	GCUCUUCAGGAUCCGCACA
2647	2648	1802-1820	GUGCGGAUCCUGAAGAGCG	CGCUCUUCAGGAUCCGCAC
2649	2650	1803-1821	UGCGGAUCCUGAAGAGCGC	GCGCUCUUCAGGAUCCGCA
2651	2652	1804-1822	GCGGAUCCUGAAGAGCGCU	AGCGCUCUUCAGGAUCCGC
2653	2654	1805-1823	CGGAUCCUGAAGAGCGCUG	CAGCGCUCUUCAGGAUCCG
2655	2656	1806-1824	GGAUCCUGAAGAGCGCUGA	UCAGCGCUCUUCAGGAUCC
2657	2658	1807-1825	GAUCCUGAAGAGCGCUGAG	CUCAGCGCUCUUCAGGAUC
2659	2660	1808-1826	AUCCUGAAGAGCGCUGAGG	CCUCAGCGCUCUUCAGGAU
2661	2662	1809-1827	UCCUGAAGAGCGCUGAGGG	CCCUCAGCGCUCUUCAGGA
2663	2664	1810-1828	CCUGAAGAGCGCUGAGGGA	UCCCUCAGCGCUCUUCAGG
2665	2666	1811-1829	CUGAAGAGCGCUGAGGGAC	GUCCCUCAGCGCUCUUCAG
2667	2668	1812-1830	UGAAGAGCGCUGAGGGACG	CGUCCCUCAGCGCUCUUCA
2669	2670	1813-1831	GAAGAGCGCUGAGGGACGG	CCGUCCCUCAGCGCUCUUC
2671	2672	1814-1832	AAGAGCGCUGAGGGACGGG	CCCGUCCCUCAGCGCUCUU
2673	2674	1815-1833	AGAGCGCUGAGGGACGGGU	ACCCGUCCCUCAGCGCUCU
2675	2676	1816-1834	GAGCGCUGAGGGACGGGUG	CACCCGUCCCUCAGCGCUC
2677	2678	1817-1835	AGCGCUGAGGGACGGGUGC	GCACCCGUCCCUCAGCGCU
2679	2680	1818-1836	GCGCUGAGGGACGGGUGCU	AGCACCCGUCCCUCAGCGC
2681	2682	1819-1837	CGCUGAGGGACGGGUGCUU	AAGCACCCGUCCCUCAGCG
2683	2684	1820-1838	GCUGAGGGACGGGUGCUUC	GAAGCACCCGUCCCUCAGC
2685	2686	1821-1839	CUGAGGGACGGGUGCUUCG	CGAAGCACCCGUCCCUCAG
2687	2688	1822-1840	UGAGGGACGGGUGCUUCGC	GCGAAGCACCCGUCCCUCA
2689	2690	1823-1841	GAGGGACGGGUGCUUCGCC	GGCGAAGCACCCGUCCCUC
2691	2692	1824-1842	AGGGACGGGUGCUUCGCCG	CGGCGAAGCACCCGUCCCU
2693	2694	1825-1843	GGGACGGGUGCUUCGCCGC	GCGGCGAAGCACCCGUCCC
2695	2696	1826-1844	GGACGGGUGCUUCGCCGCC	GGCGGCGAAGCACCCGUCC
2697	2698	1827-1845	GACGGGUGCUUCGCCGCCA	UGGCGGCGAAGCACCCGUC
2699	2700	1828-1846	ACGGGUGCUUCGCCGCCAG	CUGGCGGCGAAGCACCCGU
2701	2702	1829-1847	CGGGUGCUUCGCCGCCAGC	GCUGGCGGCGAAGCACCCG
2703	2704	1830-1848	GGGUGCUUCGCCGCCAGCA	UGCUGGCGGCGAAGCACCC
2705	2706	1831-1849	GGUGCUUCGCCGCCAGCAC	GUGCUGGCGGCGAAGCACC

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2707	2708	1832-1850	GUGCUUCGCCGCCAGCACC	GGUGCUGGCGGCGAAGCAC
2709	2710	1833-1851	UGCUUCGCCGCCAGCACCA	UGGUGCUGGCGGCGAAGCA
2711	2712	1834-1852	GCUUCGCCGCCAGCACCAG	CUGGUGCUGGCGGCGAAGC
2713	2714	1835-1853	CUUCGCCGCCAGCACCAGC	GCUGGUGCUGGCGGCGAAG
2715	2716	1836-1854	UUCGCCGCCAGCACCAGCG	CGCUGGUGCUGGCGGCGAA
2717	2718	1837-1855	UCGCCGCCAGCACCAGCGC	GCGCUGGUGCUGGCGGCGA
2719	2720	1838-1856	CGCCGCCAGCACCAGCGCA	UGCGCUGGUGCUGGCGGCG
2721	2722	1839-1857	GCCGCCAGCACCAGCGCAA	UUGCGCUGGUGCUGGCGGC
2723	2724	1840-1858	CCGCCAGCACCAGCGCAAC	GUUGCGCUGGUGCUGGCGG
2725	2726	1841-1859	CGCCAGCACCAGCGCAACG	CGUUGCGCUGGUGCUGGCG
2727	2728	1842-1860	GCCAGCACCAGCGCAACGU	ACGUUGCGCUGGUGCUGGC
2729	2730	1865-1883	CUCAUGAGACAGAUGCUAA	UUAGCAUCUGUCUCAUGAG
2731	2732	1866-1884	UCAUGAGACAGAUGCUAAU	AUUAGCAUCUGUCUCAUGA
2733	2734	1867-1885	CAUGAGACAGAUGCUAAUG	CAUUAGCAUCUGUCUCAUG
2735	2736	1868-1886	AUGAGACAGAUGCUAAUGG	CCAUUAGCAUCUGUCUCAU
2737	2738	1869-1887	UGAGACAGAUGCUAAUGGA	UCCAUUAGCAUCUGUCUCA
2739	2740	1871-1889	AGACAGAUGCUAAUGGAUG	CAUCCAUUAGCAUCUGUCU
2741	2742	1872-1890	GACAGAUGCUAAUGGAUGC	GCAUCCAUUAGCAUCUGUC
2743	2744	1873-1891	ACAGAUGCUAAUGGAUGCC	GGCAUCCAUUAGCAUCUGU
2745	2746	1874-1892	CAGAUGCUAAUGGAUGCCG	CGGCAUCCAUUAGCAUCUG
2747	2748	1875-1893	AGAUGCUAAUGGAUGCCGG	CCGGCAUCCAUUAGCAUCU
2749	2750	1876-1894	GAUGCUAAUGGAUGCCGGC	GCCGGCAUCCAUUAGCAUC
2751	2752	1877-1895	AUGCUAAUGGAUGCCGGCC	GGCCGGCAUCCAUUAGCAU
2753	2754	1878-1896	UGCUAAUGGAUGCCGGCCU	AGGCCGGCAUCCAUUAGCA
2755	2756	1879-1897	GCUAAUGGAUGCCGGCCUC	GAGGCCGGCAUCCAUUAGC
2757	2758	1880-1898	CUAAUGGAUGCCGGCCUCC	GGAGGCCGGCAUCCAUUAG
2759	2760	1881-1899	UAAUGGAUGCCGGCCUCCC	GGGAGGCCGGCAUCCAUUA
2761	2762	1882-1900	AAUGGAUGCCGGCCUCCCU	AGGGAGGCCGGCAUCCAUU
2763	2764	1883-1901	AUGGAUGCCGGCCUCCCUG	CAGGGAGGCCGGCAUCCAU
2765	2766	1884-1902	UGGAUGCCGGCCUCCCUGU	ACAGGGAGGCCGGCAUCCA
2767	2768	1885-1903	GGAUGCCGGCCUCCCUGUU	AACAGGGAGGCCGGCAUCC
2769	2770	1886-1904	GAUGCCGGCCUCCCUGUUG	CAACAGGGAGGCCGGCAUC
2771	2772	1887-1905	AUGCCGGCCUCCCUGUUGU	ACAACAGGGAGGCCGGCAU
2773	2774	1888-1906	UGCCGGCCUCCCUGUUGUC	GACAACAGGGAGGCCGGCA
2775	2776	1889-1907	GCCGGCCUCCCUGUUGUCC	GGACAACAGGGAGGCCGGC
2777	2778	1890-1908	CCGGCCUCCCUGUUGUCCA	UGGACAACAGGGAGGCCGG

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2779	2780	1891-1909	CGGCCUCCCUGUUGUCCAC	GUGGACAACAGGGAGGCCG
2781	2782	1892-1910	GGCCUCCCUGUUGUCCACU	AGUGGACAACAGGGAGGCC
2783	2784	1893-1911	GCCUCCCUGUUGUCCACUG	CAGUGGACAACAGGGAGGC
2785	2786	1894-1912	CCUCCCUGUUGUCCACUGC	GCAGUGGACAACAGGGAGG
2787	2788	1895-1913	CUCCCUGUUGUCCACUGCC	GGCAGUGGACAACAGGGAG
2789	2790	1896-1914	UCCCUGUUGUCCACUGCCC	GGGCAGUGGACAACAGGGA
2791	2792	1897-1915	CCCUGUUGUCCACUGCCCC	GGGGCAGUGGACAACAGGG
2793	2794	1898-1916	CCUGUUGUCCACUGCCCCA	UGGGGCAGUGGACAACAGG
2795	2796	1899-1917	CUGUUGUCCACUGCCCCAG	CUGGGGCAGUGGACAACAG
2797	2798	1900-1918	UGUUGUCCACUGCCCCAGC	GCUGGGGCAGUGGACAACA
2799	2800	1901-1919	GUUGUCCACUGCCCCAGCC	GGCUGGGGCAGUGGACAAC
2801	2802	1902-1920	UUGUCCACUGCCCCAGCCA	UGGCUGGGGCAGUGGACAA
2803	2804	1903-1921	UGUCCACUGCCCCAGCCAC	GUGGCUGGGGCAGUGGACA
2805	2806	1904-1922	GUCCACUGCCCCAGCCACA	UGUGGCUGGGGCAGUGGAC
2807	2808	1905-1923	UCCACUGCCCCAGCCACAU	AUGUGGCUGGGGCAGUGGA
2809	2810	1906-1924	CCACUGCCCCAGCCACAUC	GAUGUGGCUGGGGCAGUGG
2811	2812	1907-1925	CACUGCCCCAGCCACAUCA	UGAUGUGGCUGGGGCAGUG
2813	2814	1908-1926	ACUGCCCCAGCCACAUCAU	AUGAUGUGGCUGGGGCAGU
2815	2816	1909-1927	CUGCCCCAGCCACAUCAUC	GAUGAUGUGGCUGGGGCAG
2817	2818	1910-1928	UGCCCCAGCCACAUCAUCC	GGAUGAUGUGGCUGGGGCA
2819	2820	1911-1929	GCCCCAGCCACAUCAUCCC	GGGAUGAUGUGGCUGGGGC
2821	2822	1912-1930	CCCCAGCCACAUCAUCCCU	AGGGAUGAUGUGGCUGGGG
2823	2824	1913-1931	CCCAGCCACAUCAUCCCUG	CAGGGAUGAUGUGGCUGGG
2825	2826	1914-1932	CCAGCCACAUCAUCCCUGU	ACAGGGAUGAUGUGGCUGG
2827	2828	1915-1933	CAGCCACAUCAUCCCUGUG	CACAGGGAUGAUGUGGCUG
2829	2830	1916-1934	AGCCACAUCAUCCCUGUGC	GCACAGGGAUGAUGUGGCU
2831	2832	1917-1935	GCCACAUCAUCCCUGUGCG	CGCACAGGGAUGAUGUGGC
2833	2834	1918-1936	CCACAUCAUCCCUGUGCGG	CCGCACAGGGAUGAUGUGG
2835	2836	1919-1937	CACAUCAUCCCUGUGCGGG	CCCGCACAGGGAUGAUGUG
2837	2838	1920-1938	ACAUCAUCCCUGUGCGGGU	ACCCGCACAGGGAUGAUGU
2839	2840	1922-1940	AUCAUCCCUGUGCGGGUUG	CAACCCGCACAGGGAUGAU
2841	2842	1923-1941	UCAUCCCUGUGCGGGUUGC	GCAACCCGCACAGGGAUGA
2843	2844	1924-1942	CAUCCCUGUGCGGGUUGCA	UGCAACCCGCACAGGGAUG
2845	2846	1925-1943	AUCCCUGUGCGGGUUGCAG	CUGCAACCCGCACAGGGAU
2847	2848	1926-1944	UCCCUGUGCGGGUUGCAGA	UCUGCAACCCGCACAGGGA
2849	2850	1928-1946	CCUGUGCGGGUUGCAGAUG	CAUCUGCAACCCGCACAGG

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2851	2852	1929-1947	CUGUGCGGGUUGCAGAUGC	GCAUCUGCAACCCGCACAG
2853	2854	1930-1948	UGUGCGGGUUGCAGAUGCU	AGCAUCUGCAACCCGCACA
2855	2856	1931-1949	GUGCGGGUUGCAGAUGCUG	CAGCAUCUGCAACCCGCAC
2857	2858	1932-1950	UGCGGGUUGCAGAUGCUGC	GCAGCAUCUGCAACCCGCA
2859	2860	1933-1951	GCGGGUUGCAGAUGCUGCU	AGCAGCAUCUGCAACCCGC
2861	2862	1934-1952	CGGGUUGCAGAUGCUGCUA	UAGCAGCAUCUGCAACCCG
2863	2864	1935-1953	GGGUUGCAGAUGCUGCUAA	UUAGCAGCAUCUGCAACCC
2865	2866	1936-1954	GGUUGCAGAUGCUGCUAAA	UUUAGCAGCAUCUGCAACC
2867	2868	1937-1955	GUUGCAGAUGCUGCUAAAA	UUUUAGCAGCAUCUGCAAC
2869	2870	1938-1956	UUGCAGAUGCUGCUAAAAA	UUUUUAGCAGCAUCUGCAA
2871	2872	1939-1957	UGCAGAUGCUGCUAAAAAC	GUUUUUAGCAGCAUCUGCA
2873	2874	1940-1958	GCAGAUGCUGCUAAAAACA	UGUUUUUAGCAGCAUCUGC
2875	2876	1941-1959	CAGAUGCUGCUAAAAACAC	GUGUUUUUAGCAGCAUCUG
2877	2878	1961-1979	GAAGUCUGUGAUGAACUAA	UUAGUUCAUCACAGACUUC
2879	2880	1963-1981	AGUCUGUGAUGAACUAAUG	CAUUAGUUCAUCACAGACU
2881	2882	1965-1983	UCUGUGAUGAACUAAUGAG	CUCAUUAGUUCAUCACAGA
2883	2884	1966-1984	CUGUGAUGAACUAAUGAGC	GCUCAUUAGUUCAUCACAG
2885	2886	1968-1986	GUGAUGAACUAAUGAGCAG	CUGCUCAUUAGUUCAUCAC
2887	2888	1969-1987	UGAUGAACUAAUGAGCAGA	UCUGCUCAUUAGUUCAUCA
2889	2890	1970-1988	GAUGAACUAAUGAGCAGAC	GUCUGCUCAUUAGUUCAUC
2891	2892	1971-1989	AUGAACUAAUGAGCAGACA	UGUCUGCUCAUUAGUUCAU
2893	2894	1972-1990	UGAACUAAUGAGCAGACAU	AUGUCUGCUCAUUAGUUCA
2895	2896	1973-1991	GAACUAAUGAGCAGACAUA	UAUGUCUGCUCAUUAGUUC
2897	2898	1974-1992	AACUAAUGAGCAGACAUAA	UUAUGUCUGCUCAUUAGUU
2899	2900	1975-1993	ACUAAUGAGCAGACAUAAC	GUUAUGUCUGCUCAUUAGU
2901	2902	1978-1996	AAUGAGCAGACAUAACAUC	GAUGUUAUGUCUGCUCAUU
2903	2904	1979-1997	AUGAGCAGACAUAACAUCU	AGAUGUUAUGUCUGCUCAU
2905	2906	1980-1998	UGAGCAGACAUAACAUCUA	UAGAUGUUAUGUCUGCUCA
2907	2908	2000-2018	GUGCAAGCAAUCAAUUACC	GGUAAUUGAUUGCUUGCAC
2909	2910	2001-2019	UGCAAGCAAUCAAUUACCC	GGGUAAUUGAUUGCUUGCA
2911	2912	2002-2020	GCAAGCAAUCAAUUACCCU	AGGGUAAUUGAUUGCUUGC
2913	2914	2004-2022	AAGCAAUCAAUUACCCUAC	GUAGGGUAAUUGAUUGCUU
2915	2916	2024-2042	GUGCCCCGGGGAGAAGAGC	GCUCUUCUCCCCGGGGCAC
2917	2918	2025-2043	UGCCCCGGGGAGAAGAGCU	AGCUCUUCUCCCCGGGGCA
2919	2920	2026-2044	GCCCCGGGGAGAAGAGCUC	GAGCUCUUCUCCCCGGGGC
2921	2922	2027-2045	CCCCGGGGAGAAGAGCUCC	GGAGCUCUUCUCCCCGGGG

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
2923	2924	2028-2046	CCCGGGGAGAAGAGCUCCU	AGGAGCUCUUCUCCCCGGG
2925	2926	2029-2047	CCGGGGAGAAGAGCUCCUA	UAGGAGCUCUUCUCCCCGG
2927	2928	2030-2048	CGGGGAGAAGAGCUCCUAC	GUAGGAGCUCUUCUCCCCG
2929	2930	2031-2049	GGGGAGAAGAGCUCCUACG	CGUAGGAGCUCUUCUCCCC
2931	2932	2032-2050	GGGAGAAGAGCUCCUACGG	CCGUAGGAGCUCUUCUCCC
2933	2934	2033-2051	GGAGAAGAGCUCCUACGGA	UCCGUAGGAGCUCUUCUCC
2935	2936	2034-2052	GAGAAGAGCUCCUACGGAU	AUCCGUAGGAGCUCUUCUC
2937	2938	2060-2078	ACCCCUCACCACACACCCC	GGGGUGUGUGGUGAGGGGU
2939	2940	2061-2079	CCCCUCACCACACACCCCA	UGGGGUGUGUGGUGAGGGG
2941	2942	2062-2080	CCCUCACCACACACCCCAG	CUGGGGUGUGUGGUGAGGG
2943	2944	2063-2081	CCUCACCACACACCCCAGA	UCUGGGGUGUGUGGUGAGG
2945	2946	2064-2082	CUCACCACACACCCCAGAU	AUCUGGGGUGUGUGGUGAG
2947	2948	2065-2083	UCACCACACACCCCAGAUG	CAUCUGGGGUGUGUGGUGA
2949	2950	2066-2084	CACCACACACCCCAGAUGA	UCAUCUGGGGUGUGUGGUG
2951	2952	2067-2085	ACCACACACCCCAGAUGAU	AUCAUCUGGGGUGUGUGGU
2953	2954	2068-2086	CCACACACCCCAGAUGAUG	CAUCAUCUGGGGUGUGUGG
2955	2956	2069-2087	CACACACCCCAGAUGAUGA	UCAUCAUCUGGGGUGUGUG
2957	2958	2070-2088	ACACACCCCAGAUGAUGAA	UUCAUCAUCUGGGGUGUGU
2959	2960	2071-2089	CACACCCCAGAUGAUGAAC	GUUCAUCAUCUGGGGUGUG
2961	2962	2072-2090	ACACCCCAGAUGAUGAACU	AGUUCAUCAUCUGGGGUGU
2963	2964	2073-2091	CACCCCAGAUGAUGAACUA	UAGUUCAUCAUCUGGGGUG
2965	2966	2074-2092	ACCCCAGAUGAUGAACUAC	GUAGUUCAUCAUCUGGGGU
2967	2968	2076-2094	CCCAGAUGAUGAACUACUU	AAGUAGUUCAUCAUCUGGG
2969	2970	2077-2095	CCAGAUGAUGAACUACUUC	GAAGUAGUUCAUCAUCUGG
2971	2972	2078-2096	CAGAUGAUGAACUACUUCC	GGAAGUAGUUCAUCAUCUG
2973	2974	2079-2097	AGAUGAUGAACUACUUCCU	AGGAAGUAGUUCAUCAUCU
2975	2976	2080-2098	GAUGAUGAACUACUUCCUU	AAGGAAGUAGUUCAUCAUC
2977	2978	2081-2099	AUGAUGAACUACUUCCUUG	CAAGGAAGUAGUUCAUCAU
2979	2980	2082-2100	UGAUGAACUACUUCCUUGA	UCAAGGAAGUAGUUCAUCA
2981	2982	2083-2101	GAUGAACUACUUCCUUGAG	CUCAAGGAAGUAGUUCAUC
2983	2984	2084-2102	AUGAACUACUUCCUUGAGA	UCUCAAGGAAGUAGUUCAU
2985	2986	2085-2103	UGAACUACUUCCUUGAGAA	UUCUCAAGGAAGUAGUUCA
2987	2988	2086-2104	GAACUACUUCCUUGAGAAU	AUUCUCAAGGAAGUAGUUC
2989	2990	2087-2105	AACUACUUCCUUGAGAAUC	GAUUCUCAAGGAAGUAGUU
2991	2992	2088-2106	ACUACUUCCUUGAGAAUCU	AGAUUCUCAAGGAAGUAGU
2993	2994	2089-2107	CUACUUCCUUGAGAAUCUG	CAGAUUCUCAAGGAAGUAG

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2997	2998	2091-2109	ACUUCCUUGAGAAUCUGCU	AGCAGAUUCUCAAGGAAGU
2999	3000	2117-2135	UGGAAGCAAGUGGGGCUGG	CCAGCCCCACUUGCUUCCA
3001	3002	2118-2136	GGAAGCAAGUGGGGCUGGA	UCCAGCCCCACUUGCUUCC
3003	3004	2119-2137	GAAGCAAGUGGGGCUGGAA	UUCCAGCCCCACUUGCUUC
3005	3006	2120-2138	AAGCAAGUGGGGCUGGAAC	GUUCCAGCCCCACUUGCUU
3007	3008	2121-2139	AGCAAGUGGGGCUGGAACU	AGUUCCAGCCCCACUUGCU
3009	3010	2122-2140	GCAAGUGGGGCUGGAACUG	CAGUUCCAGCCCCACUUGC
3011	3012	2123-2141	CAAGUGGGGCUGGAACUGA	UCAGUUCCAGCCCCACUUG
3013	3014	2124-2142	AAGUGGGGCUGGAACUGAA	UUCAGUUCCAGCCCCACUU
3015	3016	2125-2143	AGUGGGGCUGGAACUGAAG	CUUCAGUUCCAGCCCCACU
3017	3018	2126-2144	GUGGGGCUGGAACUGAAGC	GCUUCAGUUCCAGCCCCAC
3019	3020	2127-2145	UGGGGCUGGAACUGAAGCC	GGCUUCAGUUCCAGCCCCA
3021	3022	2147-2165	CAUUCCUCAGCUGAGUGCA	UGCACUCAGCUGAGGAAUG
3023	3024	2148-2166	AUUCCUCAGCUGAGUGCAA	UUGCACUCAGCUGAGGAAU
3025	3026	2149-2167	UUCCUCAGCUGAGUGCAAC	GUUGCACUCAGCUGAGGAA
3027	3028	2150-2168	UCCUCAGCUGAGUGCAACU	AGUUGCACUCAGCUGAGGA
3029	3030	2151-2169	CCUCAGCUGAGUGCAACUU	AAGUUGCACUCAGCUGAGG
3031	3032	2152-2170	CUCAGCUGAGUGCAACUUC	GAAGUUGCACUCAGCUGAG
3033	3034	2153-2171	UCAGCUGAGUGCAACUUCU	AGAAGUUGCACUCAGCUGA
3035	3036	2154-2172	CAGCUGAGUGCAACUUCUG	CAGAAGUUGCACUCAGCUG
3037	3038	2155-2173	AGCUGAGUGCAACUUCUGC	GCAGAAGUUGCACUCAGCU
3039	3040	2156-2174	GCUGAGUGCAACUUCUGCA	UGCAGAAGUUGCACUCAGC
3041	3042	2157-2175	CUGAGUGCAACUUCUGCAG	CUGCAGAAGUUGCACUCAG
3043	3044	2158-2176	UGAGUGCAACUUCUGCAGG	CCUGCAGAAGUUGCACUCA
3045	3046	2159-2177	GAGUGCAACUUCUGCAGGA	UCCUGCAGAAGUUGCACUC
3047	3048	2160-2178	AGUGCAACUUCUGCAGGAG	CUCCUGCAGAAGUUGCACU
3049	3050	2161-2179	GUGCAACUUCUGCAGGAGG	CCUCCUGCAGAAGUUGCAC
3051	3052	2162-2180	UGCAACUUCUGCAGGAGGC	GCCUCCUGCAGAAGUUGCA
3053	3054	2163-2181	GCAACUUCUGCAGGAGGCC	GGCCUCCUGCAGAAGUUGC
3055	3056	2164-2182	CAACUUCUGCAGGAGGCCA	UGGCCUCCUGCAGAAGUUG
3057	3058	2165-2183	AACUUCUGCAGGAGGCCAC	GUGGCCUCCUGCAGAAGUU
3059	3060	2166-2184	ACUUCUGCAGGAGGCCACU	AGUGGCCUCCUGCAGAAGU
3061	3062	2167-2185	CUUCUGCAGGAGGCCACUG	CAGUGGCCUCCUGCAGAAG
3063	3064	2168-2186	UUCUGCAGGAGGCCACUGC	GCAGUGGCCUCCUGCAGAA
3065	3066	2169-2187	UCUGCAGGAGGCCACUGCA	UGCAGUGGCCUCCUGCAGA

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3069	3070	2171-2189	UGCAGGAGGCCACUGCAUU	AAUGCAGUGGCCUCCUGCA
3071	3072	2172-2190	GCAGGAGGCCACUGCAUUU	AAAUGCAGUGGCCUCCUGC
3073	3074	2173-2191	CAGGAGGCCACUGCAUUUU	AAAAUGCAGUGGCCUCCUG
3075	3076	2174-2192	AGGAGGCCACUGCAUUUUG	CAAAAUGCAGUGGCCUCCU
3077	3078	2175-2193	GGAGGCCACUGCAUUUUGA	UCAAAAUGCAGUGGCCUCC
3079	3080	2176-2194	GAGGCCACUGCAUUUUGAA	UUCAAAAUGCAGUGGCCUC
3081	3082	2177-2195	AGGCCACUGCAUUUUGAAG	CUUCAAAAUGCAGUGGCCU
3083	3084	2178-2196	GGCCACUGCAUUUUGAAGU	ACUUCAAAAUGCAGUGGCC
3085	3086	2179-2197	GCCACUGCAUUUUGAAGUG	CACUUCAAAAUGCAGUGGC
3087	3088	2180-2198	CCACUGCAUUUUGAAGUGA	UCACUUCAAAAUGCAGUGG
3089	3090	2181-2199	CACUGCAUUUUGAAGUGAU	AUCACUUCAAAAUGCAGUG
3091	3092	2182-2200	ACUGCAUUUUGAAGUGAUG	CAUCACUUCAAAAUGCAGU
3093	3094	2183-2201	CUGCAUUUUGAAGUGAUGA	UCAUCACUUCAAAAUGCAG
3095	3096	2184-2202	UGCAUUUUGAAGUGAUGAG	CUCAUCACUUCAAAAUGCA
3097	3098	2185-2203	GCAUUUUGAAGUGAUGAGU	ACUCAUCACUUCAAAAUGC
3099	3100	2186-2204	CAUUUUGAAGUGAUGAGUG	CACUCAUCACUUCAAAAUG
3101	3102	2187-2205	AUUUUGAAGUGAUGAGUGA	UCACUCAUCACUUCAAAAU
3103	3104	2188-2206	UUUUGAAGUGAUGAGUGAA	UUCACUCAUCACUUCAAAA
3105	3106	2190-2208	UUGAAGUGAUGAGUGAAAG	CUUUCACUCAUCACUUCAA
3107	3108	2191-2209	UGAAGUGAUGAGUGAAAGA	UCUUUCACUCAUCACUUCA
3109	3110	2192-2210	GAAGUGAUGAGUGAAAGAG	CUCUUUCACUCAUCACUUC
3111	3112	2193-2211	AAGUGAUGAGUGAAAGAGA	UCUCUUUCACUCAUCACUU
3113	3114	2194-2212	AGUGAUGAGUGAAAGAGAG	CUCUCUUUCACUCAUCACU
3115	3116	2195-2213	GUGAUGAGUGAAAGAGAGA	UCUCUCUUUCACUCAUCAC
3117	3118	2196-2214	UGAUGAGUGAAAGAGAGAA	UUCUCUCUUUCACUCAUCA
3119	3120	2197-2215	GAUGAGUGAAAGAGAGAAG	CUUCUCUCUUUCACUCAUC
3121	3122	2198-2216	AUGAGUGAAAGAGAGAAGU	ACUUCUCUCUUUCACUCAU
3123	3124	2199-2217	UGAGUGAAAGAGAGAAGUC	GACUUCUCUCUUUCACUCA
3125	3126	2200-2218	GAGUGAAAGAGAGAAGUCC	GGACUUCUCUCUUUCACUC
3127	3128	2201-2219	AGUGAAAGAGAGAAGUCCU	AGGACUUCUCUCUUUCACU
3129	3130	2202-2220	GUGAAAGAGAGAAGUCCUA	UAGGACUUCUCUCUUUCAC
3131	3132	2203-2221	UGAAAGAGAGAAGUCCUAU	AUAGGACUUCUCUCUUUCA
3133	3134	2204-2222	GAAAGAGAGAAGUCCUAUU	AAUAGGACUUCUCUCUUUC
3135	3136	2205-2223	AAAGAGAGAAGUCCUAUUU	AAAUAGGACUUCUCUCUUU
3137	3138	2206-2224	AAGAGAGAAGUCCUAUUUC	GAAAUAGGACUUCUCUCUU

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3141	3142	2208-2226	GAGAGAAGUCCUAUUUCUC	GAGAAAUAGGACUUCUCUC
3143	3144	2209-2227	AGAGAAGUCCUAUUUCUCA	UGAGAAAUAGGACUUCUCU
3145	3146	2210-2228	GAGAAGUCCUAUUUCUCAG	CUGAGAAAUAGGACUUCUC
3147	3148	2211-2229	AGAAGUCCUAUUUCUCAGG	CCUGAGAAAUAGGACUUCU
3149	3150	2212-2230	GAAGUCCUAUUUCUCAGGC	GCCUGAGAAAUAGGACUUC
3151	3152	2213-2231	AAGUCCUAUUUCUCAGGCU	AGCCUGAGAAAUAGGACUU
3153	3154	2214-2232	AGUCCUAUUUCUCAGGCUU	AAGCCUGAGAAAUAGGACU
3155	3156	2215-2233	GUCCUAUUUCUCAGGCUUG	CAAGCCUGAGAAAUAGGAC
3157	3158	2216-2234	UCCUAUUUCUCAGGCUUGA	UCAAGCCUGAGAAAUAGGA
3159	3160	2217-2235	CCUAUUUCUCAGGCUUGAG	CUCAAGCCUGAGAAAUAGG
3161	3162	2218-2236	CUAUUUCUCAGGCUUGAGC	GCUCAAGCCUGAGAAAUAG
3163	3164	2219-2237	UAUUUCUCAGGCUUGAGCA	UGCUCAAGCCUGAGAAAUA
3165	3166	2220-2238	AUUUCUCAGGCUUGAGCAA	UUGCUCAAGCCUGAGAAAU
3167	3168	2221-2239	UUUCUCAGGCUUGAGCAAG	CUUGCUCAAGCCUGAGAAA
3169	3170	2222-2240	UUCUCAGGCUUGAGCAAGU	ACUUGCUCAAGCCUGAGAA
3171	3172	2223-2241	UCUCAGGCUUGAGCAAGUU	AACUUGCUCAAGCCUGAGA
3173	3174	2224-2242	CUCAGGCUUGAGCAAGUUG	CAACUUGCUCAAGCCUGAG
3175	3176	2225-2243	UCAGGCUUGAGCAAGUUGG	CCAACUUGCUCAAGCCUGA
3177	3178	2226-2244	CAGGCUUGAGCAAGUUGGU	ACCAACUUGCUCAAGCCUG
3179	3180	2229-2247	GCUUGAGCAAGUUGGUAUC	GAUACCAACUUGCUCAAGC
3181	3182	2231-2249	UUGAGCAAGUUGGUAUCUG	CAGAUACCAACUUGCUCAA
3183	3184	2232-2250	UGAGCAAGUUGGUAUCUGC	GCAGAUACCAACUUGCUCA
3185	3186	2233-2251	GAGCAAGUUGGUAUCUGCU	AGCAGAUACCAACUUGCUC
3187	3188	2234-2252	AGCAAGUUGGUAUCUGCUC	GAGCAGAUACCAACUUGCU
3189	3190	2235-2253	GCAAGUUGGUAUCUGCUCA	UGAGCAGAUACCAACUUGC
3191	3192	2236-2254	CAAGUUGGUAUCUGCUCAG	CUGAGCAGAUACCAACUUG
3193	3194	2237-2255	AAGUUGGUAUCUGCUCAGG	CCUGAGCAGAUACCAACUU
3195	3196	2238-2256	AGUUGGUAUCUGCUCAGGC	GCCUGAGCAGAUACCAACU
3197	3198	2239-2257	GUUGGUAUCUGCUCAGGCC	GGCCUGAGCAGAUACCAAC
3199	3200	2240-2258	UUGGUAUCUGCUCAGGCCU	AGGCCUGAGCAGAUACCAA
3201	3202	2241-2259	UGGUAUCUGCUCAGGCCUG	CAGGCCUGAGCAGAUACCA
3203	3204	2242-2260	GGUAUCUGCUCAGGCCUGA	UCAGGCCUGAGCAGAUACC
3205	3206	2243-2261	GUAUCUGCUCAGGCCUGAG	CUCAGGCCUGAGCAGAUAC
3207	3208	2244-2262	UAUCUGCUCAGGCCUGAGC	GCUCAGGCCUGAGCAGAUA
3209	3210	2245-2263	AUCUGCUCAGGCCUGAGCA	UGCUCAGGCCUGAGCAGAU

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3213	3214	2247-2265	CUGCUCAGGCCUGAGCAUG	CAUGCUCAGGCCUGAGCAG
3215	3216	2248-2266	UGCUCAGGCCUGAGCAUGA	UCAUGCUCAGGCCUGAGCA
3217	3218	2249-2267	GCUCAGGCCUGAGCAUGAC	GUCAUGCUCAGGCCUGAGC
3219	3220	2250-2268	CUCAGGCCUGAGCAUGACC	GGUCAUGCUCAGGCCUGAG
3221	3222	2251-2269	UCAGGCCUGAGCAUGACCU	AGGUCAUGCUCAGGCCUGA
3223	3224	2252-2270	CAGGCCUGAGCAUGACCUC	GAGGUCAUGCUCAGGCCUG
3225	3226	2253-2271	AGGCCUGAGCAUGACCUCA	UGAGGUCAUGCUCAGGCCU
3227	3228	2279-2297	CACUUAACCCCAGGCCAUU	AAUGGCCUGGGGUUAAGUG
3229	3230	2280-2298	ACUUAACCCCAGGCCAUUA	UAAUGGCCUGGGGUUAAGU
3231	3232	2281-2299	CUUAACCCCAGGCCAUUAU	AUAAUGGCCUGGGGUUAAG
3233	3234	2282-2300	UUAACCCCAGGCCAUUAUC	GAUAAUGGCCUGGGGUUAA
3235	3236	2283-2301	UAACCCCAGGCCAUUAUCA	UGAUAAUGGCCUGGGGUUA
3237	3238	2284-2302	AACCCCAGGCCAUUAUCAU	AUGAUAAUGGCCUGGGGUU
3239	3240	2285-2303	ACCCCAGGCCAUUAUCAUA	UAUGAUAAUGGCCUGGGGU
3241	3242	2287-2305	CCCAGGCCAUUAUCAUAUC	GAUAUGAUAAUGGCCUGGG
3243	3244	2288-2306	CCAGGCCAUUAUCAUAUCC	GGAUAUGAUAAUGGCCUGG
3245	3246	2289-2307	CAGGCCAUUAUCAUAUCCA	UGGAUAUGAUAAUGGCCUG
3247	3248	2290-2308	AGGCCAUUAUCAUAUCCAG	CUGGAUAUGAUAAUGGCCU
3249	3250	2291-2309	GGCCAUUAUCAUAUCCAGA	UCUGGAUAUGAUAAUGGCC
3251	3252	2292-2310	GCCAUUAUCAUAUCCAGAU	AUCUGGAUAUGAUAAUGGC
3253	3254	2314-2332	CUUCAGAGUUGUCUUUAUA	UAUAAAGACAACUCUGAAG
3255	3256	2315-2333	UUCAGAGUUGUCUUUAUAU	AUAUAAAGACAACUCUGAA
3257	3258	2316-2334	UCAGAGUUGUCUUUAUAUG	CAUAUAAAGACAACUCUGA
3259	3260	2318-2336	AGAGUUGUCUUUAUAUGUG	CACAUAUAAAGACAACUCU
3261	3262	2322-2340	UUGUCUUUAUAUGUGAAUU	AAUUCACAUAUAAAGACAA
3263	3264	2323-2341	UGUCUUUAUAUGUGAAUUA	UAAUUCACAUAUAAAGACA
3265	3266	2324-2342	GUCUUUAUAUGUGAAUUAA	UUAAUUCACAUAUAAAGAC
3267	3268	2325-2343	UCUUUAUAUGUGAAUUAAG	CUUAAUUCACAUAUAAAGA
3269	3270	2326-2344	CUUUAUAUGUGAAUUAAGU	ACUUAAUUCACAUAUAAAG
3271	3272	2327-2345	UUUAUAUGUGAAUUAAGUU	AACUUAAUUCACAUAUAAA
3273	3274	2328-2346	UUAUAUGUGAAUUAAGUUA	UAACUUAAUUCACAUAUAA
3275	3276	2329-2347	UAUAUGUGAAUUAAGUUAU	AUAACUUAAUUCACAUAUA
3277	3278	2330-2348	AUAUGUGAAUUAAGUUAUA	UAUAACUUAAUUCACAUAU
3279	3280	2331-2349	UAUGUGAAUUAAGUUAUAU	AUAUAACUUAAUUCACAUA
3281	3282	2332-2350	AUGUGAAUUAAGUUAUAUU	AAUAUAACUUAAUUCACAU

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
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3285	3286	2334-2352	GUGAAUUAAGUUAUAUUAA	UUAAUAUAACUUAAUUCAC
3287	3288	2335-2353	UGAAUUAAGUUAUAUUAAA	UUUAAUAUAACUUAAUUCA
3289	3290	2336-2354	GAAUUAAGUUAUAUUAAAU	AUUUAAUAUAACUUAAUUC
3291	3292	2337-2355	AAUUAAGUUAUAUUAAAUU	AAUUUAAUAUAACUUAAUU
3293	3294	2338-2356	AUUAAGUUAUAUUAAAUUU	AAAUUUAAUAUAACUUAAU
3295	3296	2339-2357	UUAAGUUAUAUUAAAUUUU	AAAAUUUAAUAUAACUUAA
3297	3298	2340-2358	UAAGUUAUAUUAAAUUUUA	UAAAAUUUAAUAUAACUUA
3299	3300	2341-2359	AAGUUAUAUUAAAUUUUAA	UUAAAAUUUAAUAUAACUU
3301	3302	2342-2360	AGUUAUAUUAAAUUUUAAU	AUUAAAAUUUAAUAUAACU
3303	3304	2343-2361	GUUAUAUUAAAUUUUAAUC	GAUUAAAAUUUAAUAUAAC
3305	3306	2345-2363	UAUAUUAAAUUUUAAUCUA	UAGAUUAAAAUUUAAUAUA
3307	3308	2346-2364	AUAUUAAAUUUUAAUCUAU	AUAGAUUAAAAUUUAAUAU
3309	3310	2347-2365	UAUUAAAUUUUAAUCUAUA	UAUAGAUUAAAAUUUAAUA
3311	3312	2348-2366	AUUAAAUUUUAAUCUAUAG	CUAUAGAUUAAAAUUUAAU
3313	3314	2349-2367	UUAAAUUUUAAUCUAUAGU	ACUAUAGAUUAAAAUUUAA
3315	3316	2350-2368	UAAAUUUUAAUCUAUAGUA	UACUAUAGAUUAAAAUUUA
3317	3318	2351-2369	AAAUUUUAAUCUAUAGUAA	UUACUAUAGAUUAAAAUUU
3319	3320	2354-2372	UUUUAAUCUAUAGUAAAAA	UUUUUACUAUAGAUUAAAA
3321	3322	2355-2373	UUUAAUCUAUAGUAAAAAC	GUUUUUACUAUAGAUUAAA
3323	3324	2356-2374	UUAAUCUAUAGUAAAAACA	UGUUUUUACUAUAGAUUAA
3325	3326	2357-2375	UAAUCUAUAGUAAAAACAU	AUGUUUUUACUAUAGAUUA
3327	3328	2358-2376	AAUCUAUAGUAAAAACAUA	UAUGUUUUUACUAUAGAUU
3329	3330	2359-2377	AUCUAUAGUAAAAACAUAG	CUAUGUUUUUACUAUAGAU
3331	3332	2360-2378	UCUAUAGUAAAAACAUAGU	ACUAUGUUUUUACUAUAGA
3333	3334	2361-2379	CUAUAGUAAAAACAUAGUC	GACUAUGUUUUUACUAUAG
3335	3336	2362-2380	UAUAGUAAAAACAUAGUCC	GGACUAUGUUUUUACUAUA
3337	3338	2363-2381	AUAGUAAAAACAUAGUCCU	AGGACUAUGUUUUUACUAU
3339	3340	2364-2382	UAGUAAAAACAUAGUCCUG	CAGGACUAUGUUUUUACUA
3341	3342	2365-2383	AGUAAAAACAUAGUCCUGG	CCAGGACUAUGUUUUUACU
3343	3344	2366-2384	GUAAAAACAUAGUCCUGGA	UCCAGGACUAUGUUUUUAC
3345	3346	2367-2385	UAAAAACAUAGUCCUGGAA	UUCCAGGACUAUGUUUUUA
3347	3348	2368-2386	AAAAACAUAGUCCUGGAAA	UUUCCAGGACUAUGUUUUU
3349	3350	2369-2387	AAAACAUAGUCCUGGAAAU	AUUUCCAGGACUAUGUUUU
3351	3352	2370-2388	AAACAUAGUCCUGGAAAUA	UAUUUCCAGGACUAUGUUU
3353	3354	2371-2389	AACAUAGUCCUGGAAAUAA	UUAUUUCCAGGACUAUGUU

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Position on transcript NM_ 000688.4	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
3355	3356	2372-2390	ACAUAGUCCUGGAAAUAAA	UUUAUUUCCAGGACUAUGU
3357	3358	2373-2391	CAUAGUCCUGGAAAUAAAU	AUUUAUUUCCAGGACUAUG
3359	3360	2374-2392	AUAGUCCUGGAAAUAAAUU	AAUUUAUUUCCAGGACUAU
3361	3362	2375-2393	UAGUCCUGGAAAUAAAUUC	GAAUUUAUUUCCAGGACUA
3363	3364	2377-2395	GUCCUGGAAAUAAAUUCUU	AAGAAUUUAUUUCCAGGAC
3365	3366	2378-2396	UCCUGGAAAUAAAUUCUUG	CAAGAAUUUAUUUCCAGGA

Example 9. Suppression of Porphyrin Precursors Using ALAS1 siRNA in an Acute Treatment Paradigm

[0620] The AIP mouse model (see Example 5) was used to investigate whether ALAS1 siRNA would work an an acute treatment paradigm to lower already elevated levels of ALA and PBG, as would be present, for example, when a human porphyria patient suffers from an acute attack. Administration of the AD-53558 LNP11 formulation siRNA at a 1mg/kg dose 12 hours after the last dose of phenobarbital rapidly decreased the levels of both ALA and PBG in mouse plasma, whereas in Luc control treated animals the levels continued to rise (FIG. 14). These results indicate that ALAS siRNA is effective for treating an acute attack. The ALAS 1 siRNA was effective to lower and prevent further increases in ALA and PBG levels.

Example 10. siRNAs that target ALAS1

[0621] Further unmodified and modified siRNA sequences that target ALAS 1 siRNA were designed and produced as described in Example 2. The *in vitro* activity of the modified duplexes was tested as described below.

Methods

Lipid mediated transfection

[0622] For Hep3B, PMH, and primary *Cynomolgus* hepatocytes, transfection was carried out by adding 14.8 μl of Opti-MEM plus 0.2 μl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. catalog number13778-150) to 5 μl of each siRNA duplex to an individual well in a 96-well plate. The mixture was then incubated at room temperature for 20 minutes. Eighty μl of complete growth media without antibiotic containing the appropriate cell number were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification.

[0623] Single dose experiments were performed at 1 uM, 500nM, 20nM, 10nM and 0.2nM final duplex concentration for GalNAc modified.

Free uptake transfection

[0624] Cryopreserved Primary *Cynomolgus* Hepatocytes (Celsis In Vitro Technologies, M003055-P) were thawed at 37°C water bath immediately prior to usage and re-suspended at $0.26x10^6$ cells/ml in InVitroGRO CP (plating) medium (Celsis In Vitro Technologies, catalog number Z99029). During transfections, cells were plated onto a BD BioCoat 96 well collagen plate (BD, 356407) at 25,000 cells per well and incubated at 37°C in an atmosphere of 5% CO_2. Free Uptake experiments were performed by adding 10μl of siRNA duplexes in PBS per well into a 96 well (96w) plate. Ninety μl of complete growth media containing appropriate cell number for the cell type was then added to the siRNA. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 1 uM, 500nM, 20nM and 10nM final duplex.

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

[0625] Cells were harvested and lysed in 150 μl of Lysis/Binding Buffer then mixed for 5 minutes at 850 rpm using an Eppendorf Thermomixer (the mixing speed was the same throughout the process). Ten microliters of magnetic beads and 80 μl Lysis/Binding Buffer mixture were added to a round bottom plate and mixed for 1 minute. Magnetic beads

were captured using a magnetic stand and the supernatant was removed without disturbing the beads. After removing the supernatant, the lysed cells were added to the remaining beads and mixed for 5 minutes. After removing the supernatant, magnetic beads were washed 2 times with 150 μl Wash Buffer A and mixed for 1 minute. The beads were capturedagain and the supernatant was removed. The beads were then washed with 150 μl Wash Buffer B, captured and the supernatant was removed. The beads were next washed with 150 μl Elution Buffer, captured and the supernatant removed. Finally, the beads were allowed to dry for 2 minutes. After drying, 50 μl of Elution Buffer was added and mixed for 5 minutes at 70°C. The beads were captured on magnet for 5 minutes. Forty-five μl of supernatant was removed and added to another 96 well plate.

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

[0626] A master mix of 2 μl 10X Buffer, 0.8 μl 25X dNTPs, 2 μl Random primers, 1 μl Reverse Transcriptase, 1 μl RNase inhibitor and 3.2 μl of H2O per reaction as prepared. Equal volumes master mix and RNA were mixed for a final volume of 12μl for *in vitro* screened or 20μl for *in vivo* screened samples. cDNA was generated using a Bio-Rad C-1000 or S-1000 thermal cycler (Hercules, CA) through the following steps: 25°C for 10 minutes, 37°C for 120 minutes, 85°C for 5 seconds, and 4°C hold.

Real time PCR

[0627] Two μl of cDNA were added to a master mix containing 2μl of H_2O, 0.5μl GAPDH TaqMan Probe (Life Technologies catalog number 4326317E for Hep3B cells, catalog number 352339E for primary mouse hepatocytes or custom probe for cynomolgus primary hepatocytes), 0.5μl C5 TaqMan probe (Life Technologies catalog number Hs00167441_ml for Hep3B cells or Mm00457879_m1 for Primary Mouse Hepatoctyes or custom probe for cynomolgus primary hepatocytes) and 5μl Lightcycler 480 probe master mix (Roche catalog number 04887301001) per well in a 384 well (384 w) plates (Roche catalog number 04887301001). Real time PCR was performed in an Roche LC480 Real Time PCR system (Roche) using the ΔΔCt(RQ) assay. For *in vitro* screening, each duplex was tested with two biological replicates unless otherwise noted and each Real Time PCR was performed in duplicate technical replicates. For *in vivo* screening, each duplex was tested in one or more experiments (3 mice per group) and each Real Time PCR was run in duplicate technical replicates.

[0628] To calculate relative fold change in ALAS1 mRNA levels, real time data were analyzed using the ΔΔCT method and normalized to assays performed with cells transfected with 10 nM AD-1955, or mock transfected cells. IC_{50}s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 over the same dose range, or to its own lowest dose.

[0629] The sense and antisense sequences of AD-1955 are:

SENSE: cuuAcGcuGAGuAcuucGAdTsdT (SEQ ID NO:3682)

ANTISENSE: UCGAAGuACUcAGCGuAAGdTsdT (SEQ ID NO:3683).

[0630] The single strand and duplex sequences of the modified and unmodified siRNAs are provided in Table 14 and Table 15, respectively.

Table 14: Human ALAS1 Modified Single Strands and Duplex Sequences

SEQ ID NO: (sense)	SEQ ID NO: (antisens e)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_ 000688.4
3371	3372	AD-58848	CfsasUfgCfcAfaAfAfAfuGfgAfcAfuCfaUfL96	asUfsgAfuGfuCfcAfuuuUfuGfgCfaUfgsAfsc	1635-1657
3373	3374	AD-58849	AfsusUfuUfgAfaGfUfGfaUfgAfgUfgAfaAfL96	usUfsuCfaCfuCfaUfcacUfuCfaAfaAfusGfsc	2189-2211
3375	3376	AD-58850	AfsgsUfuAfuAfuUfAfAfaUfuUfuAfaUfcUfL96	asGfsaUfuAfaAfaUfuuaAfuAfuAfaCfusUfsa	2344-2366
3377	3378	AD-58851	GfscsAfuUfuUfgAfAfGfuGfaUfgAfgUfgAfL96	usCfsaCfuCfaUfcAfcuuCfaAfaAfuGfcsAfsg	2187-2209
3379	3380	AD-58852	GfsasAfcUfaAfuGfAfGfcAfgAfcAfuAfaCfL96	gsUfsuAfuGfuCfuGfcucAfuUfaGfuUfcsAfsu	1975-1997
3381	3382	AD-58853	AfsasUfgAfcCfaCfAfCfcUfaUfcGfaGfuUfL96	asAfscUfcGfaUfaGfgugUfgGfuCfaUfusCfsu	973-995
3383	3384	AD-58854	UfsasAfaUfuUfuAfAfUfcUfaUfaGfuAfaAfL96	usUfsuAfcUfaUfaGfauuAfaAfaUfuUfasAfsu	2352-2374
3385	3386	AD-58855	UfsusCfaGfuAfuGfAfUfcGfuUfuCfuUfuGfL96	csAfsaAfgAfaAfcGfaucAfuAfcUfgAfasAfsa	929-951
3387	3388	AD-58856	CfsasCfuUfuUfcAfGfUfaUfgAfuCfgUfuUfL96	asAfsaCfgAfuCfaUfacuGfaAfaAfgUfgsGfsa	924-946
3389	3390	AD-58857	AfsasAfuCfuGfuUfUfCfcAfcUfuUfuCfaGfL96	csUfsgAfaAfaGfuGfgaaAfcAfgAfuUfusUfsg	913-935
3391	3392	AD-58858	CfsasUfuUfgAfaAfCfUfgUfcCfaUfuCfaAfL96	usUfsgAfaUfgGfaCfaguUfuCfaAfaUfgsCfsc	1478-1500
3393	3394	AD-58859	CfscsUfaUfcGfaGfUfUfuUfuAfaAfaCfuGfL96	csAfsgUfuUfuAfaAfaacUfcGfaUfaGfgsUfsg	983-1005
3395	3396	AD-58861	GfsasCfcAfgAfaAfGfAfgUfgUfcUfcAfuCfL96	gsAfsuGfaGfaCfaCfucuUfuCfuGfgUfcsUfsu	872-894
3397	3398	AD-58862	AfscsCfaGfaAfaGfAfGfuGfuCfuCfaUfcUfL96	asGfsaUfgAfgAfcAfcucUfuUfcUfgGfusCfsu	873-895

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisens e)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_ 000688.4
3399	3400	AD-58863	AfscsUfaAfuGfaGfCfAfgAfcAfuAfaCfaUfL96	asUfsgUfuAfuGfuCfugcUfcAfuUfaGfusUfsc	1977-1999
3401	3402	AD-58864	UfsasGfuAfaAfaAfCfAfuAfgUfcCfuGfgAfL96	usCfscAfgGfaCfuAfuguUfuUfuAfcUfasUfsa	2366-2388
3403	3404	AD-58865	UfsasUfuUfcUfgGfAfAfcUfaGfuAfaAfuUfL96	asAfsuUfuAfcUfaGfuucCfaGfaAfaUfasUfsu	1185-1207
3405	3406	AD-58867	UfsusCfuGfcAfaAfGfCfcAfgUfcUfuGfaGfL96	csUfscAfaGfaCfuGfgcuUfuGfcAfgAfasGfsa	706-728
3407	3408	AD-58868	GfsasGfgAfaAfgAfGfGfuUfgCfuGfaAfaCfL96	gsUfsuUfcAfgCfaAfccuCfuUfuCfcUfcsAfsc	759-781
3409	3410	AD-58869	GfsgsUfaCfuAfgAfAfAfuAfuUfuCfuGfgAfL96	usCfscAfgAfaAfuAfuuuCfuAfgUfaCfcsAfsc	1174-1196
3411	3412	AD-58870	GfsasCfaUfcAfuGfCfAfaAfaGfcAfaAfgAfL96	usCfsuUfuGfcUfuUfugcAfuGfaUfgUfcsCfsu	853-875
3413	3414	AD-58871	AfsasAfuUfuUfaAfUfCfuAfuAfgUfaAfaAfL96	usUfsuUfaCfuAfuAfgauUfaAfaAfuUfusAfsa	2353-2375
3415	3416	AD-58873	CfsasUfgAfuCfcAfAfGfgGfaUfuCf	usUfsuCfgAfaUfcCfcuuGfgAfuCfaUf	1362-1384
			gAfaAfL96	gsGfsa	
3417	3418	AD-58874	AfsgsAfcCfaGfaAfAfGfaGfuGfuCfuCfaUfL96	asUfsgAfgAfcAfcUfcuuUfcUfgGfuCfusUfsu	871-893
3419	3420	AD-58875	AfsusCfcUfgAfaGfAfGfcGfcUfgAfgGfgAfL96	usCfscCfuCfaGfcGfcucUfuCfaGfgAfusCfsc	1810-1832
3421	3422	AD-58876	GfsusCfuGfuGfaUfGfAfaCfuAfaUfgAfgCfL96	gsCfsuCfaUfuAfgUfucaUfcAfcAfgAfcsUfsu	1966-1988
3423	3424	AD-58877	CfsasGfaAfaGfaGfUfGfuCfuCfaUfcUfuCfL96	gsAfsaGfaUfgAfgAfcacUfcUfuUfcUfgsGfsu	875-897
3425	3426	AD-58878	AfscsUfuUfuCfaGfUfAfuGfaUfcGfuUfuCfL96	gsAfsaAfcGfaUfcAfuacUfgAfaAfaGfusGfsg	925-947

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisens e)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_ 000688.4
3427	3428	AD-58879	UfscsAfuGfcCfaAfAfAfaUfgGfaCf aUfcAfL96	usGfsaUfgUfcCfaUfuuuUfgGfcAfuG fasCfsu	1634-1656
3429	3430	AD-58880	AfsasUfaUfuUfcUfGfGfaAfcUfaG fuAfaAfL96	usUfsuAfcUfaGfuUfccaGfaAfaUfaU fusUfsc	1183-1205
3431	3432	AD-58881	CfsusUfcUfuCfaAfGfAfuAfaCfuUf gCfcAfL96	usGfsgCfaAfgUfuAfucuUfgAfaGfaA fgsAfsu	892-914
3433	3434	AD-58882	UfsusUfcAfgUfaUfGfAfuCfgUfuU fcUfuUfL96	asAfsaGfaAfaCfgAfucaUfaCfuGfaAf asAfsg	928-950
3435	3436	AD-58883	CfscsCfaGfuGfuGfGfUfuAfgUfgU fgAfaAfL96	usUfsuCfaCfaCfuAfaccAfcAfcUfgGf gsGfsc	790-812
3437	3438	AD-58884	GfscsUfgUfgAfgAfUfUfuAfcUfcUf gAfuUfL96	asAfsuCfaGfaGfuAfaauCfuCfaCfaGf csCfsu	1325-1347
3439	3440	AD-58885	AfsgsGfcUfuGfaGfCfAfaGfuUfgG fuAfuCfL96	gsAfsuAfcCfaAfcUfugcUfcAfaGfcCf usGfsa	2229-2251
3441	3442	AD-58886	GfsasAfaGfaGfuGfUfCfuCfaUfcU fuCfuUfL96	asAfsgAfaGfaUfgAfgacAfcUfcUfuUf csUfsg	877-899
3443	3444	AD-58887	AfsusUfuCfuGfgAfAfCfuAfgUfaAf aUfuCfL96	gsAfsaUfuUfaCfuAfguuCfcAfgAfaAf usAfsu	1186-1208
3445	3446	AD-58888	UfsgsUfgAfuGfuGfGfCfcCfaUfgAf gUfuUfL96	asAfsaCfuCfaUfgGfgccAfcAfuCfaCf asCfsa	1531-1553
3447	3448	AD-58889	AfsasGfaGfaGfaAfGfUfcCfuAfuU fuCfuCfL96	gsAfsgAfaAfuAfgGfacuUfcUfcUfcUf usUfsc	2208-2230
3449	3450	AD-58890	UfsgsGfcAfgCfaCfAfGfaUfgAfaUf cAfgAfL96	usCfsuGfaUfuCfaUfcugUfgCfuGfcCf asGfsg	671-693
3451	3452	AD-58891	AfsusGfaUfcGfuUfUfCfuUfuGfaG faAfaAfL96	usUfsuUfcUfcAfaAfgaaAfcGfaUfcAf usAfsc	935-957
3453	3454	AD-58892	UfscsUfgGfaAfcUfAfGfuAfaAfuU fcCfaUfL96	asUfsgGfaAfuUfuAfcuaGfuUfcCfaG fasAfsa	1189-1211

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisens e)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_ 000688.4
3455	3456	AD-59095	GfscsCfcAfuUfcUfUfAfuCfcCfgAfgUfL96	asCfsuCfgGfgAfuAfagaAfuGfgsgsc	360-382
3457	3458	AD-59096	GfsgsAfaCfcAfuGfCfCfuCfcAfuGfaUfL96	asUfscAfuGfgAfgGfcauGfgUfuscsc	1347-1369
3459	3460	AD-59097	UfsgsGfaGfuCfuGfUfGfcGfgAfuCfcUfL96	asGfsgAfuCfcGfcAfcagAfcUfcscsa	1794-1816
3461	3462	AD-59098	CfsasCfcCfaCfgGfGfUfgUfgUfgGfgAfL96	usCfscCfaCfaCfaCfccgUfgGfgsusg	1112-1134
3463	3464	AD-59099	GfsgsAfgUfcUfgUfGfCfgGfaUfcCfuAfL96	usAfsgGfaUfcCfgCfacaGfaCfuscsc	1795-1817
3465	3466	AD-59100	CfsasAfaAfcUfgCfCfCfcAfaGfaUfgAfL96	usCfsaUfcUfuGfgGfgcaGfuUfususg	428-450
3467	3468	AD-59101	GfscsCfuCfcAfuGfAfUfcCfaAfgGfgAfL96	usCfscCfuUfgGfaUfcauGfgAfgsgsc	1355-1377
3469	3470	AD-59102	CfsasUfcAfuCfcCfUfGfuGfcGfgGfuUfL96	asAfscCfcGfcAfcAfgggAfuGfasusg	1921-1943
3471	3472	AD-59103	AfscsCfcAfcGfgGfUfGfuGfuGfgGf	usCfscCfcAfcAfcAfcccGfuGfgsgsu	1113-1135
			gAfL96		
3473	3474	AD-59104	CfsasCfaUfcAfuCfCfCfuGfuGfcGfgAfL96	usCfscGfcAfcAfgGfgauGfaUfgsusg	1919-1941
3475	3476	AD-59105	CfsasGfaAfaGfaGfUfGfuCfuCfaUfcUfL96	asGfsaUfgAfgAfcAfcucUfuUfcsusg	873-895
3477	3478	AD-59106	CfscsUfcCfaUfgAfUfCfcAfaGfgGfaUfL96	asUfscCfcUfuGfgAfucaUfgGfasgsg	1356-1378
3479	3480	AD-59107	UfsgsCfcCfaUfuCfUfUfaUfcCfcGfaAfL96	usUfscGfgGfaUfaAfgaaUfgGfgscsa	359-381
3481	3482	AD-59108	CfsusUfcAfcCfcUfGfGfcUfaAfgAfuAfL96	usAfsuCfuUfaGfcCfaggGfuGfasasg	1297-1319

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisens e)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_ 000688.4
3483	3484	AD-59109	AfsusCfaUfcCfcUfGfUfgCfgGfgUfuAfL96	usAfsaCfcCfgCfaCfaggGfa Ufgsasu	1922-1944
3485	3486	AD-59110	AfsgsAfaAfgAfgUfGfUfcUfcAfuCfuUfL96	asAfsgAfuGfaGfaCfacuCfuUfuscsu	874-896
3487	3488	AD-59111	CfsusCfcAfuGfaUfCfCfaAfgGfgAfuUfL96	asAfsuCfcCfuUfgGfaucAfuGfgsasg	1357-1379
3489	3490	AD-59112	CfscsAfuUfcUfuAfUfCfcCfgAfgUfcAfL96	usGfsaCfuCfgGfgAfuaaGfaAfusgsg	362-384
3491	3492	AD-59113	CfsasCfcCfuGfgCfUfAfaGfaUfgAfuAfL96	usAfsuCfaUfcUfuAfgccAfgGfgsusg	1300-1322
3493	3494	AD-59114	UfscsAfuCfcCfuGfUfGfcGfgGfuUfgAfL96	usCfsaAfcCfcGfcAfcagGfgAfusgsa	1923-1945
3495	3496	AD-59115	AfsasGfaGfuGfuCfUfCfaUfcUfuCfuUfL96	asAfsgAfaGfaUfgAfgacAfcUfcsusu	877-899
3497	3498	AD-59116	GfsusCfaUfgCfcAfAfAfaAfuGfgAfcAfL96	usGfsuCfcAfuUfuUfuggCfaUfgsasc	1631-1653
3499	3500	AD-59117	CfsasUfuCfuUfaUfCfCfcGfaGfuCfcAfL96	usGfsgAfcUfcGfgGfauaAfgAfasusg	363-385
3501	3502	AD-59118	AfscsCfcUfgGfcUfAfAfgAfuGfaUfgAfL96	usCfsaUfcAfuCfuUfagcCfaGfgsgsu	1301-1323
3503	3504	AD-59119	CfsusCfuUfcAfcCfCfUfgGfcUfaAfgAfL96	usCfsuUfaGfcCfaGfgguGfaAfgsasg	1295-1317
3505	3506	AD-59120	AfsusGfcCfaAfaAfAfUfgGfaCfaUfcAfL96	usGfsaUfgUfcCfaUfuuuUfgGfcsasu	1634-1656
3507	3508	AD-59121	UfsgsCfcCfcAfaGfAfUfgAfuGfgAfaUfL96	asUfsuCfcAfuCfaUfcuuGfgGfgscsa	434-456
3509	3510	AD-59122	GfsasAfcCfaUfgCfCfUfcCfaUfgAfuAfL96	usAfsuCfaUfgGfaGfgcaUfgGfususc	1348-1370

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisens e)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_ 000688.4
3511	3512	AD-59123	UfscsUfuCfaCfcCfUfGfgCfuAfaGfaUfL96	asUfscUfuAfgCfcAfgggUfgAfasgsa	1296-1318
3513	3514	AD-59124	UfsgsCfcAfaAfaAfUfGfgAfcAfuCfaUfL96	asUfsgAfuGfuCfcAfuuuUfuGfgscsa	1635-1657
3515	3516	AD-59125	CfscsAfgAfaAfgAfGfUfgUfcUfcAfuAfL96	usAfsuGfaGfaCfaCfucuUfuCfusgsg	872-894
3517	3518	AD-59126	GfsasAfaCfuGfuCfCfAfuUfcAfaUfgAfL96	usCfsaUfuGfaAfuGfgacAfgUfususc	1481-1503
3519	3520	AD-59127	UfscsAfcCfcUfgGfCfUfaAfgAfuGfaUfL96	asUfscAfuCfuUfaGfccaGfgGfusgsa	1299-1321
3521	3522	AD-59128	CfscsCfuGfgAfgUfCfUfgUfgCfgGfaUfL96	asUfscCfgCfaCfaGfacuCfcAfgsgsg	1791-1813
3523	3524	AD-59129	GfsasAfaGfaGfuGfUfCfuCfaUfcUfuAfL96	usAfsaGfaUfgAfgAfcacUfcUfususc	875-897
3525	3526	AD-59130	UfsgsGfaGfcCfcUfGfGfaGfuCfuGfuAfL96	usAfscAfgAfcUfcCfaggGfcUfcscsa	1786-1808

Table 15: Human ALAS1 Unmodified Single Strands and Duplex Sequences

SEQ ID NO: (sense)	SEQ ID NO: (antisens e)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_ 000688.4
3684	3527	AD-58848	CAUGCCAAAAAUGGACAUCAU	AUGAUGUCCAUUUUUGGCAUGAC	1635-1657
3528	3529	AD-58849	AUUUUGAAGUGAUGAGUGAAA	UUUCACUCAUCACUUCAAAAUGC	2189-2211
3530	3531	AD-58850	AGUUAUAUUAAAUUUUAAUCU	AGAUUAAAAUUUAAUAUAACUUA	2344-2366
3532	3533	AD-58851	GCAUUUUGAAGUGAUGAGUGA	UCACUCAUCACUUCAAAAUGCAG	2187-2209
3534	3535	AD-58852	GAACUAAUGAGCAGACAUAAC	GUUAUGUCUGCUCAUUAGUUCAU	1975-1997
3536	3537	AD-58853	AAUGACCACACCUAUCGAGUU	AACUCGAUAGGUGUGGUCAUUCU	973-995
3538	3539	AD-58854	UAAAUUUUAAUCUAUAGUAAA	UUUACUAUAGAUUAAAAUUUAAU	2352-2374
3540	3541	AD-58855	UUCAGUAUGAUCGUUUCUUUG	CAAAGAAACGAUCAUACUGAAAA	929-951
3542	3543	AD-58856	CACUUUUCAGUAUGAUCGUUU	AAACGAUCAUACUGAAAAGUGGA	924-946
3544	3545	AD-58857	AAAUCUGUUUCCACUUUUCAG	CUGAAAAGUGGAAACAGAUUUUG	913-935
3546	3547	AD-58858	CAUUUGAAACUGUCCAUUCAA	UUGAAUGGACAGUUUCAAAUGCC	1478-1500
3548	3549	AD-58859	CCUAUCGAGUUUUUAAAACUG	CAGUUUUAAAAACUCGAUAGGUG	983-1005
3550	3551	AD-58861	GACCAGAAAGAGUGUCUCAUC	GAUGAGACACUCUUUCUGGUCUU	872-894
3552	3553	AD-58862	ACCAGAAAGAGUGUCUCAUCU	AGAUGAGACACUCUUUCUGGUCU	873-895
3554	3555	AD-58863	ACUAAUGAGCAGACAUAACAU	AUGUUAUGUCUGCUCAUUAGUUC	1977-1999
3556	3557	AD-58864	UAGUAAAAACAUAGUCCUGGA	UCCAGGACUAUGUUUUUACUAUA	2366-2388
3558	3559	AD-58865	UAUUUCUGGAACUAGUAAAUU	AAUUUACUAGUUCCAGAAAUAUU	1185-1207
3560	3561	AD-58867	UUCUGCAAAGCCAGUCUUGAG	CUCAAGACUGGCUUUGCAGAAGA	706-728
3562	3563	AD-58868	GAGGAAAGAGGUUGCUGAAAC	GUUUCAGCAACCUCUUUCCUCAC	759-781
3564	3565	AD-58869	GGUACUAGAAAUAUUUCUGGA	UCCAGAAAUAUUUCUAGUACCAC	1174-1196
3566	3567	AD-58870	GACAUCAUGCAAAAGCAAAGA	UCUUUGCUUUUGCAUGAUGUCCU	853-875
3568	3569	AD-58871	AAAUUUUAAUCUAUAGUAAAA	UUUUACUAUAGAUUAAAAUUUAA	2353-2375
3570	3571	AD-58873	CAUGAUCCAAGGGAUUCGAAA	UUUCGAAUCCCUUGGAUCAUGGA	1362-1384
3572	3573	AD-58874	AGACCAGAAAGAGUGUCUCAU	AUGAGACACUCUUUCUGGUCUUU	871-893
3574	3575	AD-58875	AUCCUGAAGAGCGCUGAGGGA	UCCCUCAGCGCUCUUCAGGAUCC	1810-1832

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisens e)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_ 000688.4
3576	3577	AD-58876	GUCUGUGAUGAACUAAUGAGC	GCUCAUUAGUUCAUCACAGACUU	1966-1988
3578	3579	AD-58877	CAGAAAGAGUGUCUCAUCUUC	GAAGAUGAGACACUCUUUCUGGU	875-897
3580	3581	AD-58878	ACUUUUCAGUAUGAUCGUUUC	GAAACGAUCAUACUGAAAAGUGG	925-947
3582	3583	AD-58879	UCAUGCCAAAAAUGGACAUCA	UGAUGUCCAUUUUUGGCAUGACU	1634-1656
3584	3585	AD-58880	AAUAUUUCUGGAACUAGUAAA	UUUACUAGUUCCAGAAAUAUUUC	1183-1205
3586	3587	AD-58881	CUUCUUCAAGAUAACUUGCCA	UGGCAAGUUAUCUUGAAGAAGAU	892-914
3588	3589	AD-58882	UUUCAGUAUGAUCGUUUCUUU	AAAGAAACGAUCAUACUGAAAAG	928-950
3590	3591	AD-58883	CCCAGUGUGGUUAGUGUGAAA	UUUCACACUAACCACACUGGGGC	790-812
3592	3593	AD-58884	GCUGUGAGAUUUACUCUGAUU	AAUCAGAGUAAAUCUCACAGCCU	1325-1347
3594	3595	AD-58885	AGGCUUGAGCAAGUUGGUAUC	GAUACCAACUUGCUCAAGCCUGA	2229-2251
3596	3597	AD-58886	GAAAGAGUGUCUCAUCUUCUU	AAGAAGAUGAGACACUCUUUCUG	877-899
3598	3599	AD-58887	AUUUCUGGAACUAGUAAAUUC	GAAUUUACUAGUUCCAGAAAUAU	1186-1208
3600	3601	AD-58888	UGUGAUGUGGCCCAUGAGUUU	AAACUCAUGGGCCACAUCACACA	1531-1553
3602	3603	AD-58889	AAGAGAGAAGUCCUAUUUCUC	GAGAAAUAGGACUUCUCUCUUUC	2208-2230
3604	3605	AD-58890	UGGCAGCACAGAUGAAUCAGA	UCUGAUUCAUCUGUGCUGCCAGG	671-693
3606	3607	AD-58891	AUGAUCGUUUCUUUGAGAAAA	UUUUCUCAAAGAAACGAUCAUAC	935-957
3608	3609	AD-58892	UCUGGAACUAGUAAAUUCCAU	AUGGAAUUUACUAGUUCCAGAAA	1189-1211
3610	3611	AD-59095	GCCCAUUCUUAUCCCGAGU	ACUCGGGAUAAGAAUGGGC	360-382
3612	3613	AD-59096	GGAACCAUGCCUCCAUGAU	AUCAUGGAGGCAUGGUUCC	1347-1369
3614	3615	AD-59097	UGGAGUCUGUGCGGAUCCU	AGGAUCCGCACAGACUCCA	1794-1816
3616	3617	AD-59098	CACCCACGGGUGUGUGGGA	UCCCACACACCCGUGGGUG	1112-1134
3618	3619	AD-59099	GGAGUCUGUGCGGAUCCUA	UAGGAUCCGCACAGACUCC	1795-1817
3620	3621	AD-59100	CAAAACUGCCCCAAGAUGA	UCAUCUUGGGGCAGUUUUG	428-450
3622	3623	AD-59101	GCCUCCAUGAUCCAAGGGA	UCCCUUGGAUCAUGGAGGC	1355-1377

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisens e)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_ 000688.4
3624	3625	AD-59102	CAUCAUCCCUGUGCGGGUU	AACCCGCACAGGGAUGAUG	1921-1943
3626	3627	AD-59103	ACCCACGGGUGUGUGGGGA	UCCCCACACACCCGUGGGU	1113-1135
3628	3629	AD-59104	CACAUCAUCCCUGUGCGGA	UCCGCACAGGGAUGAUGUG	1919-1941
3630	3631	AD-59105	CAGAAAGAGUGUCUCAUCU	AGAUGAGACACUCUUUCUG	873-895
3632	3633	AD-59106	CCUCCAUGAUCCAAGGGAU	AUCCCUUGGAUCAUGGAGG	1356-1378
3634	3635	AD-59107	UGCCCAUUCUUAUCCCGAA	UUCGGGAUAAGAAUGGGCA	359-381
3636	3637	AD-59108	CUUCACCCUGGCUAAGAUA	UAUCUUAGCCAGGGUGAAG	1297-1319
3638	3639	AD-59109	AUCAUCCCUGUGCGGGUUA	UAACCCGCACAGGGAUGAU	1922-1944
3640	3641	AD-59110	AGAAAGAGUGUCUCAUCUU	AAGAUGAGACACUCUUUCU	874-896
3642	3643	AD-59111	CUCCAUGAUCCAAGGGAUU	AAUCCCUUGGAUCAUGGAG	1357-1379
3644	3645	AD-59112	CCAUUCUUAUCCCGAGUCA	UGACUCGGGAUAAGAAUGG	362-384
3646	3647	AD-59113	CACCCUGGCUAAGAUGAUA	UAUCAUCUUAGCCAGGGUG	1300-1322
3648	3649	AD-59114	UCAUCCCUGUGCGGGUUGA	UCAACCCGCACAGGGAUGA	1923-1945
3650	3651	AD-59115	AAGAGUGUCUCAUCUUCUU	AAGAAGAUGAGACACUCUU	877-899
3652	3653	AD-59116	GUCAUGCCAAAAAUGGACA	UGUCCAUUUUUGGCAUGAC	1631-1653
3654	3655	AD-59117	CAUUCUUAUCCCGAGUCCA	UGGACUCGGGAUAAGAAUG	363-385
3656	3657	AD-59118	ACCCUGGCUAAGAUGAUGA	UCAUCAUCUUAGCCAGGGU	1301-1323
3658	3659	AD-59119	CUCUUCACCCUGGCUAAGA	UCUUAGCCAGGGUGAAGAG	1295-1317
3660	3661	AD-59120	AUGCCAAAAAUGGACAUCA	UGAUGUCCAUUUUUGGCAU	1634-1656
3662	3663	AD-59121	UGCCCCAAGAUGAUGGAAU	AUUCCAUCAUCUUGGGGCA	434-456
3664	3665	AD-59122	GAACCAUGCCUCCAUGAUA	UAUCAUGGAGGCAUGGUUC	1348-1370
3666	3667	AD-59123	UCUUCACCCUGGCUAAGAU	AUCUUAGCCAGGGUGAAGA	1296-1318
3668	3669	AD-59124	UGCCAAAAAUGGACAUCAU	AUGAUGUCCAUUUUUGGCA	1635-1657
3670	3671	AD-59125	CCAGAAAGAGUGUCUCAUA	UAUGAGACACUCUUUCUGG	872-894

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisens e)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_ 000688.4
3672	3673	AD-59126	GAAACUGUCCAUUCAAUGA	UCAUUGAAUGGACAGUUUC	1481-1503
3674	3675	AD-59127	UCACCCUGGCUAAGAUGAU	AUCAUCUUAGCCAGGGUGA	1299-1321
3676	3677	AD-59128	CCCUGGAGUCUGUGCGGAU	AUCCGCACAGACUCCAGGG	1791-1813
3678	3679	AD-59129	GAAAGAGUGUCUCAUCUUA	UAAGAUGAGACACUCUUUC	875-897
3680	3681	AD-59130	UGGAGCCCUGGAGUCUGUA	UACAGACUCCAGGGCUCCA	1786-1808

[0631] The results of the *in vitro* assays are provided in Table 16. Table 16 also notes the target species of each of the siRNAs.

Table 16: Results of Functional Assays

			Cyno Free Uptake				Cyno Transfection		Hep3b Transfection	
Duplex ID	Target Species	Type	1uM Avg	500nM	20nM Avg	10nM	20nM Avg	0.2nM Avg	10nM Avg	0.1nM Avg
AD-58848	M/R/Rh/H	21/23	131.6	176.0	104.4	128.0	43.5	44.8	25.3	76.8
AD-58849	H/Rh	21/23	91.9	88.1	92.2	105.0	29.4	35.4	11.5	47.1
AD-58850	H/Rh	21/23	79.4	103.4	80.0	111.2	NA	62.2	31.3	72.0
AD-58851	H/Rh	21/23	99.7	74.7	94.8	104.7	NA	40.7	8.6	81.3
AD-58852	H/Rh	21/23	108.1	91.8	103.3	111.9	101.1	128.8	43.4	129.0
AD-58853	H/Rh	21/23	74.8	67.7	84.2	93.5	24.7	52.9	14.1	61.2
AD-58854	H/Rh	21/23	145.9	124.1	106.6	115.3	119.0	83.9	85.0	84.0
AD-58855	H/Rh	21/23	81.5	97.9	92.7	101.8	39.5	40.3	15.3	67.6
AD-58856	H/Rh	21/23	74.1	90.6	84.6	82.6	22.4	30.7	8.7	33.3
AD-58857	H/Rh	21/23	64.7	91.4	62.3	87.1	22.0	31.6	9.8	106.3
AD-58858	H/Rh	21/23	67.4	91.7	68.6	98.3	27.9	40.3	17.4	44.8
AD-58859	H/Rh	21/23	71.2	77.2	92.4	90.1	19.1	34.3	13.1	39.7
AD-58861	H/Rh	21/23	104.6	107.2	102.0	100.6	25.9	35.1	18.0	69.8
AD-58862	H/Rh	21/23	66.8	77.0	68.7	88.5	20.3	31.1	24.2	49.9
AD-58863	H/Rh	21/23	70.8	66.8	76.8	98.5	21.5	29.7	8.7	54.9
AD-58864	H/Rh	21/23	76.2	85.6	83.7	100.8	60.4	61.0	56.4	87.3
AD-58865	H/Rh	21/23	67.9	77.9	95.9	98.4	21.3	38.6	15.5	81.4
AD-58867	H/Rh	21/23	95.9	93.3	107.0	97.5	32.3	42.7	16.6	79.8
AD-58868	H/Rh	21/23	95.2	92.1	116.2	94.7	54.6	69.2	61.5	105.9
AD-58869	H/Rh	21/23	65.0	78.2	75.8	88.2	17.4	25.0	13.0	63.9
AD-58870	H/Rh	21/23	69.4	92.3	81.0	88.1	29.2	43.8	33.7	79.1
AD-58871	H/Rh	21/23	61.2	77.3	88.2	77.0	71.2	73.2	36.7	110.3
AD-58873	H/Rh	21/23	95.2	100.9	83.3	94.6	54.2	52.8	36.6	73.3
AD-58874	H/Rh	21/23	75.8	76.8	63.8	85.3	22.3	31.2	15.0	38.2
AD-58875	H/Rh	21/23	80.7	88.7	78.6	97.9	48.6	73.6	61.2	90.6
AD-58876	H/Rh	21/23	90.8	93.1	82.5	100.2	41.1	56.9	21.2	58.7
AD-58877	H/Rh	21/23	68.3	85.1	51.2	78.7	18.5	46.6	11.9	27.4
AD-58878	H/Rh	21/23	78.3	68.3	81.2	91.2	24.1	23.4	6.2	37.1
AD-58879	H/Rh	21/23	87.9	94.1	79.7	95.4	32.0	47.8	15.7	82.5
AD-58880	H/Rh	21/23	74.9	72.2	88.9	88.1	20.1	27.5	14.0	60.7
AD-58881	H/Rh	21/23	85.9	76.8	78.8	118.0	22.2	36.7	27.6	71.6
AD-58882	H/Rh	21/23	54.1	53.4	60.3	85.8	14.6	27.2	8.2	23.8
AD-58883	H/Rh	21/23	80.4	69.9	75.7	80.3	31.8	25.8	12.3	63.0
AD-58884	H/Rh	21/23	57.7	55.3	64.8	78.2	20.0	30.0	11.8	68.9

(continued)

			Cyno Free Uptake				Cyno Transfection		Hep3b Transfection	
Duplex ID	Target Species	Type	1uM Avg	500nM	20nM Avg	10nM	20nM Avg	0.2nM Avg	10nM Avg	0.1nM Avg
AD-58885	H/Rh	21/23	101.8	91.8	104.1	101.5	85.9	71.9	61.8	71.2
AD-58886	M/R/Rh/H	21/23	47.1	58.0	36.3	93.3	16.0	26.6	9.2	32.0
AD-58887	H/Rh	21/23	73.6	98.7	82.6	95.2	28.5	33.5	12.8	65.2
AD-58888	H/Rh	21/23	90.2	69.9	69.4	85.6	46.9	45.0	16.6	72.0
AD-58889	H/Rh	21/23	83.6	98.6	82.4	92.2	36.5	40.3	31.6	99.4
AD-58890	H/Rh	21/23	69.5	95.4	84.2	88.2	50.8	45.6	21.7	92.9
AD-58891	H/Rh	21/23	62.8	75.7	75.4	109.2	23.6	34.3	15.6	55.8
AD-58892	H/Rh	21/23	60.2	92.9	89.8	92.9	22.8	43.3	20.2	75.6
AD-59095	M/R/Rh/H	19mer	88.9	NA	132.8	NA	48.3	97.4	54.3	99.0
AD-59096	M/R/Rh/H	19mer	95.5	NA	90.5	NA	105.7	138.6	131.4	120.7
AD-59097	M/R/Rh/H	19mer	92.5	NA	84.2	NA	75.0	NA	94.7	108.5
AD-59098	M/R/Rh/H	19mer	84.0	NA	87.7	NA	109.3	NA	130.0	87.3
AD-59099	M/R/Rh/H	19mer	89.7	NA	90.0	NA	77.8	85.4	46.8	74.9
AD-59100	M/R/Rh/H	19mer	84.8	NA	144.3	NA	70.6	108.1	91.5	117.6
AD-59101	M/R/Rh/H	19mer	79.0	NA	103.8	NA	89.8	102.9	124.2	107.0
AD-59102	M/R/Rh/H	19mer	85.9	NA	100.6	NA	72.2	68.5	87.9	95.1
AD-59103	M/R/Rh/H	19mer	86.0	NA	91.1	NA	93.0	81.3	130.0	96.0
AD-59104	M/R/Rh/H	19mer	92.6	NA	96.9	NA	94.9	91.4	124.4	83.1
AD-59105	M/R/Rh/H	19mer	48.9	NA	101.7	NA	18.4	48.9	17.0	34.7
AD-59106	M/R/Rh/H	19mer	63.2	NA	76.7	NA	28.5	40.7	28.6	46.4
AD-59107	M/R/Rh/H	19mer	71.4	NA	68.7	NA	37.1	45.3	26.8	63.6
AD-59108	M/R/Rh/H	19mer	70.7	NA	85.1	NA	89.9	84.8	139.2	101.7
AD-59109	M/R/Rh/H	19mer	86.1	NA	83.4	NA	84.9	96.2	131.7	86.7
AD-59110	M/R/Rh/H	19mer	70.8	NA	119.7	NA	38.5	60.4	67.4	80.3
AD-59111	M/R/Rh/H	19mer	66.1	NA	76.5	NA	52.2	61.0	69.7	87.6
AD-59112	M/R/Rh/H	19mer	71.2	NA	80.2	NA	91.2	83.4	127.4	89.0
AD-59113	M/R/Rh/H	19mer	67.0	NA	77.8	NA	49.1	59.0	66.8	91.4
AD-59114	M/R/Rh/H	19mer	81.7	NA	79.3	NA	96.3	88.0	129.6	72.4
AD-59115	M/R/Rh/H	19mer	40.4	NA	69.6	NA	19.6	35.7	9.3	16.9
AD-59116	M/R/Rh/H	19mer	72.2	NA	78.3	NA	53.5	77.8	70.1	107.8
AD-59117	M/R/Rh/H	19mer	70.7	NA	75.6	NA	75.8	74.9	129.0	103.5
AD-59118	M/R/Rh/H	19mer	68.8	NA	75.9	NA	81.4	82.1	114.1	89.7
AD-59119	M/R/Rh/H	19mer	64.9	NA	86.5	NA	85.1	125.1	122.8	124.8
AD-59120	M/R/Rh/H	19mer	63.5	NA	75.1	NA	29.9	52.0	16.1	54.1
AD-59121	M/R/Rh/H	19mer	67.6	NA	72.0	NA	88.8	77.4	108.0	103.1

(continued)

			Cyno Free Uptake				Cyno Transfection		Hep3b Transfection	
Duplex ID	Target Species	Type	1uM Avg	500nM	20nM Avg	10nM	20nM Avg	0.2nM Avg	10nM Avg	0.1nM Avg
AD-59122	M/R/Rh/H	19mer	60.2	NA	62.3	NA	25.1	45.3	16.2	54.8
AD-59123	M/R/Rh/H	19mer	68.6	NA	108.2	NA	59.2	84.6	80.0	97.7
AD-59124	M/R/Rh/H	19mer	47.5	NA	56.5	NA	23.9	40.0	9.8	18.9
AD-59125	M/R/Rh/H	19mer	45.4	NA	47.2	NA	15.2	40.7	14.7	15.1
AD-59126	M/R/Rh/H	19mer	64.3	NA	74.6	NA	51.6	57.1	35.5	54.4
AD-59127	M/R/Rh/H	19mer	103.4	NA	105.8	NA	94.0	156.4	135.9	113.7
AD-59128	M/R/Rh/H	19mer	102.4	NA	81.4	NA	66.3	89.3	60.2	74.9
AD-59129	M/R/Rh/H	19mer	41.3	NA	38.8	NA	17.9	41.4	8.6	12.6
AD-59130	M/R/Rh/H	19mer	58.3	NA	80.8	NA	94.9	78.3	106.7	88.0

[0632] Table 17 illustrates the IC_{50}s of select ALAS 1 siRNA duplexes. The IC_{50}s were determined from the knockdown of endogenously expressed ALAS1 in the Hep3B cell line, at 24 hours following transfection of each ALAS1 modified siRNA duplex (see Table 14). At least seven duplexes, including AD-58882, AD-58878, AD-58886, AD-58877, AD-59115, AD-58856, and AD-59129, consistently demonstrated IC_{50}s of less than 0.1 nm, indicating that these duplexes were particularly effective in suppressing ALAS1 expression.

Table 17: IC_{50}s of select ALAS1 siRNA duplexes

Duplex ID	384w IC50 (nM)	96w IC50 (nM)
AD-58882	0.008	0.014
AD-58878	0.040	0.031
AD-58886	0.037	0.033
AD-58877	0.031	0.034
AD-59115	0.093	0.052
AD-58856	0.061	0.066
AD-59129	0.085	0.071
AD-59124	0.572	0.078
AD-58874	0.140	0.102
AD-59125	0.118	0.115
AD-59105	0.511	0.144
AD-59120	180.592	0.498
AD-59122	36.646	0.646
AD-59106	7.906	0.847
AD-59126	n/a	1.014
AD-59107	n/a	1.971

Example 11. ALAS1-GalNAc activity in AIP Phenobarbital induction mouse model

[0633] The AIP mouse model was used to investigate the effect of an siRNA that was an ALAS 1-GalNAc conjugate. The siRNA had the sequence of duplex AD-58632 (see Table 20).

Table 20: Sequences of ALAS1 siRNA Duplex AD-58632

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Target sites of antisense sequence	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')
4149	4150	877-899	AD-58632	GfsasAfaGfaGfuGfUfCfuCfaUfcUfuCfuUfL96	asAfsgAfaGfaUfgAfgacAfcUfcUfuUfcsusg

[0634] AIP mice were untreated (baseline), or injected subcutaneously on day 1 with saline or the ALAS1-GalNAc conjugate at a dose of 20mg/kg. On Days 2, 3, and 4 they were left untreated (baseline) or they were treated with IP injections of Phenobarbital. On Day 5 plasma was taken and levels of ALA and PBG were measured using an LC-MS assay. As shown in FIG. 15, the ALAS1-GalNAc conjugate blunted the production of plasma ALA and PBG by about 84 and 80% respectively. These results indicate that treatment with an ALAS1-GalNAc conjugate was effective in preventing increases in both plasma ALA and PBG associated with phenobarbital-induced acute attacks in this AIP animal model.

Example 12. Further siRNAs that Target ALAS1 and Inhibit ALAS1 Expression

[0635] Modified siRNA sequences that target ALAS 1 siRNA were designed and produced as described in Example 2. The sequences are provided in Table 18. The *in vitro* activity of the modified duplexes was tested as described below.

Table 18: Human ALAS1 Modified Single Strands and Duplex Sequences

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_000688.4
3685	3686	AD-59453	CAGGCAAAUCUCUGUUGUUdTdT	AACAACAGAGAUUUGCCUGdTdT	402-420
3687	3688	AD-59395	GAAAAAAAUUGAUGAGAAAdTdT	UUUCUCAUCAAUUUUUUUCdTdT	949-967
3689	3690	AD-59477	GGAAAGAUGCCGCACUCUUdTdT	AAGAGUGCGGCAUCUUUCCdTdT	1242-1260
3691	3692	AD-59492	UGUCUCAUCUUCUUCAAGAdTdT	UCUUGAAGAAGAUGAGACAdTdT	882-900
3693	3694	AD-59361	ACAUCUACGUGCAAGCAAUdTdT	AUUGCUUGCACGUAGAUGUdTdT	1992-2010
3695	3696	AD-59462	UUCUCUGAUUGACACCGUAdTdT	UACGGUGUCAAUCAGAGAAdTdT	1711-1729
3697	3698	AD-59433	GCUGCUGGCUUCAUCUUCAdTdT	UGAAGAUGAAGCCAGCAGCdTdT	1739-1757
3699	3700	AD-59424	AGCGCAACGUCAAACUCAUdTdT	AUGAGUUUGACGUUGCGCUdTdT	1851-1869
3701	3702	AD-59414	UAUUUCUGGAACUAGUAAAdTdT	UUUACUAGUUCCAGAAAUAdTdT	1183-1201
3703	3704	AD-59539	GGUUGUGUUGGAGGGUACAdTdT	UGUACCCUCCAACACAACCdTdT	1679-1697
3705	3706	AD-59400	GUGUCAGUCUGGUGCAGUAdTdT	UACUGCACCAGACUGACACdTdT	1070-1088
3707	3708	AD-59551	CUUUGUGGCCAAUGACUCAdTdT	UGAGUCAUUGGCCACAAAGdTdT	1273-1291
3709	3710	AD-59482	AGAUGCUGCUAAAAACACAdTdT	UGUGUUUUUAGCAGCAUCUdTdT	1942-1960
3711	3712	AD-59448	GAGUCAUGCCAAAAAUGGAdTdT	UCCAUUUUUGGCAUGACUCdTdT	1629-1647
3713	3714	AD-59392	CUGUGCGGAUCCUGAAGAGdTdT	CUCUUCAGGAUCCGCACAGdTdT	1800-1818
3715	3716	AD-59469	CACUUUGAAACAACAUGGUdTdT	ACCAUGUUGUUUCAAAGUGdTdT	1141-1159
3717	3718	AD-59431	AAGUGAUGAGUGAAAGAGAdTdT	UCUCUUUCACUCAUCACUUdTdT	2193-2211
3719	3720	AD-59423	AUCUGCUAGUCACAUGGAAdTdT	UUCCAUGUGACUAGCAGAUdTdT	2103-2121
3721	3722	AD-59517	UGGGGCAGGUGGUACUAGAdTdT	UCUAGUACCACCUGCCCCAdTdT	1162-1180
3723	3724	AD-59578	GCAGAUGACUAUUCAGACUdTdT	AGUCUGAAUAGUCAUCUGCdTdT	1031-1049
3725	3726	AD-59495	GCCUCAUUCCUCAGCUGAGdTdT	CUCAGCUGAGGAAUGAGGCdTdT	2143-2161
3727	3728	AD-59432	GUAUGAUCGUUUCUUUGAGdTdT	CUCAAAGAAACGAUCAUACdTdT	931-949
3729	3730	AD-59382	UAUCCAGAUGGUCUUCAGAdTdT	UCUGAAGACCAUCUGGAUAdTdT	2302-2320
3731	3732	AD-59472	UAGUGUGAAAACCGAUGGAdTdT	UCCAUCGGUUUUCACACUAdTdT	799-817
3733	3734	AD-59459	UCCCCAUGGCAGAUGACUAdTdT	UAGUCAUCUGCCAUGGGGAdTdT	1023-1041

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_000688.4
3735	3736	AD-59413	CCACUGCAGCAGUACACUAdTdT	UAGUGUACUGCUGCAGUGGdTdT	483-501
3737	3738	AD-59478	CUGUGAACCGGCGAGCACAdTdT	UGUGCUCGCCGGUUCACAGdTdT	999-1017
3739	3740	AD-59376	GGUCCUAUGCUGCUGGCUUdTdT	AAGCCAGCAGCAUAGGACCdTdT	1731-1749
3741	3742	AD-59556	AGCCUUUGGUUGUGUUGGAdTdT	UCCAACACAACCAAAGGCUdTdT	1672-1690
3743	3744	AD-59399	AAUUCCAUGUGGACUUAGAdTdT	UCUAAGUCCACAUGGAAUUdTdT	1200-1218
3745	3746	AD-59474	CCAGGGCACUGCAAGCAAAdTdT	UUUGCUUGCAGUGCCCUGGdTdT	640-658
3747	3748	AD-53542	cuuuucAGuAuGAucGuuudTsdT	AAACGAUcAuACUGAAAAGdTsdT	924-942
3749	3750	AD-59480	GAAUCAGAGAGGCAGCAGUdTdT	ACUGCUGCCUCUCUGAUUCdTdT	682-700
3751	3752	AD-59549	GCAAAGAUCUGACCCCUCAdTdT	UGAGGGGUCAGAUCUUUGCdTdT	1441-1459
3753	3754	AD-59515	GGAGAAGAGCUCCUACGGAdTdT	UCCGUAGGAGCUCUUCUCCdTdT	2033-2051
3755	3756	AD-59427	CCAUGAGUUUGGAGCAAUCdTdT	GAUUGCUCCAAACUCAUGGdTdT	1540-1558
3757	3758	AD-59390	CUUUGAGAAAAAAAUUGAUdTdT	AUCAAUUUUUUUCUCAAAGdTdT	943-961
3759	3760	AD-59511	UGAGCAGACAUAACAUCUAdTdT	UAGAUGUUAUGUCUGCUCAdTdT	1980-1998
3761	3762	AD-59532	CGUGCAAGCAAUCAAUUACdTdT	GUAAUUGAUUGCUUGCACGdTdT	1999-2017
3763	3764	AD-59562	AAAGCAAAGACCAGAAAGAdTdT	UCUUUCUGGUCUUUGCUUUdTdT	862-880
3765	3766	AD-59513	GGAUGUGCAGGAAAUGAAUdTdT	AUUCAUUUCCUGCACAUCCdTdT	733-751
3767	3768	AD-59362	CAGCAUACUUCCUGAACAUdTdT	AUGUUCAGGAAGUAUGCUGdTdT	321-339
3769	3770	AD-53541	GcAGcAcAGAuGAAucAGAdTsdT	UCUGAUUcAUCUGUGCUGCdTsdT	671-689
3771	3772	AD-59490	UCUGUUGUUCUAUGCCCAAdTdT	UUGGGCAUAGAACAACAGAdTdT	412-430
3773	3774	AD-59422	UGAGACAGAUGCUAAUGGAdTdT	UCCAUUAGCAUCUGUCUCAdTdT	1869-1887
3775	3776	AD-59467	GCCAAUGACUCAACCCUCUdTdT	AGAGGGUUGAGUCAUUGGCdTdT	1280-1298
3777	3778	AD-59579	GAGUGCAACUUCUGCAGGAdTdT	UCCUGCAGAAGUUGCACUCdTdT	2159-2177
3779	3780	AD-59426	GUGAAAGAGAGAAGUCCUAdTdT	UAGGACUUCUCUCUUUCACdTdT	2202-2220
3781	3782	AD-59363	UAACUUGCCAAAAUCUGUUdTdT	AACAGAUUUUGGCAAGUUAdTdT	901-919

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_000688.4
3783	3784	AD-59436	AAGCCAGUCUUGAGCUUCAdTdT	UGAAGCUCAAGACUGGCUUdTdT	711-729
3785	3786	AD-53536	cAcuuuucAGuAuGAucGudTsdT	ACGAUcAuACUGAAAAGUGdTsdT	922-940
3787	3788	AD-59491	GCAGCAGUGUCUUCUGCAAdTdT	UUGCAGAAGACACUGCUGCdTdT	693-711
3789	3790	AD-59500	UCCUGAACAUGGAGAGUGUdTdT	ACACUCUCCAUGUUCAGGAdTdT	330-348
3791	3792	AD-59394	AUUUCUGGAACACUUGGCAdTdT	UGCCAAGUGUUCCAGAAAUdTdT	1652-1670
3793	3794	AD-59441	CAGUACACUACCAACAGAUdTdT	AUCUGUUGGUAGUGUACUGdTdT	492-510
3795	3796	AD-59365	GCAUGACCUCAAUUAUUUCdTdT	GAAAUAAUUGAGGUCAUGCdTdT	2261-2279
3797	3798	AD-59411	AGAACUGCUGCAAAGAUCUdTdT	AGAUCUUUGCAGCAGUUCUdTdT	1432-1450
3799	3800	AD-59544	CACCCCAGAUGAUGAACUAdTdT	UAGUUCAUCAUCUGGGGUGdTdT	2073-2091
3801	3802	AD-59428	GAUCCAAGGGAUUCGAAACdTdT	GUUUCGAAUCCCUUGGAUCdTdT	1363-1381
3803	3804	AD-59471	CUCAUCACCAAAAAGCAAGdTdT	CUUGCUUUUUGGUGAUGAGdTdT	1052-1070
3805	3806	AD-59518	ACAACAUGGUGCUGGGGCAdTdT	UGCCCCAGCACCAUGUUGUdTdT	1150-1168
3807	3808	AD-53547	GAucGuuucuuuGAGAAAAdTsdT	UUUUCUcAAAGAAACGAUCdTsdT	935-953
3809	3810	AD-59573	CAGCACGAGUUCUCUGAUUdTdT	AAUCAGAGAACUCGUGCUGdTdT	1702-1720
3811	3812	AD-59473	AAUGAUGUCAGCCACCUCAdTdT	UGAGGUGGCUGACAUCAUUdTdT	1412-1430
3813	3814	AD-59412	AGUUAUGGACACUUUGAAAdTdT	UUUCAAAGUGUCCAUAACUdTdT	1132-1150
3815	3816	AD-59522	GAUGAUGAACUACUUCCUUdTdT	AAGGAAGUAGUUCAUCAUCdTdT	2080-2098
3817	3818	AD-59502	GCAGGAAAUGAAUGCCGUGdTdT	CACGGCAUUCAUUUCCUGCdTdT	739-757
3819	3820	AD-59499	UCUUCAAGAUAACUUGCCAdTdT	UGGCAAGUUAUCUUGAAGAdTdT	892-910
3821	3822	AD-59520	CGAUGGAGGGGAUCCCAGUdTdT	ACUGGGAUCCCCUCCAUCGdTdT	811-829
3823	3824	AD-59581	CCAAAAAGCAAGUGUCAGUdTdT	ACUGACACUUGCUUUUUGGdTdT	1059-1077
3825	3826	AD-59461	GAUUGGGGAUCGGGAUGGAdTdT	UCCAUCCCGAUCCCCAAUCdTdT	1612-1630
3827	3828	AD-59370	CCCUGGAGUCUGUGCGGAUdTdT	AUCCGCACAGACUCCAGGGdTdT	1791-1809
3829	3830	AD-53540	GuuGucuuuAuAuGuGAAudTsdT	AUUcAcAuAuAAAGAcAACdTsdT	2321-2339

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_000688.4
3831	3832	AD-59574	CGGGCAUUGUCCACUGCAGdTdT	CUGCAGUGGACAAUGCCCGdTdT	473-491
3833	3834	AD-59375	UAUUCAGACUCCCUCAUCAdTdT	UGAUGAGGGAGUCUGAAUAdTdT	1040-1058
3835	3836	AD-59387	CACUGCAUUUUGAAGUGAUdTdT	AUCACUUCAAAAUGCAGUGdTdT	2181-2199
3837	3838	AD-59397	CCAGAAAGAGUGUCUCAUCdTdT	GAUGAGACACUCUUUCUGGdTdT	872-890
3839	3840	AD-59396	AGGCGGAGGGAUUGGGGAUdTdT	AUCCCCAAUCCCUCCGCCUdTdT	1603-1621
3841	3842	AD-59393	AGACCUCCAUGGGAAAGAUdTdT	AUCUUUCCCAUGGAGGUCUdTdT	1231-1249
3843	3844	AD-59483	GCAGGAGGCCACUGCAUUUdTdT	AAAUGCAGUGGCCUCCUGCdTdT	2172-2190
3845	3846	AD-59430	AUCUGUUUCCACUUUUCAGdTdT	CUGAAAAGUGGAAACAGAUdTdT	913-931
3847	3848	AD-59463	AGAGAAGUCCUAUUUCUCAdTdT	UGAGAAAUAGGACUUCUCUdTdT	2209-2227
3849	3850	AD-53534	GucuucAGAGuuGucuuuAdTsdT	uAAAGAcAACUCUGAAGACdTsdT	2312-2330
3851	3852	AD-59514	GGCUGGAACUGAAGCCUCAdTdT	UGAGGCUUCAGUUCCAGCCdTdT	2130-2148
3853	3854	AD-59575	GCCAUUAUCAUAUCCAGAUdTdT	AUCUGGAUAUGAUAAUGGCdTdT	2292-2310
3855	3856	AD-59364	AGCAGGCCCCAGUGUGGUUdTdT	AACCACACUGGGGCCUGCUdTdT	781-799
3857	3858	AD-59402	UCAGCUGAGUGCAACUUCUdTdT	AGAAGUUGCACUCAGCUGAdTdT	2153-2171
3859	3860	AD-59479	GAGCACACAUCUUCCCCAUdTdT	AUGGGGAAGAUGUGUGCUCdTdT	1011-1029
3861	3862	AD-59481	ACUUCCAGGACAUCAUGCAdTdT	UGCAUGAUGUCCUGGAAGUdTdT	843-861
3863	3864	AD-59530	CCUAUCGAGUUUUUAAAACdTdT	GUUUUAAAAACUCGAUAGGdTdT	981-999
3865	3866	AD-59582	CUUCCUUGAGAAUCUGCUAdTdT	UAGCAGAUUCUCAAGGAAGdTdT	2092-2110
3867	3868	AD-59506	ACCAACAGAUCAAAGAAACdTdT	GUUUCUUUGAUCUGUUGGUdTdT	501-519
3869	3870	AD-59567	UAACCCCAGGCCAU UAUCAdTdT	UGAUAAUGGCCUGGGGUUAdTdT	2283-2301
3871	3872	AD-59485	CCAUGCCUCCAUGAUCCAAdTdT	UUGGAUCAUGGAGGCAUGGdTdT	1351-1369
3873	3874	AD-59525	UGAUGAACUAAUGAGCAGAdTdT	UCUGCUCAUUAGUUCAUCAdTdT	1969-1987
3875	3876	AD-59566	CCUGAAGAGCGCUGAGGGAdTdT	UCCCUCAGCGCUCUUCAGGdTdT	1810-1828
3877	3878	AD-59580	AACACUUGGCAAAGCCUUUdTdT	AAAGGCUUUGCCAAGUGUUdTdT	1660-1678

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_000688.4
3879	3880	AD-59512	UCUGCAGAAAGCAGGCAAAdTdT	UUUGCCUGCUUUCUGCAGAdTdT	391-409
3881	3882	AD-59475	CCGGCCUCCCUGUUGUCCAdTdT	UGGACAACAGGGAGGCCGGdTdT	1890-1908
3883	3884	AD-59438	CAUCAUCCCUGUGCGGGUUdTdT	AACCCGCACAGGGAUGAUGdTdT	1921-1939
3885	3886	AD-59442	UGUGCGGGUUGCAGAUGCUdTdT	AGCAUCUGCAACCCGCACAdTdT	1930-1948
3887	3888	AD-59516	GGAAAGAGGUUGCUGAAACdTdT	GUUUCAGCAACCUCUUUCCdTdT	759-777
3889	3890	AD-59429	AGGUCCACGCAGUGGGGCUdTdT	AGCCCCACUGCGUGGACCUdTdT	1572-1590
3891	3892	AD-59510	UGCCGUGAGGAAAGAGGUUdTdT	AACCUCUUUCCUCACGGCAdTdT	751-769
3893	3894	AD-59457	GCUAAUGGAUGCCGGCCUCdTdT	GAGGCCGGCAUCCAUUAGCdTdT	1879-1897
3895	3896	AD-59434	GAAGCAAGUGGGGCUGGAAdTdT	UUCCAGCCCCACUUGCUUCdTdT	2119-2137
3897	3898	AD-59454	CAUCUUCCGCCACAAUGAUdTdT	AUCAUUGUGGCGGAAGAUGdTdT	1399-1417
3899	3900	AD-59468	AUUUCUCAGGCUUGAGCAAdTdT	UUGCUCAAGCCUGAGAAAUdTdT	2220-2238
3901	3902	AD-59565	CCCGAGUCCCCCAGGCCUUdTdT	AAGGCCUGGGGGACUCGGGdTdT	372-390
3903	3904	AD-59416	CAAGCAAAUGCCCUUUCCUdTdT	AGGAAAGGGCAUUUGCUUGdTdT	651-669
3905	3906	AD-59420	CCCCUCAGUCCCCAAGAUUdTdT	AAUCUUGGGGACUGAGGGGdTdT	1453-1471
3907	3908	AD-59552	CUACGGUGCCCCGGGGAGAdTdT	UCUCCCCGGGGCACCGUAGdTdT	2019-2037
3909	3910	AD-59558	AAAACUGCCCCAAGAUGAUdTdT	AUCAUCUUGGGGCAGUUUUdTdT	429-447
3911	3912	AD-59404	ACAAAACUGCUAAGGCCAAdTdT	UUGGCCUUAGCAGUUUUGUdTdT	540-558
3913	3914	AD-59455	GAUUCUGGGAACCAUGCCUdTdT	AGGCAUGGUUCCCAGAAUCdTdT	1340-1358
3915	3916	AD-59496	CCAGAUGGCACACAGCUUCdTdT	GAAGCUGUGUGCCAUCUGGdTdT	593-611
3917	3918	AD-59446	AGGGAUUCGAAACAGCCGAdTdT	UCGGCUGUUUCGAAUCCCUdTdT	1369-1387
3919	3920	AD-59435	CUCUGCAGUCCUCAGCGCAdTdT	UGCGCUGAGGACUGCAGAGdTdT	109-127
3921	3922	AD-59419	CCGCCGCCUCUGCAGUCCUdTdT	AGGACUGCAGAGGCGGCGGdTdT	102-120
3923	3924	AD-59533	CUGGCUGGAGCCCUGGAGUdTdT	ACUCCAGGGCUCCAGCCAGdTdT	1781-1799
3925	3926	AD-59366	GACAUCAUGCAAAAGCAAAdTdT	UUUGCUUUUGCAUGAUGUCdTdT	851-869

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_000688.4
3927	3928	AD-59521	GCUUGAGCAAGUUGGUAUCdTdT	GAUACCAACUUGCUCAAGCdTdT	2229-2247
3929	3930	AD-59563	CAGGCUGUGAGAUUUACUCdTdT	GAGUAAAUCUCACAGCCUGdTdT	1320-1338
3931	3932	AD-59534	AGAGCUGUGUGAUGUGGCCdTdT	GGCCACAUCACACAGCUCUdTdT	1522-1540
3933	3934	AD-59407	GGAGCUGGCAGACCUCCAUdTdT	AUGGAGGUCUGCCAGCUCCdTdT	1222-1240
3935	3936	AD-59445	AUCCCAGUGGACUGCUGAAdTdT	UUCAGCAGUCCACUGGGAUdTdT	822-840
3937	3938	AD-59546	GUCAAACUCAUGAGACAGAdTdT	UCUGUCUCAUGAGUUUGACdTdT	1859-1877
3939	3940	AD-59456	CUUUCCUGGCAGCACAGAUdTdT	AUCUGUGCUGCCAGGAAAGdTdT	663-681
3941	3942	AD-59503	CCCUCCGGCCAGUGAGAAAdTdT	UUUCUCACUGGCCGGAGGGdTdT	520-538
3943	3944	AD-59536	CUACCUAGGAAUGAGUCGCdTdT	GCGACUCAUUCCUAGGUAGdTdT	1093-1111
3945	3946	AD-59385	CCCAAGAUUGUGGCAUUUGdTdT	CAAAUGCCACAAUCUUGGGdTdT	1463-1481
3947	3948	AD-59367	GAGCAAUCACCUUCGUGGAdTdT	UCCACGAAGGUGAUUGCUCdTdT	1551-1569
3949	3950	AD-59458	UGCCCAUUCUUAUCCCGAGdTdT	CUCGGGAUAAGAAUGGGCAdTdT	359-377
3951	3952	AD-59381	AAGGCCAAGGUCCAACAGAdTdT	UCUGUUGGACCUUGGCCUUdTdT	551-569
3953	3954	AD-59538	CACACAGCUUCCGUCUGGAdTdT	UCCAGACGGAAGCUGUGUGdTdT	601-619
3955	3956	AD-59421	UUAUGGGGCUCGAGGCGGAdTdT	UCCGCCUCGAGCCCCAUAAdTdT	1591-1609
3957	3958	AD-59388	UGUCUUCUGCAAAGCCAGUdTdT	ACUGGCUUUGCAGAAGACAdTdT	700-718
3959	3960	AD-59444	AGGCCUGAGCAUGACCUCAdTdT	UGAGGUCAUGCUCAGGCCUdTdT	2253-2271
3961	3962	AD-59528	AUGUGAAUUAAGUUAUAUUdTdT	AAUAUAACUUAAUUCACAUdTdT	2332-2350
3963	3964	AD-59498	ACUGCUGAAGAACUUCCAGdTdT	CUGGAAGUUCUUCAGCAGUdTdT	832-850
3965	3966	AD-59497	UGAGAAAGACAAAACUGCUdTdT	AGCAGUUUUGUCUUUCUCAdTdT	532-550
3967	3968	AD-59384	UCAGCCACCUCAGAGAACUdTdT	AGUUCUCUGAGGUGGCUGAdTdT	1419-1437
3969	3970	AD-59452	GGCAACGAGCGUUUCGUUUdTdT	AAACGAAACGCUCGUUGCCdTdT	51-69
3971	3972	AD-59379	CCUGAUGGAUCCCAGCAGAdTdT	UCUGCUGGGAUCCAUCAGGdTdT	572-590
3973	3974	AD-59529	UGUGCCCACUGGAAGAGCUdTdT	AGCUCUUCCAGUGGGCACAdTdT	1509-1527

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_000688.4
3975	3976	AD-59389	CCACAGGAGCCAGCAUACUdTdT	AGUAUGCUGGCUCCUGUGGdTdT	311-329
3977	3978	AD-59585	GUGGUACUAGAAAUAUUUCdTdT	GAAAUAUUUCUAGUACCACdTdT	1170-1188
3979	3980	AD-59570	UUCGCCGCUGCCCAUUCUUdTdT	AAGAAUGGGCAGCGGCGAAdTdT	351-369
3981	3982	AD-59415	CCGCCAGCACCAGCGCAACdTdT	GUUGCGCUGGUGCUGGCGGdTdT	1840-1858
3983	3984	AD-59505	CGCUGAGGGACGGGUGCUUdTdT	AAGCACCCGUCCCUCAGCGdTdT	1819-1837
3985	3986	AD-59557	UGGACUUCUCGACUUGAGUdTdT	ACUCAAGUCGAGAAGUCCAdTdT	69-87
3987	3988	AD-59548	AAAGAAACCCCUCCGGCCAdTdT	UGGCCGGAGGGGUUUCUUUdTdT	512-530
3989	3990	AD-59487	UUGACACCGUACGGUCCUAdTdT	UAGGACCGUACGGUGUCAAdTdT	1719-1737
3991	3992	AD-59550	CCCUCUUCACCCUGGCUAAdTdT	UUAGCCAGGGUGAAGAGGGdTdT	1293-1311
3993	3994	AD-59572	CCCCCAGGCCUUUCUGCAGdTdT	CUGCAGAAAGGCCUGGGGGdTdT	379-397
3995	3996	AD-59554	AUGCCCAAAACUGCCCCAAdTdT	UUGGGGCAGUUUUGGGCAUdTdT	423-441
3997	3998	AD-59437	CUUGAGUGCCCGCCUCCUUdTdT	AAGGAGGCGGGCACUCAAGdTdT	81-99
3999	4000	AD-59584	GGGUACAUCGCCAGCACGAdTdT	UCGUGCUGGCGAUGUACCCdTdT	1691-1709
4001	4002	AD-59373	GUGUGGGGCAGUUAUGGACdTdT	GUCCAUAACUGCCCCACACdTdT	1123-1141
4003	4004	AD-59545	ACAUAGUCCUGGAAAUAAAdTdT	UUUAUUUCCAGGACUAUGUdTdT	2372-2390
4005	4006	AD-59547	AUCCCAGCAGAGUCCAGAUdTdT	AUCUGGACUCUGCUGGGAUdTdT	580-598
4007	4008	AD-59470	CUAGAUUCUUUCCACAGGAdTdT	UCCUGUGGAAAGAAUCUAGdTdT	300-318
4009	4010	AD-59417	UUGUUUUCCUCGUGCUUUGdTdT	CAAAGCACGAGGAAAACAAdTdT	1259-1277
4011	4012	AD-59535	CCUCCUUCGCCGCCGCCUCdTdT	GAGGCGGCGGCGAAGGAGGdTdT	93-111
4013	4014	AD-59507	UGAGGCUGCUCCCGGACAAdTdT	UUGUCCGGGAGCAGCCUCAdTdT	31-49
4015	4016	AD-59519	CCAACAGACUCCUGAUGGAdTdT	UCCAUCAGGAGUCUGUUGGdTdT	562-580
4017	4018	AD-59391	UCACAUGGAAGCAAGUGGGdTdT	CCCACUUGCUUCCAUGUGAdTdT	2112-2130
4019	4020	AD-59537	CAUUCAAUGGAUGGGGCGGdTdT	CCGCCCCAUCCAUUGAAUGdTdT	1490-1508
4021	4022	AD-59450	AGGAAUGAGUCGCCACCCAdTdT	UGGGUGGCGACUCAUUCCUdTdT	1099-1117

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SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_000688.4
4023	4024	AD-59449	UGGACUUAGAGCGGGAGCUdTdT	AGCUCCCGCUCUAAGUCCAdTdT	1209-1227
4025	4026	AD-59418	CUAAAAACACAGAAGUCUGdTdT	CAGACUUCUGUGUUUUUAGdTdT	1950-1968
4027	4028	AD-59561	CCCUCACCACACACCCCAGdTdT	CUGGGGUGUGUGGUGAGGGdTdT	2062-2080
4029	4030	AD-59460	AAUCCUUGCUUCAGGGACUdTdT	AGUCCCUGAAGCAAGGAUUdTdT	171-189
4031	4032	AD-59409	UUGUGGCAUUUGAAACUGUdTdT	ACAGUUUCAAAUGCCACAAdTdT	1470-1488
4033	4034	AD-59476	UCAAUUACCCUACGGUGCCdTdT	GGCACCGUAGGGUAAUUGAdTdT	2010-2028
4035	4036	AD-59406	CAAGCCAGCCCCUCGGGCAdTdT	UGCCCGAGGGGCUGGCUUGdTdT	460-478
4037	4038	AD-59569	GAGUCUUCCCUGCCUGGAUdTdT	AUCCAGGCAGGGAAGACUCdTdT	259-277
4039	4040	AD-59451	UGGAGAGUGUUGUUCGCCGdTdT	CGGCGAACAACACUCUCCAdTdT	339-357
4041	4042	AD-59553	ACCCCUUGCCUGCCACAAGdTdT	CUUGUGGCAGGCAAGGGGUdTdT	621-639
4043	4044	AD-59372	CUGGAUGGAUGAGUGGCUUdTdT	AAGCCACUCAUCCAUCCAGdTdT	272-290
4045	4046	AD-59377	CAAGAUGAUGGAAGUUGGGdTdT	CCCAACUUCCAUCAUCUUGdTdT	439-457
4047	4048	AD-59531	UUUCGUUUGGACUUCUCGAdTdT	UCGAGAAGUCCAAACGAAAdTdT	62-80
4049	4050	AD-59560	UCAUCUUCACCACCUCUCUdTdT	AGAGAGGUGGUGAAGAUGAdTdT	1749-1767
4051	4052	AD-59489	UGCCCAGUUCUUCCCGCUGdTdT	CAGCGGGAAGAACUGGGCAdTdT	132-150
4053	4054	AD-59540	AAAAAUGGACAUCAUUUCUdTdT	AGAAAUGAUGUCCAUUUUUdTdT	1639-1657
4055	4056	AD-59378	CUUGAGCUUCAGGAGGAUGdTdT	CAUCCUCCUGAAGCUCAAGdTdT	719-737
4057	4058	AD-59403	CCUCUCUGCCACCCAUGCUdTdT	AGCAUGGGUGGCAGAGAGGdTdT	1761-1779
4059	4060	AD-59493	AAAGUCAGGAUCCCUAAGAdTdT	UCUUAGGGAUCCUGACUUUdTdT	242-260
4061	4062	AD-59374	CGACCACGGAGGAAUCCUUdTdT	AAGGAUUCCUCCGUGGUCGdTdT	159-177
4063	4064	AD-59380	UUCCGUCUGGACACCCCUUdTdT	AAGGGGUGUCCAGACGGAAdTdT	609-627
4065	4066	AD-59576	CCACCCAUGCUGCUGGCUGdTdT	CAGCCAGCAGCAUGGGUGGdTdT	1769-1787
4067	4068	AD-59425	UGAGAAAAAGAAUGACCACdTdT	GUGGUCAUUCUUUUUCUCAdTdT	961-979
4069	4070	AD-59509	UAAGAUGAUGCCAGGCUGUdTdT	ACAGCCUGGCAUCAUCUUAdTdT	1309-1327

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_000688.4
4071	4072	AD-59488	AGUUAUAUUAAAUUUUAAUdTdT	AUUAAAAUUUAAUAUAACUdTdT	2342-2360
4073	4074	AD-59486	UCUUCCCGCUGUGGGGACAdTdT	UGUCCCCACAGCGGGAAGAdTdT	140-158
4075	4076	AD-59465	UGCCACAAGCCAGGGCACUdTdT	AGUGCCCUGGCUUGUGGCAdTdT	631-649
4077	4078	AD-59484	AGCGCAGUUAUGCCCAGUUdTdT	AACUGGGCAUAACUGCGCUdTdT	122-140
4079	4080	AD-59368	GGACCAGGAGAAAGUCAGGdTdT	CCUGACUUUCUCCUGGUCCdTdT	232-250
4081	4082	AD-59464	UGUCCACUGCCCCAGCCACdTdT	GUGGCUGGGGCAGUGGACAdTdT	1903-1921
4083	4084	AD-59386	AUCGCGGCCUGAGGCUGCUdTdT	AGCAGCCUCAGGCCGCGAUdTdT	22-40
4085	4086	AD-59439	GGGGAUGUGGGGACCAGGAdTdT	UCCUGGUCCCCACAUCCCCdTdT	222-240
4087	4088	AD-59440	CUGGAAAUAAAUUCUUGCUdTdT	AGCAAGAAUUUAUUUCCAGdTdT	2380-2398
4089	4090	AD-59542	UUGAAACUGUCCAUUCAAUdTdT	AUUGAAUGGACAGUUUCAAdTdT	1479-1497
4091	4092	AD-59559	GUGGGGACACGACCACGGAdTdT	UCCGUGGUCGUGUCCCCACdTdT	150-168
4093	4094	AD-59586	CGCAGUGGGGCUUUAUGGGdTdT	CCCAUAAAGCCCCACUGCGdTdT	1579-1597
4095	4096	AD-59408	UUGUCUUUAUAUGUGAAUUdTdT	AAUUCACAUAUAAAGACAAdTdT	2322-2340
4097	4098	AD-59568	UCACCCUGGCUAAGAUGAUdTdT	AUCAUCUUAGCCAGGGUGAdTdT	1299-1317
4099	4100	AD-59398	GUAUCUGCUCAGGCCUGAGdTdT	CUCAGGCCUGAGCAGAUACdTdT	2243-2261
4101	4102	AD-59508	AUGAGUGGCUUCUUCUCCAdTdT	UGGAGAAGAAGCCACUCAUdTdT	280-298
4103	4104	AD-59523	GAAGUUGGGGCCAAGCCAGdTdT	CUGGCUUGGCCCCAACUUCdTdT	449-467
4105	4106	AD-59410	UCAGGGACUCGGGACCCUGdTdT	CAGGGUCCCGAGUCCCUGAdTdT	181-199
4107	4108	AD-59541	UCCUACGGAUUGCCCCCACdTdT	GUGGGGGCAAUCCGUAGGAdTdT	2043-2061
4109	4110	AD-59524	UUACUCUGAUUCUGGGAACdTdT	GUUCCCAGAAUCAGAGUAAdTdT	1333-1351
4111	4112	AD-59501	AUCCCUAAGAGUCUUCCCUdTdT	AGGGAAGACUCUUAGGGAUdTdT	251-269
4113	4114	AD-59383	UGCCAAAGUACAUCUUCCGdTdT	CGGAAGAUGUACUUUGGCAdTdT	1389-1407
4115	4116	AD-59577	UCCUCGGGUUUAGGGGAUGdTdT	CAUCCCCUAAACCCGAGGAdTdT	210-228
4117	4118	AD-59447	UGCUGAAACCUCAGCAGGCdTdT	GCCUGCUGAGGUUUCAGCAdTdT	769-787

(continued)

SEQ ID NO: (sense)	SEQ ID NO: (antisense)	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Target sites of antisense sequence on NM_000688.4
4119	4120	AD-59555	CCACCCACGGGUGUGUGGGdTdT	CCCACACACCCGUGGGUGGdTdT	1111-1129
4121	4122	AD-59405	UGGUGCAGUAAUGACUACCdTdT	GGUAGUCAUUACUGCACCAdTdT	1079-1097
4123	4124	AD-59371	UUCUCCACCUAGAUUCUUUdTdT	AAAGAAUCUAGGUGGAGAAdTdT	292-310
4125	4126	AD-59443	UAAGGCGCCGGCGAUCGCGdTdT	CGCGAUCGCCGGCGCCUUAdTdT	9-27
4127	4128	AD-59401	UGGAACUAGUAAAUUCCAUdTdT	AUGGAAUUUACUAGUUCCAdTdT	1189-1207
4129	4130	AD-59494	GGACCCUGCUGGACCCCUUdTdT	AAGGGGUCCAGCAGGGUCCdTdT	192-210
4131	4132	AD-59504	UCAAUUAUUUCACUUAACCdTdT	GGUUAAGUGAAAUAAUUGAdTdT	2269-2287
4133	4134	AD-59369	CCCGGACAAGGGCAACGAGdTdT	CUCGUUGCCCUUGUCCGGGdTdT	41-59
4135	4136	AD-59571	UUUUAAAACUGUGAACCGGdTdT	CCGGUUCACAGUUUUAAAAdTdT	991-1009
4137	4138	AD-59527	GUGCUUCGCCGCCAGCACCdTdT	GGUGCUGGCGGCGAAGCACdTdT	1832-1850
4139	4140	AD-59466	UGGACCCCUUCCUCGGGUUdTdT	AACCCGAGGAAGGGGUCCAdTdT	201-219
4141	4142	AD-59526	CUGUAUAUUAAGGCGCCGGdTdT	CCGGCGCCUUAAUAUACAGdTdT	1-19
4143	4144	AD-59543	UUGCCCCCACCCCUCACCAdTdT	UGGUGAGGGGUGGGGGCAAdTdT	2052-2070
4145	4146	AD-59564	AUGGGGCGGUGUGCCCACUdTdT	AGUGGGCACACCGCCCCAUdTdT	1500-1518
4147	4148	AD-59583	CUAUAGUAAAAACAUAGUCdTdT	GACUAUGUUUUUACUAUAGdTdT	2361-2379

[0636] The *in vitro* activity of the siRNAs in suppressing ALAS 1 mRNA was tested in a single dose screen in Hep3B cells that were transfected using Lipofectamine2000 as a transfection reagent. Single dose experiments were performed at 10nM duplex concentration and analyzed by branched DNA (bDNA) assay. The results are shown in Table 19 and are expressed as percent remaining mRNA.

Table 19: Suppression of ALAS1 mRNA as assessed by bDNA assay

Duplex	% remaining mRNA	SD
AD-59453	11.2	1.5
AD-59395	12.7	1.1
AD-59477	14.5	2.0
AD-59492	14.8	2.1
AD-59361	15.1	4.9
AD-59462	15.4	2.6
AD-59433	15.8	2.7
AD-59424	16.0	1.7
AD-59414	16.1	1.3
AD-59539	16.2	2.6
AD-59400	16.2	1.8
AD-59551	16.3	2.3
AD-59482	16.6	2.1
AD-59448	16.6	3.7
AD-59392	16.9	3.5
AD-59469	16.9	2.2
AD-59431	17.0	2.0
AD-59423	17.1	3.8
AD-59517	17.2	1.5
AD-59578	17.3	3.1
AD-59495	17.7	3.7
AD-59432	17.7	2.8
AD-59382	17.9	3.2
AD-59472	18.6	3.5
AD-59459	18.7	3.8
AD-59413	18.8	2.4
AD-59478	18.9	3.0
AD-59376	18.9	3.2
AD-59556	18.9	2.4
AD-59399	19.0	4.1
AD-59474	19.4	1.6
AD-53542	19.4	1.7
AD-59480	19.6	1.6
AD-59549	19.7	2.1
AD-59515	19.8	4.4

(continued)

Duplex	% remaining mRNA	SD
AD-59427	19.9	3.2
AD-59390	19.9	3.4
AD-59511	19.9	2.2
AD-59532	20.0	2.4
AD-59562	20.2	2.6
AD-59513	20.3	3.9
AD-59362	20.6	2.5
AD-53541	20.6	2.2
AD-59490	20.7	2.3
AD-59422	20.8	4.5
AD-59467	21.2	2.3
AD-59579	21.2	3.3
AD-59426	21.7	2.3
AD-59363	21.7	2.7
AD-59436	21.7	2.7
AD-53536	21.9	1.5
AD-59491	21.9	2.6
AD-59500	22.2	2.8
AD-59394	22.3	10.1
AD-59441	22.3	2.6
AD-59365	22.4	4.2
AD-59411	22.5	2.9
AD-59544	22.5	2.1
AD-59428	22.7	4.7
AD-59471	22.9	5.0
AD-59518	22.9	2.3
AD-53547	22.9	1.5
AD-59573	23.0	4.2
AD-59473	23.2	1.8
AD-59412	23.4	2.5
AD-59522	23.4	3.3
AD-59502	23.6	2.7
AD-59499	23.6	1.6
AD-59520	23.8	3.8
AD-59581	23.9	6.0
AD-59461	24.3	4.2
AD-59370	24.3	5.6
AD-53540	24.4	2.1

(continued)

Duplex	% remaining mRNA	SD
AD-59574	24.5	2.0
AD-59375	24.6	2.3
AD-59387	24.8	7.2
AD-59397	24.9	9.6
AD-59396	25.0	10.2
AD-59393	25.3	11.6
AD-59483	25.4	3.8
AD-59430	25.5	1.8
AD-59463	25.6	4.8
AD-53534	25.9	3.1
AD-59514	26.2	5.7
AD-59575	26.2	3.2
AD-59364	26.2	4.5
AD-59402	26.3	3.1
AD-59479	26.3	2.5
AD-59481	26.4	2.2
AD-59530	26.4	4.4
AD-59582	26.6	3.9
AD-59506	27.0	4.1
AD-59567	27.3	1.1
AD-59485	27.7	4.7
AD-59525	28.3	3.1
AD-59566	28.5	0.6
AD-59580	28.7	7.1
AD-59512	29.5	2.5
AD-59475	29.6	4.2
AD-59438	29.6	3.3
AD-59442	29.9	2.8
AD-59516	30.4	3.8
AD-59429	30.8	4.3
AD-59510	31.3	1.9
AD-59457	31.4	1.2
AD-59434	31.6	3.5
AD-59454	32.0	1.9
AD-59468	32.2	3.2
AD-59565	32.4	1.5
AD-59416	32.7	1.7
AD-59420	33.2	3.1

(continued)

Duplex	% remaining mRNA	SD
AD-59552	33.2	2.2
AD-59558	33.8	3.8
AD-59404	34.0	5.4
AD-59455	34.8	1.3
AD-59496	34.9	5.2
AD-59446	35.5	1.7
AD-59435	35.9	1.2
AD-59419	36.0	1.4
AD-59533	36.7	3.7
AD-59366	36.7	6.0
AD-59521	36.9	4.3
AD-59563	36.9	4.1
AD-59534	36.9	3.3
AD-59407	37.1	4.7
AD-59445	37.2	3.2
AD-59546	37.9	4.9
AD-59456	38.3	4.0
AD-59503	38.8	5.0
AD-59536	39.8	4.2
AD-59385	39.9	13.7
AD-59367	40.0	3.6
AD-59458	40.0	3.4
AD-59381	40.3	9.9
AD-59538	40.8	4.9
AD-59421	40.9	6.4
AD-59388	41.0	9.1
AD-59444	41.1	2.7
AD-59528	41.9	3.3
AD-59498	42.2	3.3
AD-59497	42.4	4.9
AD-59384	42.7	17.6
AD-59452	42.7	3.1
AD-59379	43.6	2.6
AD-59529	43.8	4.8
AD-59389	44.1	6.4
AD-59585	44.3	3.2
AD-59570	45.1	4.0
AD-59415	46.6	2.3

(continued)

Duplex	% remaining mRNA	SD
AD-59505	47.5	6.2
AD-59557	48.1	4.4
AD-59548	49.9	4.0
AD-59487	50.7	3.2
AD-59550	50.8	5.8
AD-59572	51.1	4.0
AD-59554	51.3	6.0
AD-59437	52.2	4.8
AD-59584	54.9	2.7
AD-59373	55.3	20.1
AD-59545	55.4	3.4
AD-59547	55.9	4.7
AD-59470	56.0	2.7
AD-59417	56.4	7.7
AD-59535	57.6	5.1
AD-59507	58.8	4.7
AD-59519	59.1	5.6
AD-59391	60.1	12.5
AD-59537	60.6	9.1
AD-59450	60.7	7.2
AD-59449	61.6	6.8
AD-59418	61.8	8.4
AD-59561	62.2	7.2
AD-59460	62.8	4.7
AD-59409	64.4	9.0
AD-59476	65.2	5.6
AD-59406	65.6	3.5
AD-59569	66.7	7.6
AD-59451	66.9	2.9
AD-59553	67.2	8.8
AD-59372	67.3	25.6
AD-59377	68.7	5.1
AD-59531	68.7	9.0
AD-59560	68.7	12.7
AD-59489	69.6	8.9
AD-59540	70.1	10.1
AD-59378	70.6	14.1
AD-59403	71.4	3.3

(continued)

Duplex	% remaining mRNA	SD
AD-59493	72.3	3.5
AD-59374	75.9	5.1
AD-59380	76.4	11.1
AD-59576	77.5	16.2
AD-59425	77.9	10.6
AD-59509	78.0	3.2
AD-59488	78.6	7.1
AD-59486	79.4	5.0
AD-59465	79.5	5.1
AD-59484	79.8	3.2
AD-59368	80.0	11.9
AD-59464	80.2	9.3
AD-59386	80.6	33.2
AD-59439	80.9	4.0
AD-59440	82.2	1.9
AD-59542	83.3	10.6
AD-59559	83.7	9.1
AD-59586	83.8	11.5
AD-59408	86.3	2.8
AD-59568	86.8	4.2
AD-59398	87.4	24.9
AD-59508	87.5	2.5
AD-59523	87.6	11.8
AD-59410	88.8	8.3
AD-59541	88.9	10.8
AD-59524	89.5	12.1
AD-59501	89.9	5.1
AD-59383	90.8	27.4
AD-59577	91.1	2.3
AD-59447	91.3	12.9
AD-59555	91.7	3.4
AD-59405	92.5	5.7
AD-59371	93.5	31.7
AD-59443	93.8	9.0
AD-59401	94.5	7.1
AD-59494	95.1	9.1
AD-59504	96.8	11.7
AD-59369	96.8	4.8

(continued)

Duplex	% remaining mRNA	SD
AD-59571	97.4	7.0
AD-59527	98.6	7.8
AD-59466	99.7	14.0
AD-59526	102.9	4.6
AD-59543	103.7	3.0
AD-59564	103.7	12.1
AD-59583	112.4	13.2

[0637] The two hundred thirty-two duplexes that were tested suppressed ALAS 1 mRNA to varying extents in this single dose assay. According to this assay, at least four of the duplexes (AD-59453, AD-59395, AD-59477, and AD-59492) suppressed ALAS 1 mRNA by 85% or more, 39 of the duplexes suppressed ALAS 1 mRNA by 80% or more, 101 of the duplexes suppressed ALAS 1 mRNA by 70% or more, and 152 of the duplexes suppressed ALAS 1 mRNA by 50% or more. In contrast, some duplexes did not show appreciable suppression in this assay.

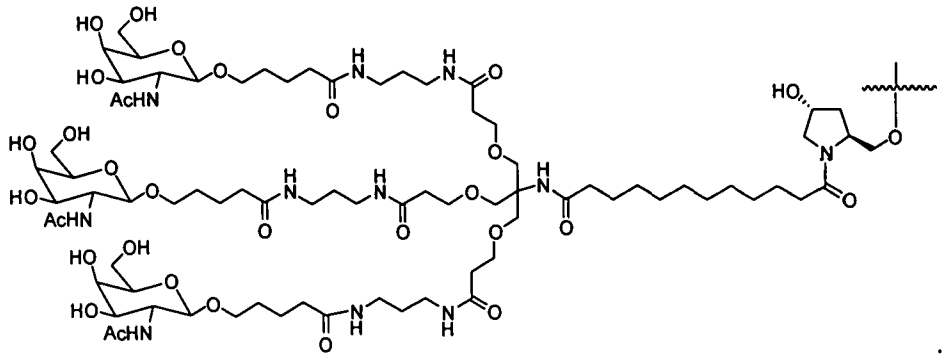
Claims

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1, wherein the dsRNA comprises:

   (i) an antisense strand complementary to at least nucleotides 871-889 of SEQ ID NO:1;
   (ii) a sense strand comprising at least 15 contiguous nucleotides from SEQ ID NO:1295; and
   (iii) a ligand comprising one or more N-acetylgalactosamine (GalNAc) derivatives.

2. The dsRNA of claim 1, wherein said dsRNA comprises at least one modified nucleotide, wherein at least one of the modified nucleotides is chosen from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified 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.

3. The dsRNA of claim 1 or 2, wherein the GalNAc derivative structure is as shown below and is attached to the 3' end of the sense strand of the dsRNA



4. The dsRNA of claim 1, 2 or 3, wherein the antisense strand comprises the sequence of SEQ ID NO: 1296.

5. The dsRNA of claim 4, wherein the sense strand comprises the sequence of SEQ ID NO:1295.

6. The dsRNA of any one of claims 1 to 5, comprising a duplex region of 15 to 30 base pairs in length.

7. The dsRNA of claim 6, wherein said duplex region is 19 to 23 base pairs in length.

8. The dsRNA of any one of claims 1 to 7, wherein each strand is no more than 30 nucleotides in length.

9. A pharmaceutical composition for inhibiting expression of an ALAS1 gene, the composition comprising the dsRNA of any one of claims 1 to 8, wherein preferably said composition is to be administered intravenously or subcutaneously.

10. A method of inhibiting ALAS1 expression in a cell, the method comprising:

(a) introducing into the cell the dsRNA of any one of claims 1 to 8, and
(b) maintaining the cell of step (a) for a time sufficient to obtain degradation of the mRNA transcript of an ALAS1 gene, thereby inhibiting expression of the ALAS1 gene in the cell,

wherein any method of treatment of the human or animal body by therapy is excluded.

11. A method for decreasing a level of a porphyrin or a porphyrin precursor in a cell, comprising contacting the cell with the dsRNA of any one of claims 1 to 8, in an amount effective to decrease the level of the porphyrin or the porphyrin precursor in the cell, wherein any method of treatment of the human or animal body by therapy is excluded.

12. A dsRNA of any one of claims 1 to 8 or a pharmaceutical composition of claim 9 for use in a method of treating a disorder related to ALAS1 expression, wherein a therapeutically effective amount of said dsRNA or said composition is to be administered to a subject in need of such treatment.

13. The dsRNA for use of claim 12, wherein

(a) the subject is at risk for developing, or is diagnosed with, a porphyria;
(b) said method

(i) ameliorates a symptom associated with an ALAS1 related disorder (e.g., a porphyria),
(ii) inhibits ALAS1 expression in the subject,
(iii) decreases a level of a porphyrin precursor or a porphyrin in the subject,
(iv) decreases frequency of acute attacks of symptoms associated with a porphyria in the subject, or
(v) decreases incidence of acute attacks of symptoms associated with a porphyria in the subject when the subject is exposed to a precipitating factor;

(c) the porphyria is a hepatic porphyria selected from acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP), ALA dehydratase deficiency porphyria (ADP), and hepatoerythropoietic porphyria;
(d) the dsRNA is to be administered before, during, or after an acute attack of porphyria;
(e) the dsRNA is to be administered during a prodrome, wherein preferably the prodrome is **characterized by** pain, nausea, psychological symptoms, restlessness or insomnia; or
(f) the subject has an elevated level of ALA and/or PBG.

14. An in vitro or ex vivo cell comprising the dsRNA of any one of claims 1 to 8.

Patentansprüche

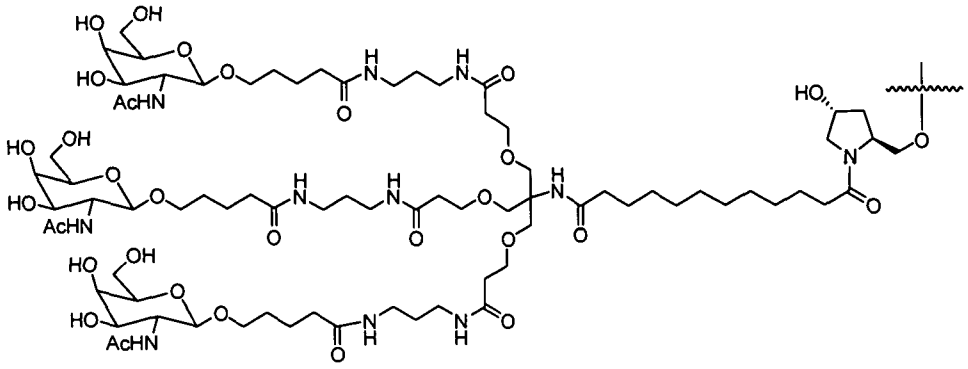
1. Doppelsträngige Ribonukleinsäure (dsRNA) zum Inhibieren der Expression von ALAS1, wobei die dsRNA umfasst:

(i) einen Antisinnstrang, der zumindest zu den Nukleotiden 871-889 der SEQ ID Nr. 1 komplementär ist;
(ii) einen Sinnstrang, der mindestens 15 benachbarte Nukleotide der SEQ ID Nr. 1295 umfasst; und
(iii) einen Ligand, der ein oder mehrere N-Acetylgalactosamin(GalNAc)-Derivate umfasst.

2. DsRNA nach Anspruch 1, wobei die dsRNA mindestens ein modifiziertes Nukleotid umfasst, wobei das mindestens eine modifizierte Nukleotid ausgewählt ist aus der Gruppe bestehend aus: einem 2'-O-Methyl-modifizierten Nukleotid, einem Nukleotid, das eine 5'-Phosphorothioat-Gruppe umfasst, einem terminalen Nukleotid, das mit einem Cholesterinderivat oder einer Dodekansäurebisdezylamidgruppe verbunden ist, einem 2'-Deoxy-2'-Fluor-modifi-

zierten Nukleotid, einem 2'-Deoxy-modifizierten Nukleotid, einem verbrückten ("locked") Nukleotid, einem abasischen Nukleotid, einem 2'-Aminomodifizierten Nukleotid, einem 2'-Alkyl-modifizierten Nukleotid, einem Morpholinonukleotid, einem Phosphoramidat, und einem Nukleotid, das eine nicht-natürliche Base umfasst.

3. DsRNA nach Anspruch 1 oder 2, wobei die GalNAc Derivatstruktur im folgenden dargestellt ist und an das 3' Ende des Sinnstrangs der dsRNA gebunden ist



4. DsRNA nach Anspruch 1, 2 oder 3, wobei der Antisinnstrang die Sequenz der SEQ ID Nr. 1296 umfasst.

5. DsRNA nach Anspruch 4, wobei der Sinnstrang die Sequenz der SEQ ID Nr. 1295 umfasst.

6. DsRNA nach einem der Ansprüche 1 bis 5, umfassend eine Duplexregion mit einer Länge von 15 bis 30 Basenpaaren.

7. DsRNA nach Anspruch 6, wobei die Duplexregion 19 bis 23 Basenpaare lang ist.

8. DsRNA nach einem der Ansprüche 1 bis 7, wobei jeder Strang nicht länger als 30 Nukleotide ist.

9. Pharmazeutische Zusammensetzung zum Inhibieren der Expression eines ALAS1 Gens, wobei die Zusammensetzung die dsRNA nach einem der Ansprüche 1 bis 8 umfasst, und wobei die Zusammensetzung vorzugsweise intravenös oder subkutan zu verabreichen ist.

10. Verfahren zum Inhibieren der ALAS1 Expression in einer Zelle, wobei das Verfahren umfasst:

(a) Einbringen der dsRNA nach einem der Ansprüche 1 bis 8 in die Zelle, und
(b) Aufrechterhalten der Zelle aus Schritt (a) für eine Zeit, die ausreicht, um Abbau des mRNA Transkripts eines ALAS1 Gens zu erhalten, wodurch die Expression des ALAS1 Gens in der Zelle inhibiert wird,

wobei jedes Verfahren zur Behandlung des menschlichen oder Tierkörpers mittels Therapie ausgeschlossen ist.

11. Verfahren zum Vermindern des Spiegels eines Porphyrins oder eines Porphyrinvorläufers in einer Zelle, umfassend das Inkontaktbringen der Zelle mit der dsRNA nach einem der Ansprüche 1 bis 8, und zwar in einer Menge, die wirksam ist, den Spiegel des Porphyrins oder des Porphyrinvorläufers in der Zelle zu reduzieren, wobei jedes Verfahren zur Behandlung des menschlichen oder Tierkörpers mittels Therapie ausgeschlossen ist.

12. DsRNA nach einem der Ansprüche 1 bis 8 oder pharmazeutische Zusammensetzung nach Anspruch 9 für die Verwendung in einem Verfahren zum Behandeln einer Störung, die mit ALAS1 Expression in Zusammenhang steht, wobei eine therapeutisch wirksame Menge der dsRNA oder der Zusammensetzung an ein Individuum zu verabreichen ist, das einer solchen Behandlung bedarf.

13. DsRNA für die Verwendung nach Anspruch 12, wobei

(a) das Individuum ein Risiko aufweist, Porphyrie zu entwickeln oder es wurde Porphyrie in dem Individuum diagnostiziert;
(b) das Verfahren

(i) ein Symptom verbessert, das mit einer mit ALAS1 in Zusammenhang stehenden Störung (bspw. einer Porphyrie) assoziiert ist,
(ii) ALAS1 Expression in dem Individuum inhibiert,
(iii) einen Spiegel eines Porphyrinvorläufers oder eines Porphyrins in dem Individuum reduziert,
(iv) die Frequenz akuter Attacken von Symptomen reduziert, die mit einer Porphyrie in dem Individuum assoziiert sind, oder
(v) das Vorkommen akuter Attacken von Symptomen reduziert, die mit einer Porphyrie in dem Individuum in Zusammenhang stehen, wenn das Individuum einem auslösenden Faktor ausgesetzt ist;

(c) die Porphyrie eine Leberporphyrie ist, die ausgewählt ist aus akuter intermittierender Porphyrie (AIP), hereditärer Coproporphyrie (HCP), variegater Porphyrie (VP), Porphyrie mit ALA-Dehydratase-Defizienz (ADP), und Hepatoerythropoietischer Porphyrie;
(d) die dsRNA vor, während oder nach einem akuten Anfall von Porphyrie zu verabreichen ist;
(e) die dsRNA während eines Prodroms zu verabreichen ist, wobei das Prodrom vorzugsweise durch Schmerz, Schwindel, psychologische Symptome, Ruhelosigkeit oder Schlaflosigkeit gekennzeichnet ist; oder
(f) das Individuum einen erhöhten Spiegel von ALA und/oder PBG hat.

14. In vitro oder ex vivo Zelle, umfassend die dsRNA nach einem der Ansprüche 1 bis 8.

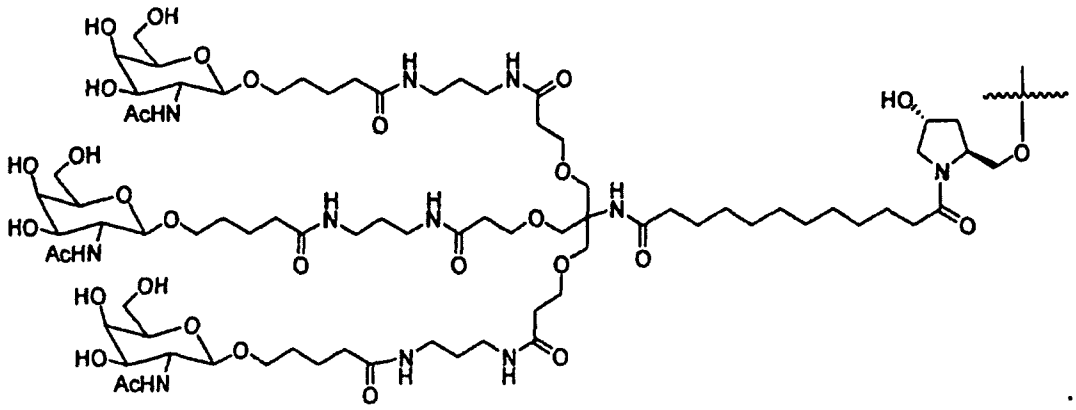
Revendications

1. Acide ribonucléique double brin (ARNdb) pour l'inhibition de l'expression d'ALAS1, dans lequel l'ARNdb comprend :

(i) un brin antisens complémentaire au moins des nucléotides 871-889 de la SEQ ID NO: 1 ;
(ii) un brin sens comprenant au moins 15 nucléotides contigus de la SEQ ID NO: 1295 ; et
(iii) un ligand comprenant un ou plusieurs dérivés de N-acétylgalactosamine (GalNAc).

2. ARNdb selon la revendication 1, dans lequel ledit ARNdb comprend au moins un nucléotide modifié, dans lequel au moins un des nucléotides modifiés est choisi dans le groupe constitué par : un nucléotide modifié par un 2'-O-méthyle, un nucléotide comprenant un groupe 5'-phosphorothioate, un nucléotide terminal lié à un dérivé cholestéryle ou à un groupe bisdécylamide d'acide dodécanoïque, un nucléotide modifié par un 2'-désoxy-2'-fluoro, un nucléotide modifié par un 2'-désoxy, un nucléotide verrouillé, un nucléotide abasique, un nucléotide modifié par un 2'-amino, un nucléotide modifié par un 2'-alkyle, un nucléotide morpholino, un phosphoramidate, et un nucléotide comprenant une base non naturelle.

3. ARNdb selon la revendication 1 ou 2, dans lequel la structure dérivée d'un GalNAc est telle que représentée ci-dessous et est liée à l'extrémité 3' du brin sens de l'ARNdb.



4. ARNdb selon la revendication 1, 2 ou 3, dans lequel le brin antisens comprend la séquence de SEQ ID NO: 1296.

5. ARNdb selon la revendication 4, dans lequel le brin sens comprend la séquence de SEQ ID NO: 1295.

6. ARNdb selon l'une quelconques des revendications 1 à 5, comprenant une région duplex d'une longueur de 15 à

30 paires de bases.

7. ARNdb selon la revendication 6, dans lequel ladite région duplex est d'une longueur de 19 à 23 paires de bases.

8. ARNdb selon l'une quelconque des revendications 1 à 7, dans lequel chaque brin n'est pas d'une longueur supérieure à 30 nucléotides.

9. Composition pharmaceutique destinée à inhiber l'expression d'un gène ALAS1, la composition comprenant l'ARNdb selon l'une quelconque des revendications 1 à 8, dans laquelle de préférence ladite composition doit être administrée par voie intraveineuse ou sous-cutanée.

10. Méthode destinée à inhiber l'expression d'ALAS1 dans une cellule, la méthode comprenant :

(a) l'introduction dans la cellule de l'ARNdb selon l'une quelconque des revendications 1 à 8, et
(b) le maintien de la cellule de l'étape (a) pendant un temps suffisant pour obtenir la dégradation du transcrit d'ARNm d'un gène ALAS1, inhibant ainsi l'expression du gène ALAS1 dans la cellule,

dans laquelle toute méthode de traitement du corps humain ou animal par thérapie est exclue.

11. Méthode destinée à abaisser le niveau d'une porphyrine ou d'un précurseur de porphyrine dans une cellule, comprenant la mise en contact de la cellule avec l'ARNdb selon l'une quelconque des revendications 1 à 8, en une quantité efficace pour abaisser le niveau de la porphyrine ou du précurseur de porphyrine dans la cellule, dans laquelle toute méthode de traitement du corps humain ou animal par thérapie est exclue.

12. ARNdb selon l'une quelconque des revendications 1 à 8 ou composition pharmaceutique selon la revendication 9 pour une utilisation dans une méthode de traitement d'une affection liée à l'expression d'ALAS1, dans lequel une quantité thérapeutiquement efficace dudit ARNdb ou de ladite composition est destinée à être administrée à un sujet ayant besoin d'un tel traitement.

13. ARNdb pour son utilisation selon la revendication 12, dans lequel

(a) le sujet est à risque de développer une porphyrie, ou est diagnostiqué comme atteint de porphyrie ;
(b) ladite méthode

(i) améliore un symptôme associé à une affection liée à ALAS1 (p. ex., porphyrie),
(ii) inhibe l'expression d'ALAS1 chez le sujet,
(iii) abaisse le niveau d'un précurseur de porphyrine ou d'une porphyrine chez le sujet,
(iv) réduit la fréquence des crises symptomatiques aiguës associées à la porphyrie chez le sujet, ou
(v) réduit l'incidence des crises symptomatiques aiguës associées à la porphyrie chez le sujet quand le sujet est exposé à un facteur précipitant ;

(c) la porphyrie est une porphyrie hépatique choisie parmi la porphyrie aiguë intermittente (AIP), la coproporphyrie héréditaire (HCP), la porphyrie variegata (VP), la porphyrie de type déficience en ALA déshydratase (ADP), et la porphyrie hépatoérythropoïétique ;
(d) l'ARNdb doit être administré avant, pendant, ou après une crise aiguë de porphyrie ;
(e) l'ARNdb doit être administré pendant un prodrome, dans lequel de préférence le prodrome est **caractérisé par** la douleur, des nausées, des symptômes psychologiques, l'agitation ou l'insomnie ; ou
(f) le sujet présente un niveau élevé d'ALA et/ou de PBG.

14. Cellule in vitro ou ex vivo comprenant l'ARNdb selon l'une quelconque des revendications 1 à 8.

FIG. 1

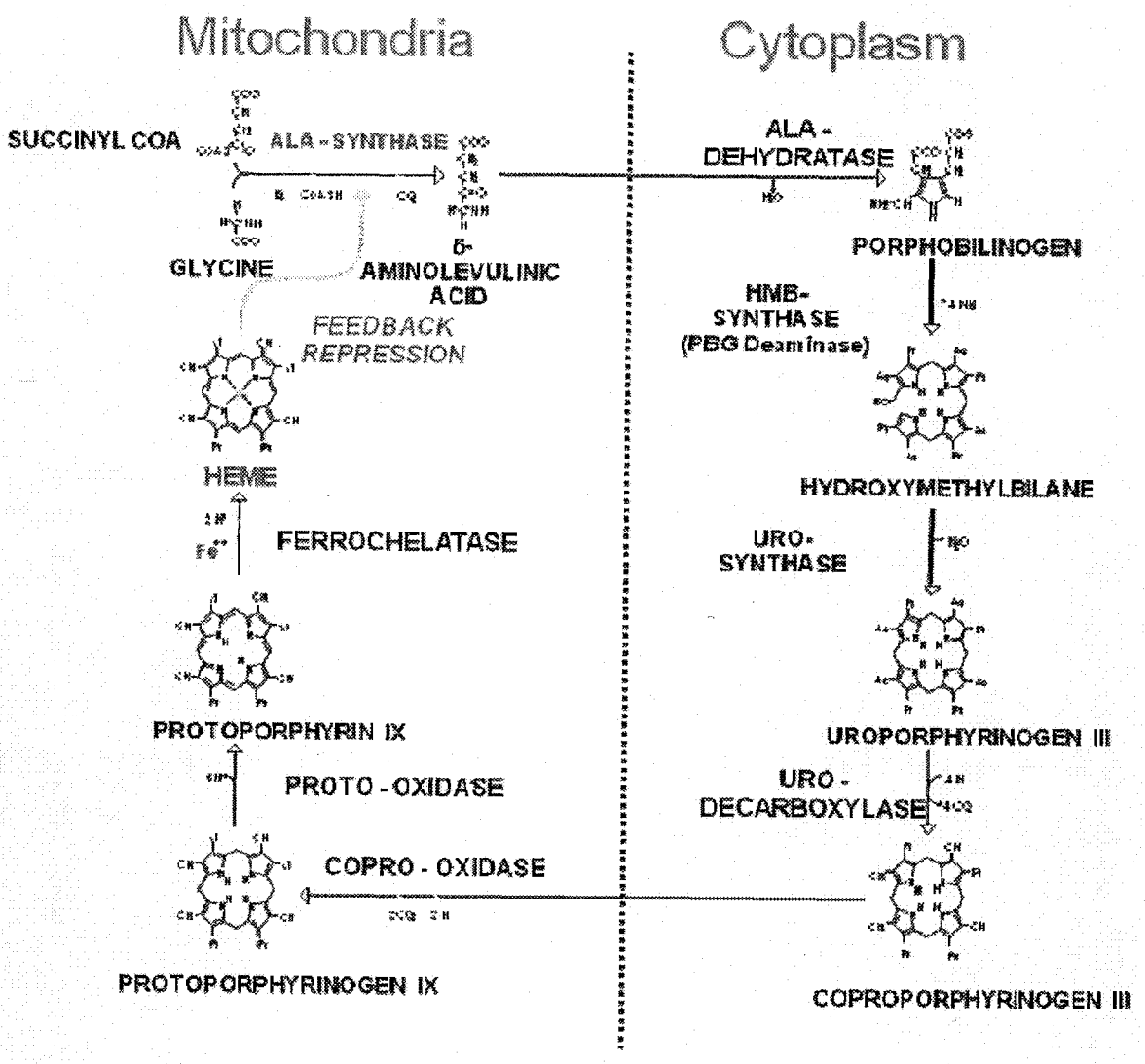

Mitochondria
Cytoplasm
SUCCINYL COA
ALA - SYNTHASE
GLYCINE
AMINOLEVULINIC ACID
FEEDBACK REPRESSION
HEME
FERROCHELATASE
PROTOPORPHYRIN IX
PROTO - OXIDASE
COPRO - OXIDASE
PROTOPORPHYRINOGEN IX
ALA - DEHYDRATASE
PORPHOBILINOGEN
HMB-SYNTHASE (PBG Deaminase)
HYDROXYMETHYLBILANE
URO-SYNTHASE
UROPORPHYRINOGEN III
URO - DECARBOXYLASE
COPROPORPHYRINOGEN III

FIG. 2A

Enzyme, Chromosomal location	Reaction Catalyzed	Associated Porphyria	Type of Porphyria	Typical Inheritance Pattern	Typical Symptoms
δ-aminolevulinate (ALA) synthase 1 3p21	Glycine + SuccinylCoA ↓ δ-aminolevulinic acid (ALA)				
δ-aminolevulinate (ALA) synthase 2 (ALAS2) (erythroid specific) Xp11.21	Glycine + SuccinylCoA ↓ δ-aminolevulinic acid (ALA)	X-linked sideroblastic anemia (XLSA), X-linked protoporphyria (XLP)	Erythropoietic	X-linked	
δ-aminolevulinate dehydratase (ALAD) 9q34	δ-aminolevulinic acid (ALA) ↓ Porphobilinogen (PBG)	ALA dehydratase deficiency porphyria (ADP or Doss porphyria)	Hepatic	Autosomal recessive	Abdominal pain, neuropathy
PBG deaminase (PBGD) or Hydroxymethylbilane synthase (HMBS) 11q23	Porphobilinogen (PBG) ↓ Hydroxymethylbilane (HMB)	Acute intermittent porphyria (AIP)	Hepatic	Autosomal dominant	Periodic abdominal pain, peripheral neuropathy, psychiatric disorders, tachycardia

FIG. 2B

Uroporphyrinogen III Synthase (UROS) 10q26	Hydroxymethylbilane ↓ Uroporphyrinogen III (URO)	Congenital erythropoietic porphyria (CEP)	Erythropoietic	Autosomal recessive	Severe photosensitivity with erythema, swelling and blistering. Hemolytic anemia, splenomegaly
Uroporphyrinogen decarboxylase (UROD) 1q34	Uroporphyrinogen III (URO) ↓ Coprophyrinogen III	Porphyria cutanea tarda (PCT)	Hepatic	Autosomal dominant or sporadic	Photosensitivity with vesicles and bullae
Coproporphyrinogen III oxidase (CPOX)3q12	Coprophyrinogen III (COPRO) ↓ Protoporphyrinogen IX	Hereditary coproporphyria (HCP)	Hepatic	Autosomal dominant	Photosensitivity, neurologic symptoms, colic
Protoporphyrinogen oxidase (PPOX) 1q14	Protoporphyrinogen IX (PROTO) ↓ Protoporphyrin IX	Variegate prophyria (VP)	Mixed	Autosomal dominant	Photosensitivity, neurologic symptoms, developmental delay
Ferrochelatase 18q21.3	Protoporphyrin IX ↓ Heme	Erythropoietic protoporphyria (EPP)	Erythropoietic	Autosomal recessive	Photosensitivity with skin lesions. Gallstones, mild liver dysfunction

FIG. 3A

```
   1 ctgtatatta aggcgccggc gatcgcggcc tgaggctgct cccggacaag ggcaacgagc
  61 gtttcgtttg gacttctcga cttgagtgcc cgcctccttc gccgccgcct ctgcagtcct
 121 cagcgcagtt atgcccagtt cttcccgctg tggggacacg accacggagg aatccttgct
 181 tcagggactc gggaccctgc tggacccctt cctcgggttt aggggatgtg gggaccagga
 241 gaaagtcagg atccctaaga gtcttccctg cctggatgga tgagtggctt cttctccacc
 301 tagattcttt ccacaggagc cagcatactt cctgaacatg gagagtgttg ttcgccgctg
 361 cccattctta tcccgagtcc cccaggcctt tctgcagaaa gcaggcaaat ctctgttgtt
 421 ctatgcccaa aactgcccca agatgatgga agttggggcc aagccagccc ctcgggcatt
 481 gtccactgca gcagtacact accaacagat caaagaaacc cctccggcca gtgagaaaga
 541 caaaactgct aaggccaagg tccaacagac tcctgatgga tcccagcaga gtccagatgg
 601 cacacagctt ccgtctggac acccccttgcc tgccacaagc cagggcactg caagcaaatg
 661 ccctttcctg gcagcacaga tgaatcagag aggcagcagt gtcttctgca aagccagtct
 721 tgagcttcag gaggatgtgc aggaaatgaa tgccgtgagg aaagaggttg ctgaaacctc
 781 agcaggcccc agtgtggtta gtgtgaaaac cgatggaggg gatcccagtg gactgctgaa
 841 gaacttccag gacatcatgc aaaagcaaag accagaaaga gtgtctcatc ttcttcaaga
 901 taacttgcca aaatctgttt ccacttttca gtatgatcgt ttctttgaga aaaaaattga
 961 tgagaaaaag aatgaccaca cctatcgagt ttttaaaact gtgaaccggc gagcacacat
1021 cttccccatg gcagatgact attcagactc cctcatcacc aaaaagcaag tgtcagtctg
1081 gtgcagtaat gactacctag gaatgagtcg ccacccacgg gtgtgtgggg cagttatgga
1141 cactttgaaa caacatggtg ctggggcagg tggtactaga aatatttctg gaactagtaa
1201 attccatgtg gacttagagc gggagctggc agacctccat gggaaagatg ccgcactctt
1261 gttttcctcg tgctttgtgg ccaatgactc aaccctcttc accctggcta agatgatgcc
1321 aggctgtgag atttactctg attctgggaa ccatgcctcc atgatccaag ggattcgaaa
1381 cagccgagtg ccaaagtaca tcttccgcca caatgatgtc agccacctca gagaactgct
1441 gcaaagatct gacccctcag tccccaagat tgtggcattt gaaactgtcc attcaatgga
1501 tggggcggtg tgcccactgg aagagctgtg tgatgtggcc catgagtttg gagcaatcac
1561 cttcgtggat gaggtccacg cagtggggct ttatggggct cgaggcggag ggattgggga
1621 tcgggatgga gtcatgccaa aaatggacat catttctgga acacttggca aagcctttgg
1681 ttgtgttgga gggtacatcg ccagcacgag ttctctgatt gacaccgtac ggtcctatgc
1741 tgctggcttc atcttcacca cctctctgcc acccatgctg ctggctggag ccctggagtc
1801 tgtgcggatc ctgaagagcg ctgagggacg ggtgcttcgc cgccagcacc agcgcaacgt
1861 caaactcatg agacagatgc taatggatgc cggcctccct gttgtccact gccccagcca
1921 catcatccct gtgcgggttg cagatgctgc taaaaacaca gaagtctgtg atgaactaat
1981 gagcagacat aacatctacg tgcaagcaat caattaccct acggtgcccc ggggagaaga
2041 gctcctacgg attgccccca ccctcacca cacaccccag atgatgaact acttccttga
2101 gaatctgcta gtcacatgga agcaagtggg gctggaactg aagcctcatt cctcagctga
2161 gtgcaacttc tgcaggaggc cactgcattt tgaagtgatg agtgaaagag agaagtccta
2221 tttctcaggc ttgagcaagt tggtatctgc tcaggcctga gcatgacctc aattatttca
```

FIG. 3B

```
2281 cttaacccca ggccattatc atatccagat ggtcttcaga gttgtcttta tatgtgaatt
2341 aagttatatt aaattttaat ctatagtaaa aacatagtcc tggaaataaa ttcttgctta
2401 aatggtg
     (SEQ ID NO:1)
```

FIG. 4A

```
   1 cagaagaagg cagcgcccaa ggcgcatgcg cagcggtcac tcccgctgta tattaaggcg
  61 ccggcgatcg cggcctgagg ctgctcccgg acaagggcaa cgagcgtttc gtttggactt
 121 ctcgacttga gtgcccgcct ccttcgccgc cgcctctgca gtcctcagcg cagttatgcc
 181 cagttcttcc cgctgtgggg acacgaccac ggaggaatcc ttgcttcagg gactcgggac
 241 cctgctggac cccttcctcg ggtttagggg atgtggggac caggagaaag tcaggatccc
 301 taagagtctt ccctgcctgg atggatgagt ggcttcttct ccacctagat tctttccaca
 361 ggagccagca tacttcctga acatggagag tgttgttcgc cgctgcccat tcttatcccg
 421 agtcccccag gcctttctgc agaaagcagg caaatctctg ttgttctatg cccaaaactg
 481 ccccaagatg atggaagttg gggccaagcc agcccctcgg gcattgtcca ctgcagcagt
 541 acactaccaa cagatcaaag aaacccctcc ggccagtgag aaagacaaaa ctgctaaggc
 601 caaggtccaa cagactcctg atggatccca gcagagtcca gatggcacac agcttccgtc
 661 tggacacccc ttgcctgcca caagccaggg cactgcaagc aaatgccctt tcctggcagc
 721 acagatgaat cagagaggca gcagtgtctt ctgcaaagcc agtcttgagc ttcaggagga
 781 tgtgcaggaa atgaatgccg tgaggaaaga ggttgctgaa acctcagcag gccccagtgt
 841 ggttagtgtg aaaaccgatg gaggggatcc cagtggactg ctgaagaact tccaggacat
 901 catgcaaaag caaagaccag aaagagtgtc tcatcttctt caagataact tgccaaaatc
 961 tgtttccact tttcagtatg atcgtttctt tgagaaaaaa attgatgaga aaaagaatga
1021 ccacacctat cgagttttta aaactgtgaa ccggcgagca cacatcttcc ccatggcaga
1081 tgactattca gactccctca tcaccaaaaa gcaagtgtca gtctggtgca gtaatgacta
1141 cctaggaatg agtcgccacc cacgggtgtg tggggcagtt atggacactt tgaaacaaca
1201 tggtgctggg gcaggtggta ctagaaatat ttctggaact agtaaattcc atgtggactt
1261 agagcgggag ctggcagacc tccatgggaa agatgccgca ctcttgtttt cctcgtgctt
1321 tgtggccaat gactcaaccc tcttcaccct ggctaagatg atgccaggct gtgagattta
1381 ctctgattct gggaaccatg cctccatgat ccaagggatt cgaaacagcc gagtgccaaa
1441 gtacatcttc cgccacaatg atgtcagcca cctcagagaa ctgctgcaaa gatctgaccc
1501 ctcagtcccc aagattgtgg catttgaaac tgtccattca atggatgggg cggtgtgccc
1561 actggaagag ctgtgtgatg tggcccatga gtttggagca atcaccttcg tggatgaggt
1621 ccacgcagtg gggctttatg gggctcgagg cggagggatt ggggatcggg atggagtcat
1681 gccaaaaatg gacatcattt ctggaacact tggcaaagcc tttggttgtg ttggagggta
1741 catcgccagc acgagttctc tgattgacac cgtacggtcc tatgctgctg gcttcatctt
1801 caccacctct ctgccaccca tgctgctggc tggagccctg gagtctgtgc ggatcctgaa
1861 gagcgctgag ggacgggtgc ttcgccgcca gcaccagcgc aacgtcaaac tcatgagaca
1921 gatgctaatg gatgccggcc tccctgttgt ccactgcccc agccacatca tccctgtgcg
1981 ggttgcagat gctgctaaaa acacagaagt ctgtgatgaa ctaatgagca gacataacat
2041 ctacgtgcaa gcaatcaatt accctacggt gccccgggga gaagagctcc tacggattgc
2101 ccccacccct caccacacac cccagatgat gaactacttc cttgagaatc tgctagtcac
2161 atggaagcaa gtggggctgg aactgaagcc tcattcctca gctgagtgca acttctgcag
2221 gaggccactg cattttgaag tgatgagtga aagagagaag tcctatttct caggcttgag
```

FIG. 4B

2281 caagttggta tctgctcagg cctgagcatg acctcaatta tttcacttaa ccccaggcca
2341 ttatcatatc cagatggtct tcagagttgt ctttatatgt gaattaagtt atattaaatt
2401 ttaatctata gtaaaaacat agtcctggaa ataaattctt gcttaaatgg tgaaaaaa
(SEQ ID NO:382)

FIG. 5

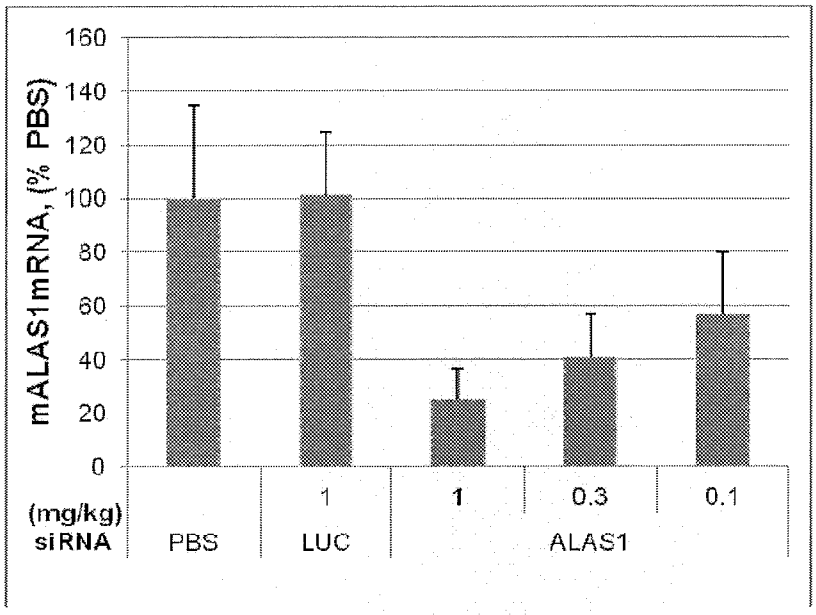

mALAS1mRNA, (% PBS)
160
140
120
100
80
60
40
20
0
(mg/kg)
siRNA
PBS
1
LUC
1
0.3
0.1
ALAS1

FIG. 6

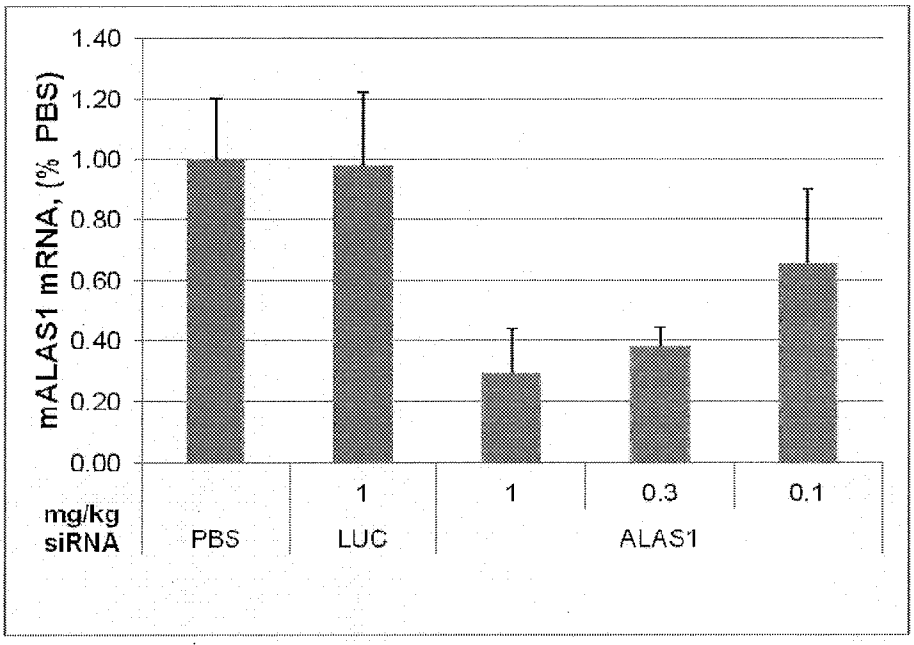

mALAS1 mRNA, (% PBS)
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
mg/kg siRNA
PBS
1
LUC
1
0.3
0.1
ALAS1

FIG. 7

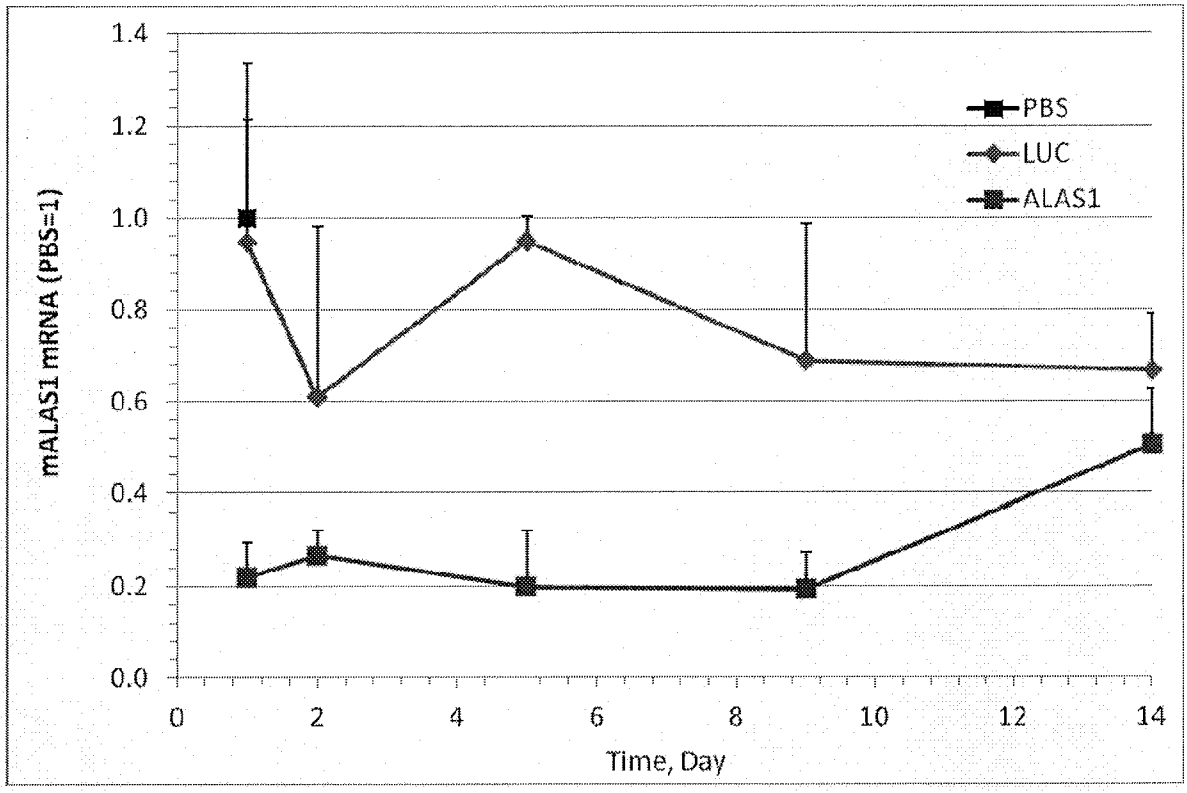

mALAS1 mRNA (PBS=1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
Time, Day
PBS
LUC
ALAS1

FIG. 8

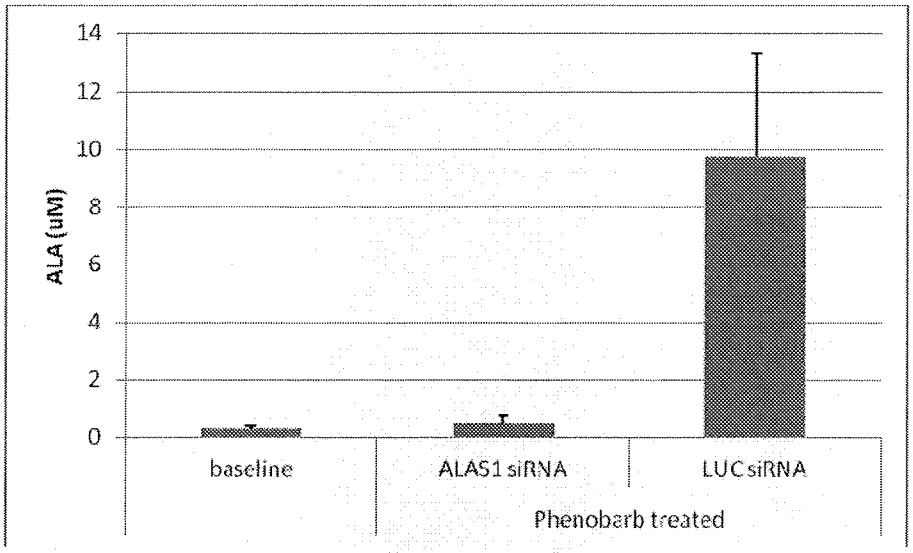

14
12
10
8
6
4
2
0
ALA (uM)
baseline
ALAS1 siRNA
LUC siRNA
Phenobarb treated

FIG. 9

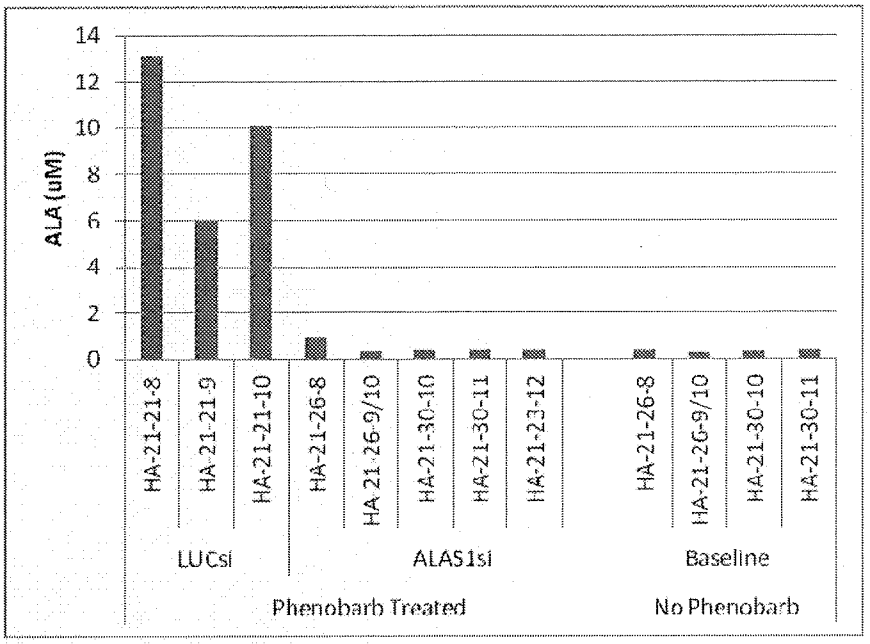

14
12
10
8
6
4
2
0
ALA (uM)
HA-21-21-8
HA-21-21-9
HA-21-21-10
HA-21-26-8
HA-21-26-9/10
HA-21-30-10
HA-21-30-11
HA-21-23-12
HA-21-26-8
HA-21-26-9/10
HA-21-30-10
HA-21-30-11
LUCsi
ALAS1si
Baseline
Phenobarb Treated
No Phenobarb

FIG. 10

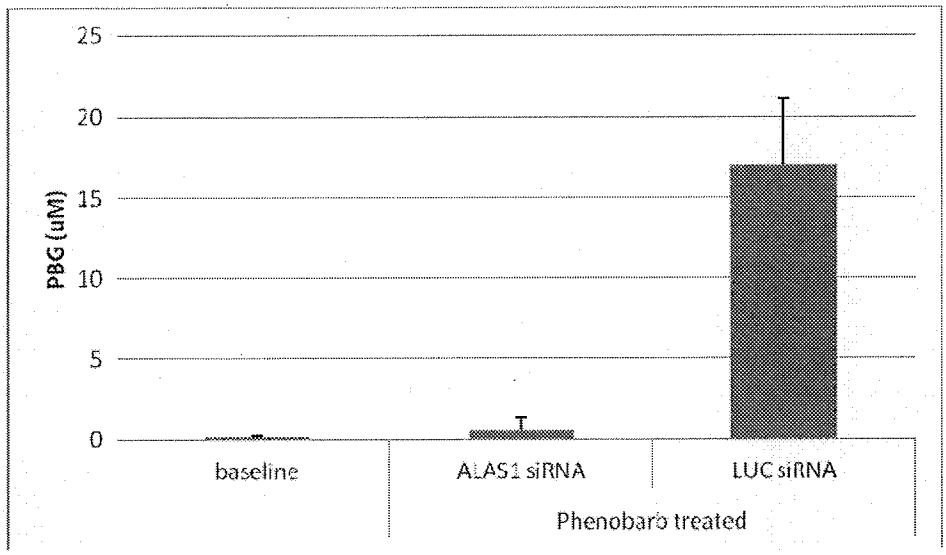

25
20
15
10
5
0
PBG (uM)
baseline
ALAS1 siRNA
LUC siRNA
Phenobarb treated

FIG. 11

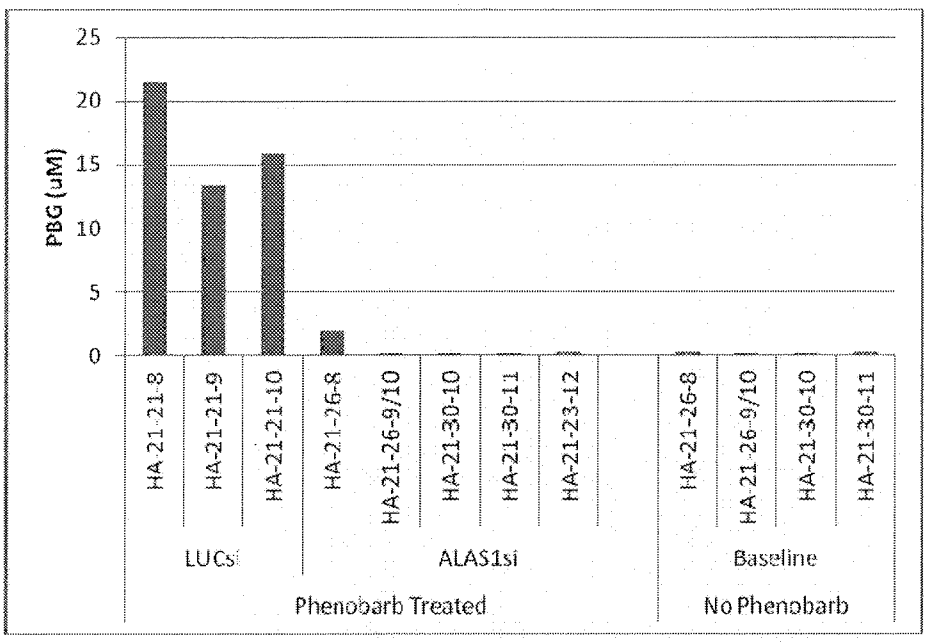

25
20
15
10
5
0
PBG (uM)
HA-21-21-8
HA-21-21-9
HA-21-21-10
HA-21-26-8
HA-21-26-9/10
HA-21-30-10
HA-21-30-11
HA-21-23-12
HA-21-26-8
HA-21-26-9/10
HA-21-30-10
HA-21-30-11
LUCsi
ALAS1si
Baseline
Phenobarb Treated
No Phenobarb

FIG. 12

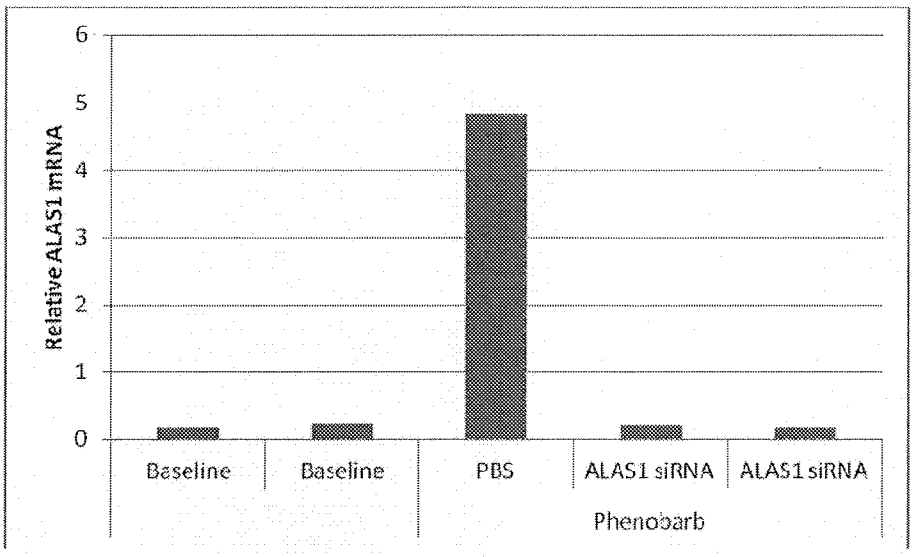

Relative ALAS1 mRNA
6
5
4
3
2
1
0
Baseline
Baseline
PBS
ALAS1 siRNA
ALAS1 siRNA
Phenobarb

FIG. 13

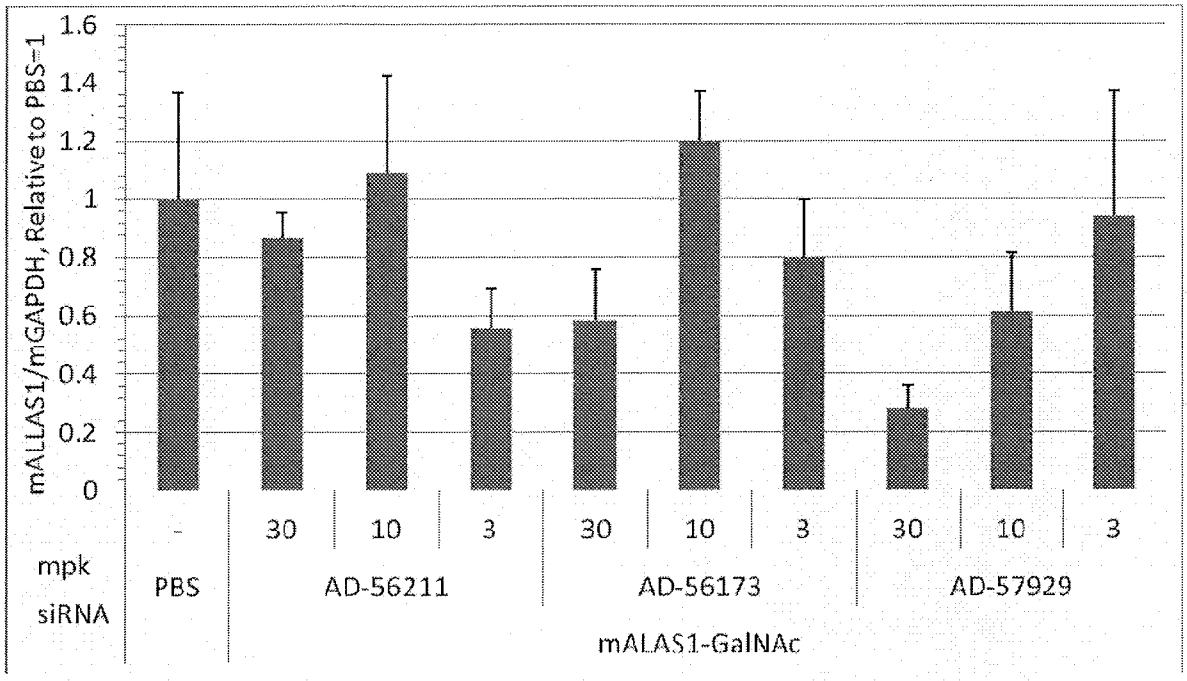
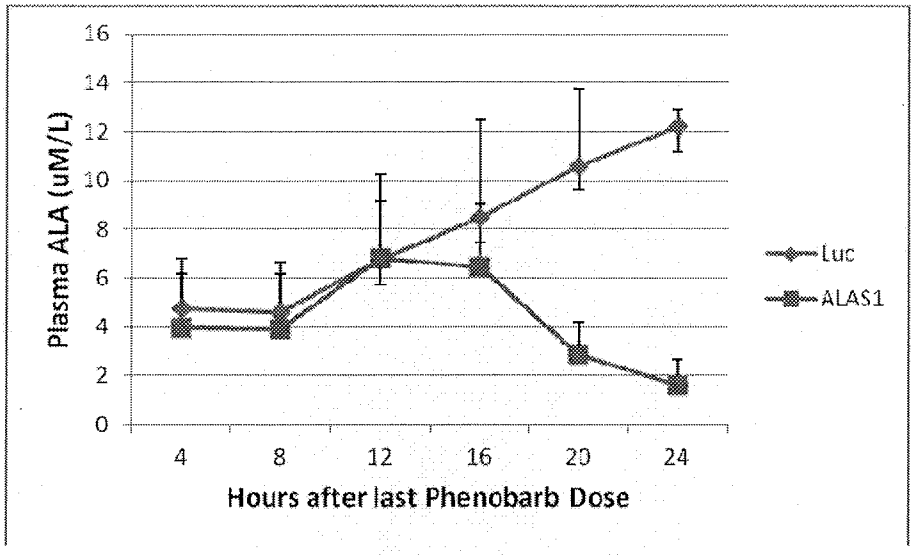

mALLAS1/mGAPDH, Relative to PBS=1
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-
30
10
3
30
10
3
30
10
3
mpk
siRNA
PBS
AD-56211
AD-56173
AD-57929
mALAS1-GalNAc

FIG. 14


Plasma ALA (uM/L)
16
14
12
10
8
6
4
2
0
4
8
12
16
20
24
Hours after last Phenobarb Dose
Luc
ALAS1

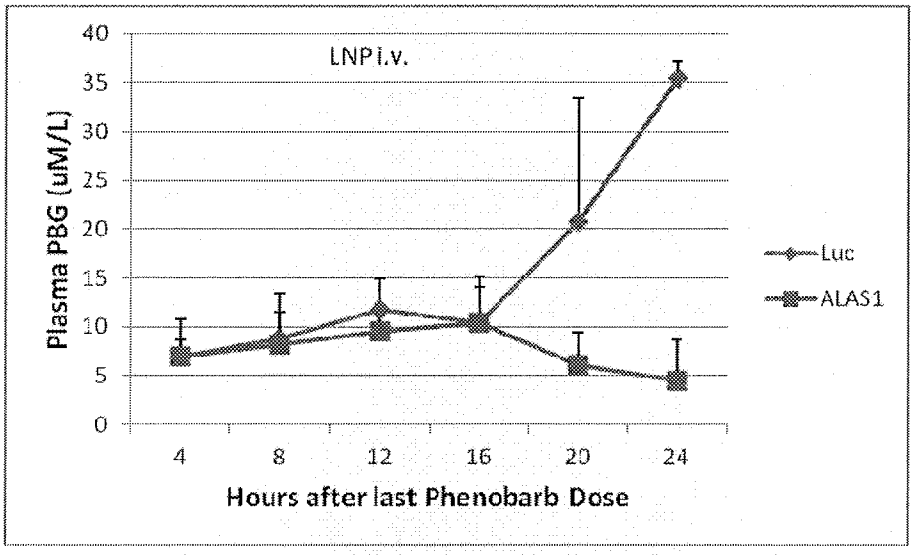
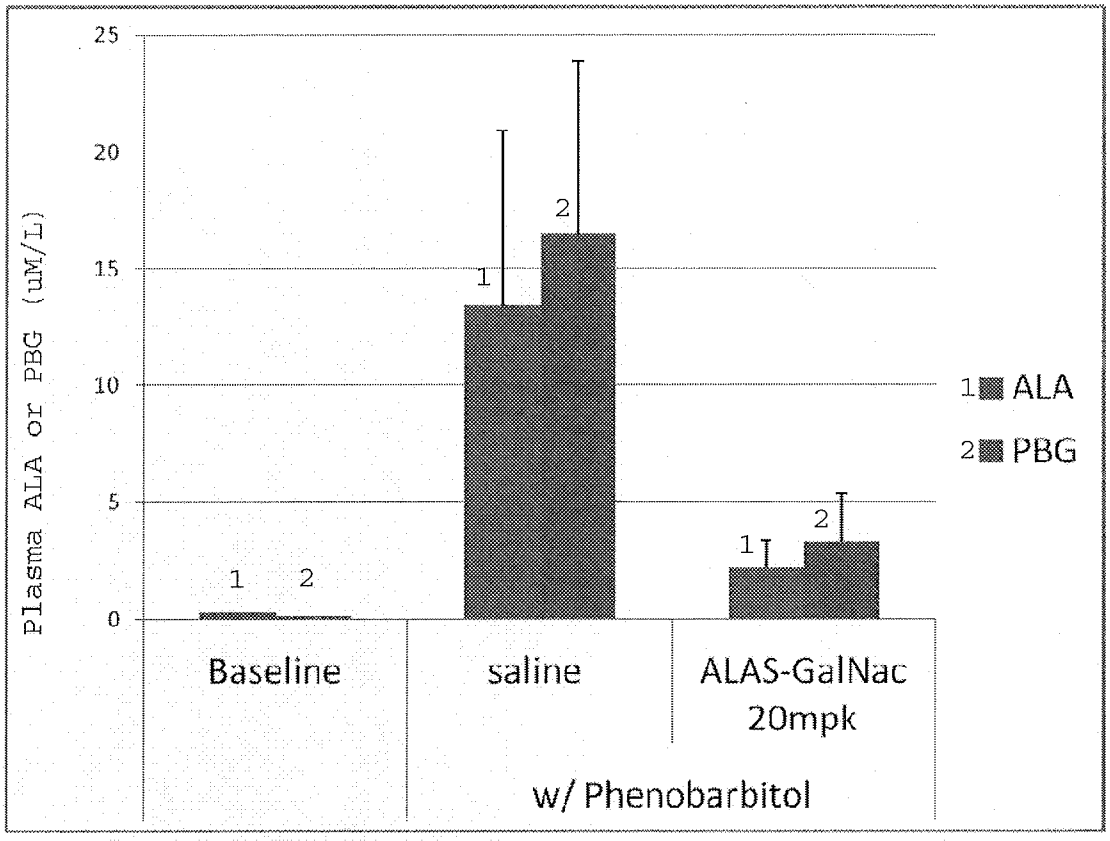

LNP i.v.
Plasma PBG (uM/L)
40
35
30
25
20
15
10
5
0
4
8
12
16
20
24
Hours after last Phenobarb Dose
Luc
ALAS1

FIG. 15


Plasma ALA or PBG (uM/L)
25
20
15
10
5
0
1
2
1
2
1
2
1 ALA
2 PBG
Baseline
saline
ALAS-GalNac
20mpk
w/ Phenobarbitol

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Készítmények és eljárások az ALAS1 gén expressziójának gátlására

Szabadalmi igénypontok

1. Duplaszálú ribonukleinsav (dsRNS) ALAS1 expressziójának gátlására, ahol a dsRNS tartalmaz:

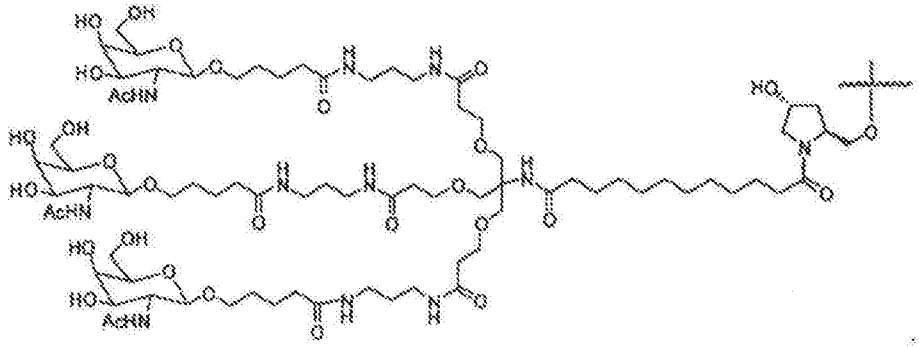
(i) antiszensz szálat, amely komplementer SEQ ID NO:1 szerinti szekvencia legalább 871-889. nukleotidjaival;

(ii) szensz szálat, amely tartalmazza a SEQ ID NO:1295 szekvencia legalább 15 szomszédos nukleotidját; és

(iii) ligandumot, amely tartalmaz egy vagy több N-acetilgalaktózamin (GalNAc) származékot.

2. Az 1. igénypont szerinti dsRNS, ahol a dsRNS tartalmaz legalább egy módosított nukleotidot, ahol a módosított nukleotidok legalább egyike a következőkből álló csoportból van kiválasztva: 2'-O-metil-módosított nukleotid, 5'-foszforotioát-csoportot tartalmazó nukleotid, terminális nukleotid, amely koleszterilszármazékhoz vagy dodekánsav-biszdecilamid-csoporthoz van kapcsolva, 2'-deoxi-2'-fluoro-módosított nukleotid, 2'-deoxi-módosított nukleotid, lakatolt nukleotid, abázisos nukleotid, 2'-amino-módosított nukleotid, 2'-alkil-módosított nukleotid, morfolino-nukleotid, foszforamidát és nem-természetes bázist tartalmazó nukleotid.

3. Az 1. vagy 2. igénypont szerinti dsRNS, ahol a GalNAc-származék szerkezete az alább bemutatott, és a dsRNS szensz szálának 3' végéhez van kapcsolva:



4. Az 1., 2. vagy 3. igénypont szerinti dsRNS, ahol az antiszensz szál tartalmazza a SEQ ID NO: 1296 szerinti szekvenciát.

5. A 4. igénypont szerinti dsRNS, ahol a szensz szál tartalmazza a SEQ ID NO: 1295 szerinti szekvenciát.

6. Az 1-5. igénypontok bármelyike szerinti dsRNS, amely 15-30 bázispár hosszúságú duplexrégiót tartalmaz.

7. A 6. igénypont szerinti dsRNS, ahol a duplexrégió 19-23 bázispár hosszúságú.

8. Az 1-7. igénypontok bármelyike szerinti dsRNS, ahol mindegyik szál legfeljebb 30 nukleotid hosszúságú.

9. Gyógyászati készítmény ALAS1 gén expressziójának gátlására, ahol a készítmény tartalmazza az 1-8. igénypontok bármelyike szerinti dsRNS-t, ahol előnyösen a készítményt intravénásan vagy szubkután adandó be.

10. Eljárás ALAS1 expressziójának gátlására sejtben, ahol az eljárás tartalmazza a következőket:

(a) 1-8. igénypontok bármelyike szerinti dsRNS bejuttatása a sejtbe és

(b) az (a) szerinti sejt fenntartása elegendő ideig ahhoz, hogy ALAS1 gén mRNS-átiratának degradációját kapjuk, ezáltal az ALAS1 gén expressziójának gátlása a sejtben,

ahol bármely, emberi vagy állati test kezelésére szolgáló eljárás ki van zárva az oltalmi igényből.

11. Eljárás sejtben porfirin vagy porfirinprekurzor szintjének csökkentésére, amely magában foglalja a sejt érintkeztetését 1-8. igénypontok bármelyike szerinti dsRNS-sel olyan mennyiségben, amely hatékonyan csökkenti a sejtben a porfirin vagy porfirinprekurzor szintjét, ahol bármely, emberi vagy állati test kezelésére szolgáló eljárás ki van zárva az oltalmi igényből.

12. Az 1-8. igénypontok bármelyike szerinti dsRNS vagy a 9. igénypont szerinti gyógyászati készítmény ALAS1 expressziójával kapcsolatos rendellenesség kezelésére szolgáló eljárásban történő alkalmazásra, ahol a dsRNS vagy a készítmény terápiásan hatékony mennyisége adandó be ilyen kezelésre szoruló alanynak.

13. A 12. igénypont szerinti dsRNS az ott meghatározott alkalmazásra, ahol

(a) az alanyt porfíria kifejlődése veszélyezteti vagy porfíriát diagnosztizáltak nála;

(b) az eljárás

(i) ALAS1-gyel kapcsolatos rendellenességgel (pl. porfíria) összefüggő tünetet enyhít,

(ii) gátolja ALAS1 expresszióját az alanyban,

(iii) csökkenti porfirinprekurzor vagy porfirin szintjét az alanyban,

(iv) porfíriával összefüggő tünetek akut attakjának gyakoriságát csökkenti az alanyban vagy

(v) porfíriával összefüggő tünetek akut attakjának incidenciáját csökkenti az alanyban, amikor az alany ki van téve egy precipitáló faktornak,

(c) a porfíria a következők közül kiválasztott hepatikus porfíria: akut intermittáló porfíria (AIP), örökletes koproporfíria (HCP), variegata porfíria, ALA-dehidratázhiányos porfíria (ADP) és hepatoeritropoetikus porfíria,

(d) a dsRNS porfíria akut attakja előtt, közben vagy utána adandó be,

(e) a dsRNS prodroma alatt adandó be, ahol előnyösen a prodroma a következővel van jellemezve: fájdalom, émelygés, pszichológiai tünetek, nyugtalanság vagy álmatlanság; vagy

(f) az alany ALA- és/vagy PBG-szintje emelkedett.

14. *In vitro* vagy *ex vivo* sejt, amely 1-8. igénypontok bármelyike szerinti dsRNS-t tartalmaz.