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.

Summary

In one aspect, the present invention refers to a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to an ALAS1 RNA transcript (e.g., SEQ ID NO:1), which antisense strand comprises usAfsAfGfaUfgAfgAfcAfcUfcUfuUfcUfgsgsu (SEQ ID NO: 4161), wherein c, a, g, u = 2′-OMe ribonucleosides; Af, Cf, Gf, Uf = 2′F ribonucleosides; s = phosphortioate, or a sequence differing by no more than 1, 2, or 3 nucleotides thereof. In another aspect, the present invention refers to a cell comprising the dsRNA of the present invention. In yet another aspect, the present invention refers to a pharmaceutical composition comprising the dsRNA of the present invention. In still another aspect, the present invention refers to the use of the dsRNA of the present invention, or the composition of the present invention, in the manufacture of a medicament for treating a porphyria in a subject. In another aspect, the present invention refers to the dsRNA of the present invention, or a pharmaceutical composition of the present invention for use in therapy. The present disclosure describes methods and iRNA compositions for modulating the expression of an ALAS1 gene. In certain examples, 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 expression, such as porphyrias.

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. In some examples, the disorder is an acute hepatic porphyria, *e.g.*, ALA deyhdratase deficiency porphyria (ADP), AIP, HCP, or VP. In certain examples, the disorder is ALA deyhdratase deficiency porphyria (ADP) or AIP.

In examples, 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. In examples, the porphyria is a homozygous dominant hepatic porphyria (e.g., homozygous dominant AIP, HCP, or VP) or hepatoerythropoietic porphyria, In examples, the porphyria is a dual porphyria.

As used herein, the term "iRNA," "RNAi", "iRNA agent," "RNAi agent," or "iRNA molecule," 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. In one example, an iRNA as described herein effects inhibition of ALAS1 expression in a cell or mammal.

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 ALAS1 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 ALAS1 gene (*e.g.*, a human variant 1 or 2 of an ALAS1 gene) (also referred to herein as a "ALAS1-specific iRNA").

In examples, the iRNA (e.g, dsRNA) described herein comprises an antisense strand having a region that is substantially complementary to a region of a human ALAS1. In examples, the human ALAS1 has the sequence of NM_000688.4 (SEQ ID NO:1) or NM_000688.5 (SEQ ID NO:382). In examples, the human ALAS1 has the sequence of NM_199166.1.

In examples, the antisense sequence of the iRNA (e.g., dsRNA) targets within the region 871 to 895 (plus or minus 5, 4, 3, 2, or 1 nucleotides in either or both directions on the 5' and/or 3' end) on the ALAS1 transcript NM_000688.4. In examples, the antisense sequence targets the nucleotides 871 to 893, 871 to 892, or 873 to 895 on the ALAS1 transcript NM_000688.4. In examples, the antisense sequence comprises or consists of a sequence that is fully complementary or substantially complementary to nucleotides 871 to 893, 871 to 892, or 873 to 895 on the ALAS1 transcript NM_000688.4.

In one example, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, 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 (e.g., at least 16, 17, 18, 19, 20, 21, 22, or 23) contiguous nucleotides differing by no more than 3, 2 or 1 nucleotides from the sequence of

UAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 4153) or UAAGAUGAGACACUCTUUCUGGU (SEQ ID NO: 4154). In examples, the antisense strand comprises the sequence of UAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 4153) or UAAGAUGAGACACUCTUUCUGGU (SEQ ID NO: 4154). In examples, the sense strand comprises the sequence of CAGAAAGAGUGUCUCAUCUUA (SEQ ID NO: 4155). In examples, one or more nucleotides of the antisense strand and/or sense strand are modified as described herein. In examples, the dsRNA comprises (i) an antisense strand that comprises, or consists of, the antisense sequence of AD-60489, AD-60519, or AD-61193 and/or (ii) a sense strand that comprises, or consists of, the sense sequence of AD-60489, AD-60519, or AD-61193 (including one or more (*e.g.*, all) of the modifications of the antisense strand and/or antisense strand of AD-60489, AD-60519, or AD-61193).

In one example, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, 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 (e.g., at least 16, 17, 18, 19, 20, 21, 22, or 23) contiguous nucleotides differing by no more than 3 (e.g., by no more than 0, 1 or 2) nucleotides from an antisense sequence listed in any one of Tables 21 to 40, or an unmodified version of an antisense sequence (e.g., a version having the same nucleotide sequence except that some or all of the nucleotides are unmodified) listed in any one of Tables 21 to 40. In one example, the antisense sequence comprises at least 15 (e.g., at least 16, 17, 18, 19, 20, 21, 22, or 23) contiguous nucleotides differing by no more than 3 (e.g., by no more than 0, 1 or 2) nucleotides from (i) the antisense sequence of AD-60489, AD-60519, or AD-61193 or (ii) an unmodified version of any one of these sequences. In some examples, the antisense strand comprises at least 15 (e.g., at least 16, 17, 18, 19, 20, 21, 22, or 23) contiguous nucleotides differing by no more than 3 (e.g., by no more than 0, 1 or 2) nucleotides from the sequence of UAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 4153) or UAAGAUGAGACACUCTUUCUGGU (SEQ ID NO: 4154). In an example, the antisense sequence targets positions 871-893 of NM_000688.4 (SEQ ID NO:1). In some examples, the sense strand comprises the sequence of CAGAAAGAGUGUCUCAUCUUA (SEQ ID NO:

4155). In some examples, one or more nucleotides of the antisense strand and/or sense strand are modified as described herein.

In some examples, the dsRNA is not a sense and/or antisense sequence listed in any one of Tables 2, 3, 6, 7, 8, 9, 14, 15, 18 or 20.

In one example, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, 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 (e.g., at least 16, 17, 18, 19, 20, 21, 22, or 23) contiguous nucleotides differing by no more than 3 nucleotides, no more than 2 nucleotides, or no more than one nucleotide, from the antisense sequence of AD-60519. In some examples, one or more nucleotides are modified as described herein.

In one example, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, 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 (e.g., at least 16, 17, 18, 19, 20, 21, 22, or 23) contiguous nucleotides differing by no more than 3 (e.g., by no more than 0, 1 or 2) nucleotides from the antisense sequence of AD-60489, or a derivative of AD-60489 as described herein. In some examples, one or more nucleotides are modified as described herein, *e.g.*, one or more (or all) nucleotides of AD-60489 are modified as described herein. In some examples, the derivative of AD-60489 is AD-60501, AD-60519, AD-60901, AD-60495, AD-60900, AD-60935, AD-60879, AD-61190, AD-61191, AD-60865, AD-60861, AD-60876, AD-61193, AD-60519, AD-60519, or AD-60901. In some examples, the derivative of AD-60489 is AD-60519. In some examples, the derivative of AD-60489 is AD-61193.

In one example, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, 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 (e.g., at least 16, 17, 18, 19, 20, 21, 22, or 23) contiguous nucleotides differing by no more than 3 (e.g., by no more than 0, 1 or 2) nucleotides from a derivative of AD-58632 described herein. In some examples, one or more nucleotides are modified as described herein, *e.g.*, one or more (or all) nucleotides of AD-58632

are modified as described herein. In some examples, the derivative of AD-58632 is AD-60405, AD-60887, AD-60923, AD-60434, AD-60892, AD-60419, AD-60924, AD-60445, AD-60925, and AD-60926, AD-60820, AD-60843, AD-60819, AD-61140, AD-61141, AD-61142, AD-60835, AD-60839, AD-61143, AD-61144, AD-61145, AD-61146, AD-60892, or AD-60419. In some examples, the derivative of AD-58632 is AD-60819.

In some examples, the dsRNA has an IC_{50} of less than 1nM. In some examples, the dsRNA has an IC_{50} in the range of 0.01-1nM. In some examples, the dsRNA has an IC_{50} of less than 0.05 nM. In some examples, the dsRNA has an IC_{50} of less than 0.02 nM. In some examples, the dsRNA has an IC_{50} of less than 0.01 nM. In some examples, the IC_{50} is determined as described herein in the Examples.

In some examples, the dsRNA has a single dose ED50 of less than about 10 mg/kg. In some examples, the dsRNA has a single dose ED50 of less than about 5 mg/kg. In some examples, the EC50 is determined as described herein in the Examples.

In some examples, the dsRNA shows improved activity compared with AD-58632. In some examples, the dsRNA shows improved activity compared with AD-60489. In some examples, the dsRNA shows improved activity compared with AD-58632 and AD-60489.

In some examples, the dsRNA is AD-60501, AD-60519, AD-60901, AD-60495, AD-60900, AD-60935, AD-60879, AD-61190, AD-61191, AD-60865, AD-60861, AD-60876, AD-61193, AD-60519, AD-60519, AD-60901, AD-60405, AD-60887, AD-60923, AD-60434, AD-60892, AD-60419, AD-60924, AD-60445, AD-60925, AD-60926, AD-60820, AD-60843, AD-60819, AD-61140, AD-61141, AD-61142, AD-60835, AD-60839, AD-61143, AD-61144, AD-61145, AD-61146, AD-60892, or AD-60419 (*e.g.*, including the nucleotide sequence and/or one or more (*e.g.*, all) of the modifications of the aforesaid dsRNAs). In some examples, the dsRNA comprises an antisense strand that comprises, or consists of, an antisense sequence (and/or one or more (*e.g.*, all) of the modifications)) selected from AD-60501, AD-60519, AD-60901, AD-60495, AD-60900, AD-60935, AD-60879, AD-61190, AD-61191, AD-60865, AD-60861, AD-60876, AD-61193, AD-60519, AD-60519, AD-60901, AD-60405, AD-60887, AD-60923, AD-60434, AD-60892, AD-60419, AD-60924, AD-60445, AD-60925, AD-60926, AD-60820, AD-60843, AD-60819, AD-61140, AD-61141, AD-61142, AD-60835, AD-60839, AD-61143, AD-61144, AD-61145, AD-61146, AD-60892, or AD-60419. In some examples, the dsRNA

comprises a sense strand that comprises, or consists of, a sense sequence (and/or one or more (*e.g.*, all) of the modifications)) selected from AD-60501, AD-60519, AD-60901, AD-60495, AD-60900, AD-60935, AD-60879, AD-61190, AD-61191, AD-60865, AD-60861, AD-60876, AD-61193, AD-60519, AD-60519, AD-60901, AD-60405, AD-60887, AD-60923, AD-60434, AD-60892, AD-60419, AD-60924, AD-60445, AD-60925, AD-60926, AD-60820, AD-60843, AD-60819, AD-61140, AD-61141, AD-61142, AD-60835, AD-60839, AD-61143, AD-61144, AD-61145, AD-61146, AD-60892, or AD-60419.

In some examples, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, wherein the dsRNA comprises (i) an antisense strand that comprises, or consists of, the sequence of UAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 4153) or UAAGAUGAGACACUCTUUCUGGU (SEQ ID NO: 4154) and/or (ii) a sense strand that comprises, or consists of, the sequence of CAGAAAGAGUGUCUCAUCUUA (SEQ ID NO: 4155). In some examples, one or more nucleotides of the antisense strand and/or sense strand are modified as described herein.

In some examples, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, wherein the dsRNA comprises (i) an antisense strand that comprises, or consists of, the antisense sequence of AD-60489 and/or (ii) a sense strand that comprises, or consists of, the sense sequence of AD-60489 (wherein the sense and/or antisense sequence includes one or more (*e.g.*, all) of the modifications of the sense strand and/or antisense strand of AD-60489).

In some examples, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, wherein the dsRNA comprises (i) an antisense strand that comprises, or consists of, the antisense sequence of AD-60519 and/or (ii) a sense strand that comprises, or consists of, the sense sequence of AD-60519 (wherein the sense and/or antisense sequence includes one or more (*e.g.*, all) of the modifications of the sense strand and/or antisense strand of AD-60519).

In some examples, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, wherein the dsRNA comprises (i) an antisense strand that comprises, or consists of, the antisense sequence of AD-61193 and/or (ii) a sense strand that comprises, or consists of, the sense sequence of AD-61193 (wherein the sense and/or antisense sequence

includes one or more (*e.g.*, all) of the modifications of the sense strand and/or antisense strand of AD-61193).

In some examples, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, wherein the dsRNA comprises (i) an antisense strand that comprises, or consists of, the antisense sequence of AD-60819 and/or (ii) a sense sequence that comprises, or consists of, the sense sequence of AD-60819 (wherein the sense and/or antisense sequence includes one or more (*e.g.*, all) of the modifications of the sense strand and/or antisense strand of AD-60819).

In some examples, a dsRNA for inhibiting expression of ALAS1 is provided, wherein the dsRNA comprises (i) an antisense strand that comprises, or consists of, the antisense sequence of AD-60489, AD-60519, AD-61193, or AD-60819 (or a corresponding unmodified antisense sequence) and/or (ii) a sense strand that comprises, or consists of, the sense sequence of AD-60489, AD-60519, AD-61193, or AD-60819 (or a corresponding unmodified antisense sequence). In some examples, the dsRNA comprises (i) an antisense strand that consists of the antisense sequence of AD-60489, AD-60519, AD-61193, or AD-60819 and/or (ii) a sense strand that consists of the sense sequence of AD-60489, AD-60519, AD-61193, or AD-60819, except that the antisense strand and/or sense strand of the dsRNA differs by 1, 2, or 3 nucleotides from the corresponding antisense and/or sense sequence of AD-60489, AD-60519, AD-61193, or AD-60819.

The sequences and modifications of AD-60489, AD-60519, AD-61193, and AD-60819 are shown in Table 44 below.

Table 44: Sequences and Modifications of AD-60489, AD-60519, AD-61193, AD-60819

Target sites of antisense sequence on NM_000688.4	Duplex Name	Sense Sequence (5'-3')	Antisense Sequence (5'-3')	Corresponding unmodified sense sequence	Corresponding unmodified antisense sequence
871-893	AD-60489	CfsasGfaAfaGfaGfUfGfuCfuCfaUfcUfuAfL96 (SEQ ID NO: 4156)	usAfsaGfaUfgAfgAfcacUfcUfuUfcUfgsgsu (SEQ ID NO: 4157)	CAGAAAGAGUGUCUCAUCUUA (SEQ ID NO: 4158)	UAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 4159)
871-893	AD-60519	csasgaaaGfaGfuGfuCfuCfaucuuaL96 (SEQ ID NO: 4160)	usAfsAfGfaUfgAfgAfcAfcUfcUfuUfcUfgsgsu (SEQ ID NO: 4161)	CAGAAAGAGUGUCUCAUCUUA (SEQ ID NO: 4162)	UAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 4163)
871-893	AD-61193	csasgaaaGfaGfuGfuCfuCfaucuuaL96 (SEQ ID NO: 4164)	usAfsaGfaUfgAfgAfcacUfcdTuUfcUfgsgsu (SEQ ID NO: 4165)	CAGAAAGAGUGUCUCAUCUUA (SEQ ID NO: 4166)	UAAGAUGAGACACUCTUUCUGGU (SEQ ID NO: 4167)
873-895	AD-60819	GfsasAfaGfaGfuGfuCfuCfaucuuCfuuL96 (SEQ ID NO: 4168)	asAfsgAfaGfaugAfgAfcAfcucuuucsusg (SEQ ID NO: 4169)	GAAAGAGUGUCUCAUCUUCUU (SEQ ID NO: 4170)	AAGAAGAUGAGACACUCUUUCUG (SEQ ID NO: 4171)

wherein c, a, g, u = 2'-OMe ribonucleosides; Af, Cf, G, Uf = 2'F ribonucleosides; S = phosphorothioate; L96 has the structure depicted in Table 1.

In some examples, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, wherein the dsRNA comprises (i) an antisense strand that comprises, or consists of, the antisense sequence of AD-60489, AD-60519, or AD-61193 and/or (ii) a sense strand that comprises, or consists of, the sense sequence of AD-60489, AD-60519, or AD-61193 (including the nucleotide sequence and one or more (*e.g.*, all) of the modifications of the sense strand and/or antisense strand of AD-60489, AD-60519, or AD-61193).

In some examples, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, wherein the dsRNA is AD-60489, AD-60519, AD-61193, or AD-60819. In some examples, a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1 is provided, wherein the dsRNA is AD-60489, AD-60519, or AD-61193 (*e.g.*, including the nucleotide sequence and/or one or more (*e.g.*, all) of the modifications of AD-60489, AD-60519, or AD-61193).

In some examples, the dsRNA is, comprises, or consists of, AD-60489 (*e.g.*, including the nucleotide sequence and/or one or more (*e.g.*, all) of the modifications of AD-60489).

In some examples, the dsRNA is, comprises, or consists of, AD-60519 (*e.g.*, including the nucleotide sequence and/or one or more (*e.g.*, all) of the modifications of AD-60519).

In some examples, the dsRNA is, comprises, or consists of, AD-61193 (*e.g.*, including the nucleotide sequence and/or one or more (*e.g.*, all) of the modifications of AD-61193).

In some examples, the dsRNA is, comprises, or consists of, AD-60819 (*e.g.*, including the nucleotide sequence and/or one or more (*e.g.*, all) of the modifications of AD-60819).

In some examples, the dsRNA (e.g., AD-60489, AD-60519, AD-61193, AD-60819, or another dsRNA disclosed herein in any one of Tables 21 to 40) is effective to suppress the liver level of ALAS1 mRNA, e.g., to achieve silencing of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% (e.g., such that ALAS1 mRNA levels are decreased to 90% or less, 80% or less, 70% or less, 60% or less, 50% or less, 40% or less, 30% or less, or 20% or less of a control level of liver ALAS1 mRNA, e.g., the level in an untreated individual or group of individuals, e.g., an individual or group of individuals treated with PBS only). In some examples, the effectiveness of the dsRNA in suppressing the liver level of ALAS1 mRNA is assessed using a non-human primate model, e.g., as described herein in the Examples.

In some examples, the dsRNA (e.g., AD-60489, AD-60519, AD-61193, AD-60819, or another dsRNA disclosed herein in any one of Tables 21 to 40) is effective to suppress the circulating level of ALAS1 mRNA, e.g., to achieve silencing of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% (e.g., such that ALAS1 mRNA levels are decreased to 90% or less, 80% or less, 70% or less, 60% or less, 50% or less, 40% or less, 30% or less, or 20% or less of a control level of circulating ALAS1 mRNA, e.g., the level prior to treatment with the dsRNA, or the level in an untreated individual or group of individuals). In some examples, the effectiveness of the dsRNA in suppressing the circulating level of ALAS1 mRNA is assessed using a non-human primate model, e.g., as described herein in the Examples. In some examples, the circulating level of ALAS1 mRNA is assessed using a circulating extracellular RNA detection (cERD) assay, *e.g.*, as described herein or in Sehgal, A. et al. Quantitation of tissue-specific target gene modulation using circulating RNA (Poster presented on February 9, 2012 at the Keystone Gene Silencing by small RNAs symposium (Vancouver, February 7-12, 2012) or

Sehgal, A. et al. Tissue-specific gene silencing monitored in circulating RNA, *RNA*, 20: 1-7, published online December 19, 2013.

The cERD method can be applied to any appropriate biological sample. In some examples, the circulating level of ALAS1 mRNA is assessed using a blood sample, e.g., a serum sample. In some examples, the circulating level of ALAS1 mRNA is assessed using a urine sample.

In some examples, the dsRNA is a derivative of AD-60489 that is disclosed herein, e.g., in any one of the tables herein. In some examples, the dsRNA shows improved activity compared with AD-60489. In some such examples, the dsRNA is AD-60519.

In some examples, the dsRNA is a derivative of AD-58632 that is disclosed herein, e.g., in any one of the tables herein. In some examples, the dsRNA shows improved activity compared with AD-58632.

In some examples, improved activity is indicated by a lower IC50, e.g., as determined based on in vitro assays, e.g., as described herein, e.g., in the Examples.

In some examples, improved activity is indicated by a lower effective dose. The effective dose may be determined based on the administration of a single dose or multiple repeated doses. In some examples, the effective dose is determined based on the single dose ED50. In some examples, the effective dose or the single dose ED50 is determined based on an in vivo assay. In some examples, the in vivo assay is conducted in a non-human animal, e.g., in a rat, in a non-human primate, or in a mouse.

In some examples, the effective dose is determined based on the dose required to obtain a reduction of in a level of ALAS1 mRNA (e.g., a liver level of ALAS1 mRNA and/or a circulating level of ALAS1 mRNA), e.g., as described herein in the Examples. In some examples, circulating mRNA is assessed using the cERD assay.

In some examples, the effective dose is determined based on the dose required to obtain a reduction of a level (e.g., a urine and/or plasma level) of ALA and/or PBG.

In some examples, the effective dose is determined based on the dose required to obtain a particular treatment effect described herein, e.g., prevention or reduction of symptoms associated with a porphyria.

In some examples, improved activity is indicated by the achievement of a higher liver level of the dsRNA. In some examples, a higher liver level is obtained after a single dose of dsRNA

(e.g., a dose of 1, 2.5, 3, 5, or 10 mg/kg). In some examples, a higher liver level is obtained after multiple doses of dsRNA have been administered (e.g., 2-10 daily or weekly doses of 1, 2.5, 3, 5, or 10 mg/kg).

In one example, the iRNA encompasses 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 ALAS1 mRNA (*e.g.*, a human ALAS1 mRNA as provided in SEQ ID NO:1 or SEQ ID NO:382).

In one example, 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.

In some examples, the iRNA is 19-21 nucleotides in length. In some examples, the iRNA is 19-21 nucleotides in length and is in a lipid formulation, e.g. a lipid nanoparticle (LNP) formulation (e.g., an LNP11 formulation).

In some examples, the iRNA is 21-23 nucleotides in length. In some exampless, the iRNA is 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.

In some examples the iRNA is from about 15 to about 25 nucleotides in length, and in other examples the iRNA is 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 herein. In one example, the iRNA targeting ALAS1 is formulated in a stable nucleic acid lipid particle (SNALP).

In one example, 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 21 to 40 and a second sequence that is selected from the group consisting of the corresponding antisense sequences of Tables 21 to 40.

The iRNA molecules featured herein can include naturally occurring nucleotides or can include at least one modified nucleotide. In some examples, the at least one modified nucleotide include one or more of a modification on the nucleotide chosen from the group consisting of a locked nucleic acid (LNA), an acyclic nucleotide, a hexitol or hexose nucleic acid (HNA), a cyclohexene nucleic acid (CeNA), 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy, 2'-hydroxyl, or any combination thereof. In one example, the at least one modified nucleotide includes, but is not limited to a 2'-O-methyl modified nucleotide, 2′-fluoro modified nucleotide, a nucleotide having a 5'-phosphorothioate group, and a terminal nucleotide linked to a ligand, *e.g.*, an N-acetylgalactosamine (GalNAc) or 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 acyclic 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 disclosed in Tables 21-40, and a second sequence selected from the group consisting of the corresponding antisense sequences disclosed in Tables 21-40.

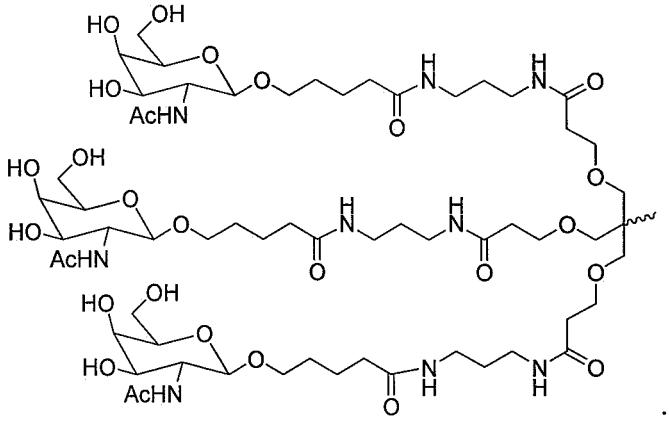
In one example, an iRNA as described herein targets a wildtype ALAS1 RNA transcript variant, and in another example, the iRNA targets a mutant transcript (*e.g.*, an ALAS1 RNA carrying an allelic variant). For example, an iRNA featured in the disclosure can target a polymorphic variant, such as a single nucleotide polymorphism (SNP), of ALAS1. In another example, the iRNA targets both a wildtype and a mutant ALAS1 transcript. In yet another example, the iRNA targets a particular transcript variant of ALAS1 (*e.g.*, human ALAS1 variant 1). In yet another example, the iRNA agent targets multiple transcript variants (*e.g.*, both variant 1 and variant 2 of human ALAS1).

In one example, an iRNA featured in the disclosure targets a non-coding region of an ALAS1 RNA transcript, such as the 5' or 3' untranslated region of a transcript.

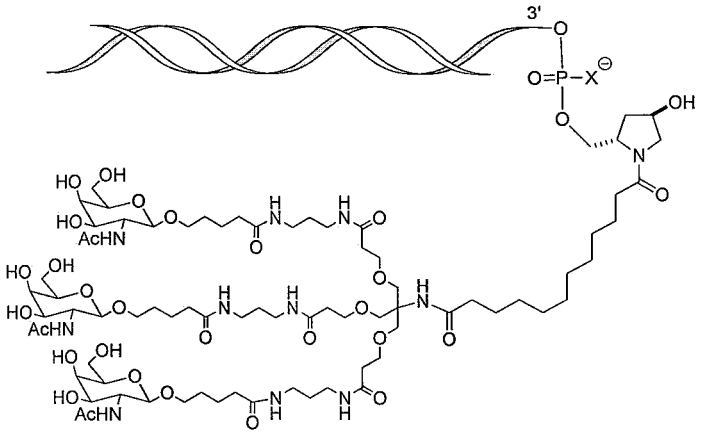
In some examples, an iRNA as described herein is in the form of a conjugate, e.g., a carbohydrate conjugate, which may serve as a targeting moiety and/or ligand, as described herein. In one example, the conjugate is attached to the 3' end of the sense strand of the

dsRNA. In some examples, the conjugate is attached via a linker, e.g., via a bivalent or trivalent branched linker.

In some examples, the conjugate comprises one or more N-acetylgalactosamine (GalNAc) derivatives. Such a conjugate is also referred to herein as a GalNAc conjugate. In some examples, the conjugate targets 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. In particular examples, the conjugate is

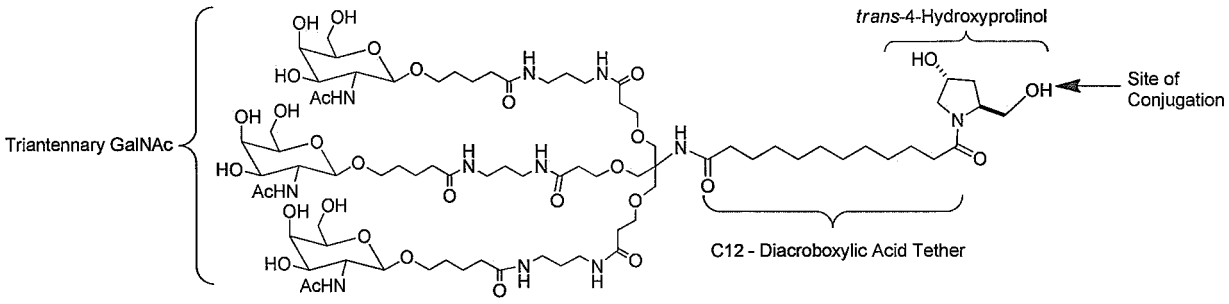


In some examples, the RNAi agent is attached to the carbohydrate conjugate via a linker, e.g., a linker as shown in the following schematic, wherein X is O or S



In some examples, X is O. In some examples, X is S.

In some examples, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below



In one example, the dsRNA has one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen or all of the following:

(i) is chemically synthesized, *e.g.*, is synthesized by solid phase oligonucleotide synthesis;

(ii) all the nucleotides in the dsRNA are modified, *e.g.*, all the nucleotides are 2'-OMe or 2'-F modified, or a combination of 2'-OMe and 2'-F modified;

(iii) all nucleotides are connected through 3'-5' phosphodiester linkages;

(iv) the sense strand comprises or consists of 21 nucleotides;

(v) the antisense sense strand comprises or consists of 23 nucleotides;

(vi) has a blunt-end at the 3'-end of sense strand;

(vii) has a 3'-overhang, *e.g.*, has a two-nucleotide overhang, at the 3'-end of the antisense strand;

(viii) is covalently attached to a ligand containing three N-acetylgalactosamine (GalNAc) moieties;

(ix) the 3'-end of the sense strand is conjugated to the triantennary GalNAc moiety (*e.g.*, referred to herein as L96 as defined in Table 1). In one example, the 3'-end is linked to the triantennary GalNAc moiety through a phosphodiester linkage;

(x) has an antisense strand that comprises one or more (*e.g.*, four) phosphorothioate linkages. In one example, the phosphorothioate linkages are located at the 3' end and at the 5' end of the antisense strand. In one example, two phosphorothioate linkages are located at the 3' end and two phosphorothioate linkages are located at the 5' end of the antisense strand;

(xi) has a sense strand that comprises one or more (*e.g.*, two) phosphorothioate linkages. In one example, the one or more (*e.g.*, two) phosphorothioate linkages are located at the 5' end of the sense strand;

(xii) 21 nucleotides of the sense strand hybridize to the complementary 21 nucleotides of the antisense strand;

(xiii) forms 21 nucleotide base pairs and a two-base overhang at the 3'-end of the antisense strand;

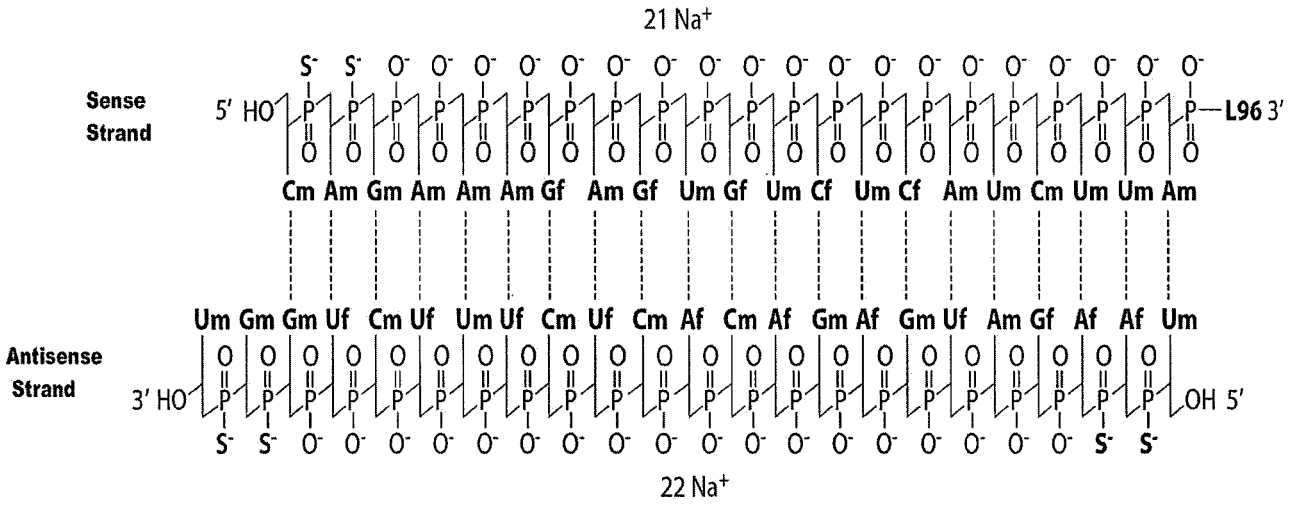
(xiv) comprises, or consists of, a sense and antisense strand having the sequence of AD-60519;

(xv) has a sense strand with 10, 12, 14, 16, 18, 19, 20 or all of the modifications of the sense strand of AD-60519;

(xvi) has an antisense strand with 10, 12, 14, 16, 18, 19, 20 or all of the modifications of the antisense strand of AD-60519; or

(xvii) has the duplex sequence and all the modifications of AD-60519.

In some examples, the dsRNA is in the form of a conjugate having the following structure (also referred to herein as AD-60519 or ALN-60519) (SEQ ID NOS 5238-5239, respectively, in order of appearance):



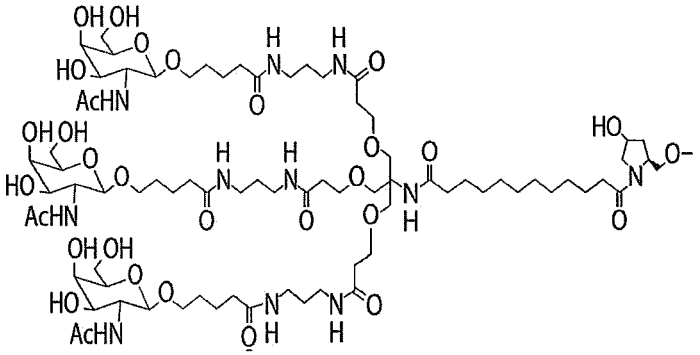
ALN-60519

Af, Cf, Gf, Uf = 2'-F ribonucleosides

Am, Cm, Gm, Um = 2'-OMe ribonucleosides

S = phosphorothioate

L96 =



Also described herein is a composition, e.g., a pharmaceutical composition, that includes one or more of the iRNAs described herein and a pharmaceutically acceptable carrier or delivery vehicle. In one example, the composition is used for inhibiting the expression of an

ALAS1 gene in an organism, generally a human subject. In one example, the composition is used for treating a porphyria, *e.g.*, AIP.

In one example, an iRNA provided herein 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.

In a further example, an iRNA provided herein 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 RNA transcript, wherein each strand has about 14 to about 30 nucleotides, wherein said double stranded RNAi agent is represented by formula (III):

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

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$

(III)

wherein:

i, j, k, and l 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'.

In some examples, the sense strand is conjugated to at least one ligand.

In some examples, i is 1; j is 1; or both i and j are 1.

In some examples, k is 1; l is 1; or both k and l are 1.

In some examples, XXX is complementary to X'X'X', YYY is complementary to Y'Y'Y', and ZZZ is complementary to Z'Z'Z'.

In some examples, the Y'Y'Y' motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end.

In some examples, the Y' is 2'-O-methyl.

In some examples, the duplex region is 15-30 nucleotide pairs in length.

In some examples, the duplex region is 17-23 nucleotide pairs in length.

In some examples, the duplex region is 19-21 nucleotide pairs in length.

In some examples, the duplex region is 21-23 nucleotide pairs in length.

In some examples, the modification on the nucleotide is selected from the group consisting of a locked nucleic acid (LNA), an acyclic nucleotide, a hexitol or hexose nucleic acid (HNA), a cyclohexene nucleic acid (CeNA), 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl, 2'-C- allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, and any combination thereof.

In some examples, 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.

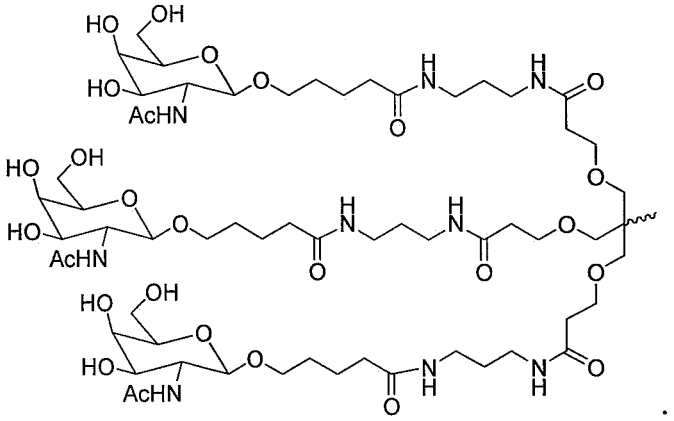
In some examples, the modifications on the nucleotides are 2′-O-methyl, 2′-fluoro or both.

In some examples, the ligand comprises a carbohydrate.

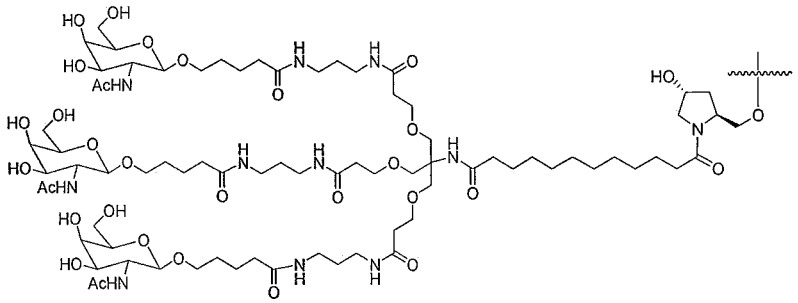
In some examples, the ligand is attached via a linker.

In some examples, the linker is a bivalent or trivalent branched linker.

In some examples, the ligand is



In some examples, the ligand and linker are as shown in Formula XXIV:



In some examples, the ligand is attached to the 3’ end of the sense strand.

In some examples, the dsRNA consists of or comprises a nucleotide sequence selected from the group of sequences provided in Tables 21-40.

In a further example, an iRNA provided herein is a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1, 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 21-40. In some examples, the nucleotides of the antisense strand have fewer modifications, more modifications, or different modifications compared with the antisense sequences listed in any one of Tables 21-40.

In some examples, the sense and antisense sequences are those of a duplex disclosed herein that suppresses ALAS1 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.

In some examples, ALAS1 mRNA expression is assessed based on an ALAS1 mRNA level in the liver, e.g., as assessed using a liver biopsy sample. In some examples, ALAS1 mRNA expression is assessed based on an ALAS1 mRNA level in a biological fluid, e.g., blood, serum, plasma, cerebrospinal fluid, or urine. In some examples, ALAS1 mRNA expression is assessed using a circulating extracellular RNA detection (cERD) assay, e.g., a cERD assay as described herein or in Sehgal, A. et al. Quantitation of tissue-specific target gene modulation using circulating RNA (Poster presented on February 9, 2012 at the Keystone Gene Silencing by small RNAs symposium (Vancouver, February 7-12, 2012) or Sehgal, A. et al. Tissue-specific gene silencing monitored in circulating RNA, *RNA*, 20: 1-7, published online December 19, 2013.

In some examples, the dsRNA comprises at least one modified nucleotide.

In some examples, 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.

In some examples, 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

acyclic nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

In some examples, the region of complementarity is at least 17 nucleotides in length.

In some examples, the region of complementarity is between 19 and 21 nucleotides in length.

In some examples, the region of complementarity is 19 nucleotides in length.

In some examples, each strand is no more than 30 nucleotides in length.

In some examples, at least one strand comprises a 3' overhang of at least 1 nucleotide. In examples, the antisense strand comprises a 3' overhang of at least 1 nucleotide.

In some examples, at least one strand comprises a 3' overhang of at least 2 nucleotides. In examples, the antisense strand comprises a 3' overhang.of at least 2 nucleotides. In examples, the antisense strand comprises a 3' overhang.of 2 nucleotides.

In some examples, a dsRNA described herein further comprises a ligand.

In some examples, the ligand is a GalNAc ligand.

In some examples, the ligand targets the dsRNA to hepatocytes.

In some examples, the ligand is conjugated to the 3' end of the sense strand of the dsRNA.

In some examples, the region of complementarity consists of an antisense sequence selected from the antisense sequences listed in Tables 21-40, or a corresponding antisense sequence in which some or all of the nucleotides are unmodified. In some examples, the region of complementarity consists of the sequence UAAGAUGAGACACUCUUUCUGGU (SEQ ID NO: 4153) or UAAGAUGAGACACUCTUUCUGGU (SEQ ID NO: 4154). In some examples, the region of complementarity consists of the antisense sequence of the duplex AD-60489. In some examples, the region of complementarity consists of the antisense sequence of the duplex AD-60519.

In some examples, the region of complementarity consists of an antisense sequence selected from a duplex disclosed herein that suppresses ALAS1 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.

In some examples, the dsRNA comprises a sense strand consisting of a sense strand sequence selected from Tables 21-40, and an antisense strand consisting of an antisense sequence selected from Tables 21-40. In some examples, the dsRNA comprises a pair of corresponding sense and antisense sequences selected from those of the duplexes disclosed in Tables 21-40.

In one example, the disclosure provides a cell containing at least one of the iRNAs (e.g., dsRNAs) featured herein. The cell is generally a mammalian cell, such as a human cell. In some examples, the cell is an erythroid cell. In other examples, the cell is a liver cell (*e.g.*, a hepatocyte).

Also described herein is a pharmaceutical composition for inhibiting expression of an ALAS1 gene, the composition comprising an iRNA (e.g., a dsRNA) described herein.

In some examples of the pharmaceutical compositions described herein, the iRNA (e.g., dsRNA) is administered in an unbuffered solution. In some examples, the unbuffered solution is saline or water, e.g., water for injection.

In some examples, the pharmaceutical composition comprises AD-60519 and water for injection. In some examples, the composition comprises about 100 to 300 mg/mL, *e.g.*, 200 mg/mL, of AD-60519. In some examples, the composition has a pH of 6.0-7.5, e.g., about 7.0. In some examples, the composition is for subcutaneous injection. In some examples, the pharmaceutical composition is packaged in a container (e.g., a glass vial, e.g., a 2 mL glass vial,) at a volume of about 0.3 to 1 mL, *e.g.*, 0.55 mL. In some examples, the pharmaceutical composition is ALN-AS1 as described herein in the examples.

In some examples of the pharmaceutical compositions described herein, the iRNA (e.g., dsRNA is administered with a buffer solution. In some examples, the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof. In some examples, the buffer solution is phosphate buffered saline (PBS).

In some examples of the pharmaceutical compositions described herein, the iRNA (e.g., dsRNA) is targeted to hepatocytes.

In some examples of the pharmaceutical compositions described herein, the composition is administered intravenously.

In some examples of the pharmaceutical compositions described herein, the composition is administered subcutaneously.

In some examples, a pharmaceutical composition comprises 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.

In some examples, a pharmaceutical composition comprises an iRNA (e.g., a dsRNA) described herein that comprises a ligand (e.g., a GalNAc ligand), and the pharmaceutical composition is administered subcutaneously. In some examples, the ligand targets the iRNA (e.g., dsRNA) to hepatocytes.

In certain examples, a pharmaceutical composition, e.g., a composition described herein, includes a lipid formulation. In some examples, the RNAi agent is in a LNP formulation, e.g., a MC3 formulation. In some examples, the LNP formulation targets the RNAi agent to a particular cell, e.g., a liver cell, e.g., a hepatocyte. In some examples, the lipid formulation is a LNP11 formulation. In some examples, the composition is administered intravenously.

In another example, the pharmaceutical composition is 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. In another example, 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.

In another example, a composition containing an iRNA featured in the disclosure, *e.g.*, a dsRNA targeting ALAS1, is 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). In another example, a composition containing an iRNA featured in the disclosure, *e.g.*, a dsRNA targeting AIP, is 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 disclosure 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 disclosure 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.

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 examples in which glucose is administered by other means are also encompassed.

In one example, an ALAS1 iRNA is 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). In another example, an ALAS1 iRNA and the non-iRNA therapeutic agent or therapeutic regimen are administered at the same time.

Also 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 ALAS1 gene, thereby inhibiting expression of the ALAS1 gene in the cell.

Also 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 ALAS1, 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 ALAS1, inhibits expression of an ALAS1 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 ALAS1 gene, thereby reducing or inhibiting expression of an ALAS1gene in the cell.

In some examples of the foregoing methods of inhibiting ALAS1 expression in a cell, the cell is treated *ex vivo*, *in vitro*, or *in vivo*. In some examples, the cell is a hepatocyte.

In some examples, the cell is present in a subject in need of treatment, prevention and/or management of a disorder related to ALAS1 expression.

In some examples, the disorder is a porphyria. In some examples, the porphyria is acute intermittent porphyria or ALA-dehydratase deficiency porphyria.

In some examples, 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. In some examples, the porphyria is a homozygous dominant hepatic porphyria (e.g., homozygous dominant AIP, HCP, or VP) or hepatoerythropoietic porphyria. In some examples, the porphyria is a dual porphyria.

In some examples, the expression of ALAS1 is inhibited by at least 30%.

In some examples, the iRNA (e.g., dsRNA) has an IC_{50} in the range of 0.01-1nM.

In certain examples, the cell (*e.g.*, the hepatocyte) is a mammalian cell (*e.g.*, a human, non-human primate, or rodent cell).

In one example, 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).

In one example, the subject is 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. In some examples, the disorder is an acute hepatic porphyria, *e.g.*, ALA deyhdratase deficiency porphyria (ADP), AIP, HCP, or VP. In specific some examples, the disorder is ALA deyhdratase deficiency porphyria (ADP) or AIP.

In some examples, 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. In some examples, the porphyria is a homozygous dominant hepatic porphyria (e.g., homozygous dominant AIP, HCP, or VP) or hepatoerythropoietic porphyria, In some examples, the porphyria is a dual porphyria.

In one example, the dsRNA introduced reduces or inhibits expression of an ALAS1 gene in the cell.

In one example, the dsRNA introduced reduces or inhibits expression of an ALAS1 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 ALAS1 gene is likely to reduce the level of one or more porphyrin precursors, porphyrins or porphyrin products or metabolites.

In other examples, 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). In one example, the method includes 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.

Also described herein is a method of treating and/or preventing a disorder related to ALAS1 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.

Also 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.

In one example, subject (e.g., the patient) has a porphyria. In another example, the subject (e.g., patient) is at risk for developing a porphyria. In some examples, administration of the iRNA targeting ALAS1 alleviates or relieves the severity of at least one symptom of a disorder related to ALAS1 in the patient.

In one example, the subject is 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. In a further example, the porphyria is an acute hepatic porphyria, *e.g.*, ALA deyhdratase deficiency porphyria (ADP), AIP, HCP, or VP. In some such examples, the disorder is ALA deyhdratase deficiency porphyria (ADP) or AIP.

In some examples the subject has, or is at risk for developing, a porphyria. In examples, 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. In some examples, the porphyria is a homozygous dominant hepatic porphyria (e.g., homozygous dominant AIP, HCP, or VP) or hepatoerythropoietic porphyria, In some examples, the porphyria is a dual porphyria.

In some examples, a porphyria, a symptom of porphyria, a prodrome, or an attack of porphyria is induced by exposure to a precipitating factor, as described herein. In some examples, the precipitating factor is a chemical exposure. In some examples, the precipitating factor is a drug, e.g., a prescription drug or an over the counter drug. In some examples, the precipitating factor is the menstrual cycle, e.g., a particular phase of the menstrual cycle, e.g., the luteal phase.

In some examples, the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered after an acute attack of porphyria.

In some examples, the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered during an acute attack of porphyria.

In some examples, the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered prophylactically to prevent an acute attack of porphyria.

In some examples, the iRNA (e.g., dsRNA) is formulated as an LNP formulation.

In some examples, the iRNA (e.g., dsRNA) is in the form of a GalNAc conjugate.

In some examples, iRNA (e.g., dsRNA) is administered at a dose of 0.05-50 mg/kg.

In some examples, the iRNA (e.g., dsRNA) is administered at a concentration of 0.01 mg/kg-5 mg/kg bodyweight of the subject.

In some examples, the iRNA (e.g., dsRNA) is formulated as an LNP formulation and is administered at a dose of 0.05-5 mg/kg.

In some examples, 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. In certain examples, the iRNA in the GalNAc conjugate is administered at a dose of less than 10 mg/kg (e.g., 5 mg/kg or less) e.g., once per week; e.g., a dose of 1 mg/kg or less, 2.5 mg/kg or less, or 5 mg/kg or less, e.g., once per week. In one example, iRNA in the GalNAc conjugate is administered at a dose of about 2.5 mg/kg or less, e.g., once per week. In one example, the administration of the iRNA in the GalNAc conjugate is subcutaneous.

In some examples, the iRNA (e.g., dsRNA) is in the form of a GalNAc conjugate and is administered, e.g., subcutaneously, at a dose of 0-5 mg/kg, e.g. 0-2.5 mg/kg or 1-2.5 mg/kg. In some examples, the iRNA is administered weekly. In some examples, the iRNA is administered as a composition comprising the iRNA and water for injection. In some examples, the iRNA is AD-60519. In some examples, the composition comprises the iRNA, e.g., AD-60519, at a concentration of about 200 mg/mL.

In some examples, the method decreases a level of a porphyrin or a porphyrin precursor in the subject.

In some examples, 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%.

In some examples, the porphyrin precursor is δ-aminolevulinic acid (ALA) or porphopilinogen (PBG).

In some examples, the iRNA (e.g., dsRNA) has an IC_{50} in the range of 0.01-1nM.

In some examples, a method described herein

(i) ameliorates a symptom associated with an ALAS1 related disorder (e.g., a porphyria)

(ii) inhibits ALAS1 expression in the subject (e.g., as assessed using the cERD assay),

(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).

In some examples, the method ameliorates pain and/or progressive neuropathy.

In some examples, the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered according to a dosing regimen.

In some examples, the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered before or during an acute attack of porphyria. In some examples, the iRNA is administered before an acute attack of porphyria.

In some examples, the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered during a prodrome. In some examples, the prodrome is characterized by abdominal pain, nausea, psychological symptoms (e.g., anxiety), restlessness and/or insomnia.

In some examples, 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.

In some examples, the method ameliorates or prevents cyclical attacks of porphyria, e.g., by reducing the severity, duration, or frequency of attacks. In some examples, the cyclical attacks are associated with a precipitating factor. In some examples, the precipitating factor is the menstrual cycle, e.g., a particular phase of the menstrual cycle, e.g., the luteal phase.

In some examples, the subject has an elevated level of ALA and/or PBG. In some examples, the level of ALA and/or PBG is elevated in plasma or urine from the subject. In some examples,

the subject has or is at risk for developing a porphyria, e.g., a hepatic porphyria. In some examples, the subject is asymptomatic. In some examples, the subject carries a genetic alteration (e.g., a gene mutation) associated with a porphyria, as described herein. In some examples, 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). In some examples, 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). In some examples, the pain is abdominal pain.

In some examples, 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). In some examples, the pain is abdominal pain.

In some examples, the subject has a plasma level and/ or a urine level of ALA and/or PBG that is elevated. In some examples, 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). In some examples, the pain is abdominal pain. In some examples, the subject is asymptomatic. In some examples, the subject has a genetic mutation associated with a porphyria, e.g., a mutation as described herein.

In some examples, 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. In some examples, the level is greater than the reference value. In some examples, the reference value is two standard deviations above the mean level in a sample of healthy individuals. In some examples, the reference value is an upper reference limit.

In some examples, 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. In some examples, 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. In some examples, the subject has a urine level of ALA and/or PBG that is greater than 4 times that of an upper reference limit.

In some examples, the reference value for plasma PBG is 0.12 μmol/L. In some examples, 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. In some examples, 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.

In some examples, the reference value for urine PBG is 1.2 mmol/mol creatinine. In some examples, 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. In some examples, 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.

In some examples, the reference value for plasma ALA is 0.12 μmol/L. In some examples, 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. In some examples, 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.

In some examples, the reference value for urine ALA is 3.1 mmol/mol creatinine. In some examples, 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.

In some examples, the method decreases one or more signs or symptoms of a porphyria. In some examples, the method decreases an elevated level of ALA and/or PBG. In some examples, the method decreases pain (e.g., chronic pain, e.g. chronic neuropathic pain) and/or neuropathy (e.g., progressive neuropathy). In some examples, the pain is abdominal pain. In some examples, 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. In some examples, the decrease in pain is assessed based on the subject's use of pain medication.

In some examples, the method ameliorates or prevents acute attacks of porphyria, e.g., by reducing the severity, duration, or frequency of attacks.

In some examples, the method decreases or prevents nerve damage.

In some examples, 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.

In some examples, the method decreases heme use by the subject.

In some examples, the method decreases pain medication use by the subject.

In some examples, the method reduces hospitalization.

In some examples, 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). In some examples, the method is effective to produce a predetermined reduction in the elevated level of ALA and/or PBG.

In some examples, the predetermined reduction is a reduction to a value that is less than or equal to a reference value. In some examples, the reference value is an upper reference limit. In some examples, the reference value is the value that is two standard deviations above the mean level in a reference sample.

In some examples, the method is effective to reduce the level of ALA and/or PBG in a subject to a level that is below two times the upper reference limit. In some examples, the method is effective to reduce the level of ALA to below two times the upper reference limit. In some examples, the method is effective to reduce the level of PBG to below two times the upper reference limit.

In some examples, the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered as a single dose or at multiple doses, *e.g.*, according to a dosing regimen.

In some examples, the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered prophylactically to a subject who is at risk for developing a porphyria. In some examples, the iRNA (e.g., dsRNA) or composition comprising the iRNA is administered prophylactically beginning at puberty. In some examples, 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). In some examples, 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). In some examples, the mutation is associated with elevated levels of a porphyrin or a porphyrin precursor (e.g., ALA and/or PBG). In some examples, the mutation is associated with chronic pain (e.g., chronic neuropathic pain) and/or neuropathy (e.g., progressive neuropathy).

In some examples, the mutation is a mutation in the ALAS1 gene. In some examples, the mutation is a mutation in the ALAS1 gene promoter, or in regions upstream or downstream from the ALAS1 gene. In some examples, the mutation is a mutation in transcription factors or other genes that interact with ALAS1. In some examples, the mutation is a mutation in a gene that encodes an enzyme in the heme biosynthetic pathway.

In some examples, the iRNA (e.g., dsRNA or a conjugate thereof) or composition comprising the iRNA is administered subcutaneously. In some examples, the iRNA is in the form of a GalNAc conjugate. In some examples, the iRNA (e.g., the dsRNA) is administered at a dose of 0.5-50 mg/kg. In certain examples, the iRNA is administered at a dose of less than 10 mg/kg (e.g., 5 mg/kg or less) once per week; e.g., a dose of 1 mg/kg or less, 2.5 mg/kg or less, or 5 mg/kg or less, e.g., once per week. In one embodiment, iRNA is administered at a dose of about 2.5 mg/kg or less, e.g., once per week.

In some examples, the subject to be treated is asymptomatic and has an elevated level of ALA and/or PBG. In some examples, the subject has a porphyria, e.g., AIP. In some examples, the patient suffers from recurrent porphyric attacks.

In some examples, the iRNA (*e.g.*, AD-60519) is administered at a dose of less than 5 mg/kg, *e.g.*, at 0.1, 0.35, 1.0, or 2.5 mg/kg. In some examples, the iRNA (*e.g.*, AD-60519) is administered in repeated doses, e.g., weekly doses.

In one example, the subject is asymptomatic and has an elevated level of ALA and/or PBG, and the iRNA (*e.g.*, AD-60519) is administered at single doses, *e.g.*, at 0.1, 0.35, 1.0, or 2.5 mg/kg; or in repeatedly weekly dosages, *e.g.*, of 1 and 2.5 mg/kg for several weeks (e.g., for 4 weeks).

In one example, the subject has AIP, *e.g.*, is an AIP patient, the iRNA (*e.g.*, AD-60519) is administered at a dose of 1-2.5 mg/kg weekly.

In some examples, a treatment regimen is employed in which the iRNA is initially administered more frequently, followed by less frequent administration. In some examples, the

iRNA is initially administered once per day for multiple days (e.g., for 2-14 days, e.g., for 2, 3, 4, 5, 6, or 7 days). In some examples, the iRNA is subsequently administered once per week. In some examples, the iRNA is subsequently administered once every two weeks. In some examples, the iRNA is subsequently administered at a frequency that is effective to reduce one or more signs or symptoms of a porphyria.

Also 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.

Also 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.

In some examples, the methods described herein are effective to decrease the level of ALA and/or PBG. In some examples, 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 some examples, the subject to be treated is asymptomatic and has an elevated level of ALA and/or PBG. In some examples, the subject has a porphyria, e.g., AIP.

In some examples, the iRNA is administered at a dose of less than 5 mg/kg, e.g., at 0.1, 0.35 1.0, or 2.5 mg/kg. In some examples, the iRNA is administered in repeated doses, e.g., weekly doses.

In another example, the disclosure 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). In one example, 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 ALAS1, *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 ALAS1 expression, such as porphyrias, e.g., AIP or ALA dehydratase deficiency porphyria.

In one example, 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. In an example, the porphyria is an acute hepatic porphyria. In some examples, 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. In some examples, the porphyria is a homozygous dominant hepatic porphyria (e.g., homozygous dominant AIP, HCP, or VP) or hepatoerythropoietic porphyria, In some examples, the porphyria is a dual porphyria.

Also described herein 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.

In some examples, the cell is a hepatocyte. In some examples, 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. In some examples, the porphyrin precursor is ALA or PBG.

In one example, the cell is an erythroid cell. In a further example, the cell is a liver cell (*e.g.*, a hepatocyte).

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

Also described herein 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 ALAS1, wherein said dsRNA is 30 base pairs or less in length, and wherein said dsRNA targets said mRNA for cleavage.

In some examples, the region of complementarity is at least 15 nucleotides in length.

In some examples, the region of complementarity is 19 to 21 nucleotides in length. Also described herein is a vector for inhibiting the expression of an ALAS1 gene in a cell. In one example, the vector comprises an iRNA as described herein. In one example, 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. In one examples the vector comprises at least one strand of an ALAS1 iRNA.

Also described herein is a cell comprising a vector as described herein. Also described herein is a cell containing a vector for inhibiting the expression of an ALAS1 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. In one example, the cell is a liver cell (*e.g.*, a hepatocyte). In another example, the cell is an erythroid cell.

Also described herein is a method is provided for assaying the level of circulating extracellular ALAS1 mRNA in a subject, said method comprising detecting (*e.g.*, measuring) the level of ALAS1 mRNA in a biological fluid sample (*e.g.*, a blood sample (e.g., a serum or plasma sample), a cerebrospinal fluid sample, or a urine from the subject, said biological fluid sample comprising the ALAS1 mRNA, thereby assaying the level of circulating extracellular ALAS1 mRNA in the subject.

Also described herein is a method is provided for assaying the level of circulating extracellular ALAS1 mRNA in a subject, said method comprising (i) providing RNA (*e.g.*, extracellular RNA) from a biological fluid sample (e.g., blood or plasma sample) from the subject, said biological fluid sample comprising the ALAS1 mRNA; (ii) obtaining an ALAS1 cDNA from the ALAS1 mRNA; (iii) contacting the ALAS1 cDNA with a nucleic acid complementary (e.g., probe and/or primer) to the ALAS1 cDNA or a portion thereof, thereby producing a reaction mix; and (iv) detecting (e.g., measuring) the level of ALAS1 cDNA in the reaction mix, wherein the ALAS1 cDNA level is indicative of the ALAS1 mRNA level, thereby assaying the level of circulating extracellular ALAS1 mRNA in the subject.

In some examples, said biological fluid sample is a blood sample. In some examples, said biological fluid sample is a serum sample. In some examples, said biological fluid sample is a urine sample.

In some examples, the the method comprises PCR, qPCR or 5'-RACE.

In some examples, said nucleic acid is a probe or primer.

In some examples, said nucleic acid comprises a detectable moiety and the level of ALAS1 mRNA is determined by detection of the amount of the detectable moiety.

In some examples, said method further comprises obtaining the biological fluid sample from the subject. In some examples, the biological fluid sample is separate from the tissue and contains exosomes. In some examples of these methods, the efficacy of a porphyria treatment is assessed based on a comparison of the level of circulating extracellular ALAS1 mRNA in the subject relative to a reference value.

In some examples, a decrease in the level of circulating extracellular ALAS1 mRNA in the subject in response to the porphyria treatment, relative to the reference value, indicates that the porphyria treatment is efficacious. In some examples, the reference value is the level of circulating extracellular ALAS1 mRNA in the subject prior to the porphyria treatment.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

The details of various embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims.

Description of the Drawings

FIG. 1 depicts the heme biosynthetic pathway.

FIG. 2A and FIG. 2B show a table summarizing certain porphyrias associated with genetic errors in heme metabolism.

FIG. 3A and FIG. 3B depict a human ALAS1 mRNA sequence transcript (Ref. Seq. NM_000688.4 (GI:40316942, record dated November 19, 2011), SEQ ID NO: 1).

FIG. 4A and FIG. 4B depict a human ALAS1 mRNA sequence transcript (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.

We claim:

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of ALAS1, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to an ALAS1 RNA transcript (e.g., SEQ ID NO:1), which antisense strand comprises usAfsAfGfaUfgAfgAfcAfcUfcUfuUfcUfgsgsu (SEQ ID NO: 4161), wherein c, a, g, u = 2′-OMe ribonucleosides; Af, Cf, Gf, Uf = 2′F ribonucleosides; s = phosphortioate, or a sequence differing by no more than 1, 2, or 3 nucleotides thereof.

2. The dsRNA of claim 1, which further comprises a sense strand comprising csasgaaaGfaGfuGfuCfuCfaucuua (SEQ ID NO: 4160), or a sequence differing by no more than 1, 2, or 3 nucleotides thereof.

3. The dsRNA of claim 1 or 2, wherein said dsRNA has one or more of the following:

(a) comprises a duplex region which is 17-23 nucleotide pairs in length;

(b) at least one strand comprises a 3′ overhang of at least 2 nucleotides; or

(c) each strand is no more than 26 nucleotides in length.

4. The dsRNA of claim 3, wherein the duplex region is 21-23 nucleotide pairs or 21 nucleotide paris in length.

5. The dsRNA of any one of the preceding claims, further comprising a ligand.

6. The dsRNA of claim 5, wherein the ligand has one or more of the following:

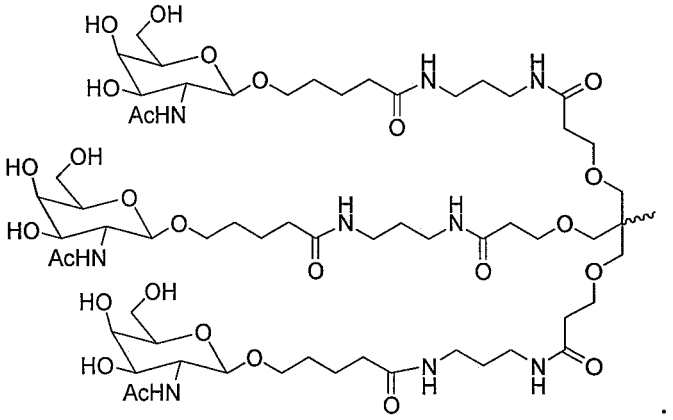
(a) is conjugated to the 3′ end of the sense strand of the dsRNA;

(b) comprises a carbohydrate;

(c) targets the dsRNA to hepatocytes.

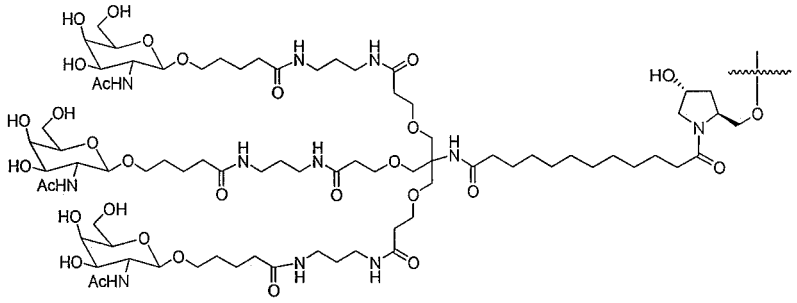
7. The dsRNA of claim 5, wherein the ligand is a GalNAc ligand.

8. The dsRNA of claim 5, wherein the ligand is:



9. The dsRNA of claim 5, wherein the ligand is attached via a bivalent or trivalent branched linker.

10. The dsRNA of claim 9, wherein the ligand and linker are as shown in Formula XXIV:



11. The dsRNA of any one of the preceding claims, wherein the antisense strand comprises or consists of usAfsAfGfaUfgAfgAfcAfcUfcUfuUfcUfgsgsu (SEQ ID NO: 4161), and/or the sense strand comprises or consists of csasgaaaGfaGfuGfuCfuCfaucuuaL96 (SEQ ID NO: 4160), wherein c, a, g, u = 2′-OMe ribonucleosides; Af, Cf, Gf, Uf = 2′F ribonucleosides; s = phosphortioate and L96= N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

12. The dsRNA of any of the preceding claims, wherein the dsRNA has one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or all of the following:

(i) is chemically synthesized;

(ii) all the nucleotides in the dsRNA are modified;

(iii) all nucleotides are connected through 3'-5' phosphodiester linkages;

(iv) the sense strand comprises or consists of 21 nucleotides;

(v) the antisense sense strand comprises or consists of 23 nucleotides;

(vi) has a blunt-end at the 3'-end of sense strand;

(vii) has a 3'-overhang;

(viii) is covalently attached to a ligand containing three N-acetylgalactosamine (GalNAc) moieties;

(ix) the 3'-end of the sense strand is conjugated to the triantennary GalNAc moiety;

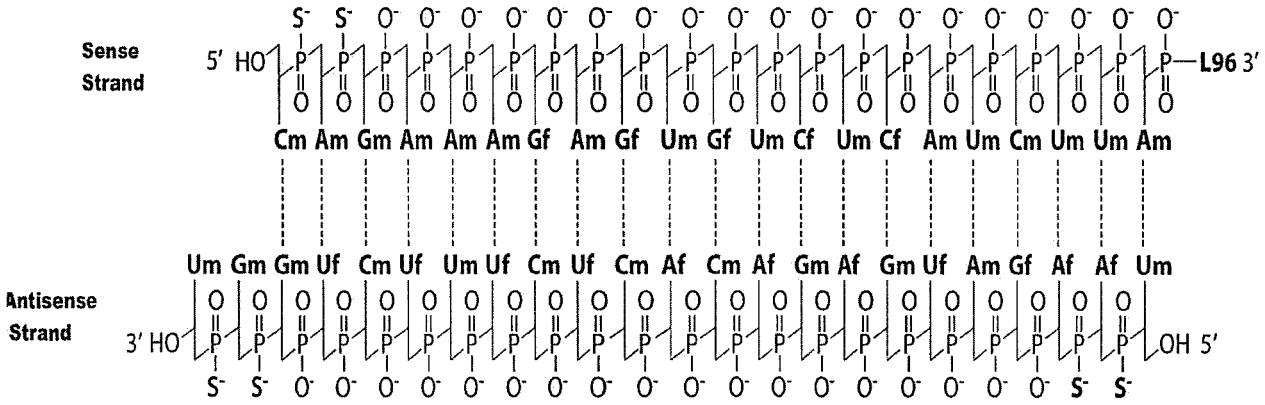
(x) has a sense strand that comprises one or more phosphorothioate linkages;

(xi) 21 nucleotides of the sense strand hybridize to the complementary 21 nucleotides of the antisense strand;

(xii) forms 21 nucleotide base pairs and a two-base overhang at the 3'-end of the antisense strand; or

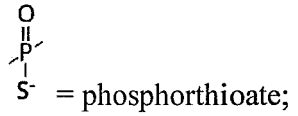
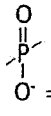
(xiii) comprises, or consists of, a sense strand having csasgaaaGfaGfuGfuCfuCfaucuuaL96 (SEQ ID NO: 4160) and an antisense strand having usAfsAfGfaUfgAfgAfcAfcUfcUfuUfcUfgsgsu (SEQ ID NO: 4161).

13. The dsRNA of claim 1, wherein said dsRNA is in the form of a conjugate having the structure of:

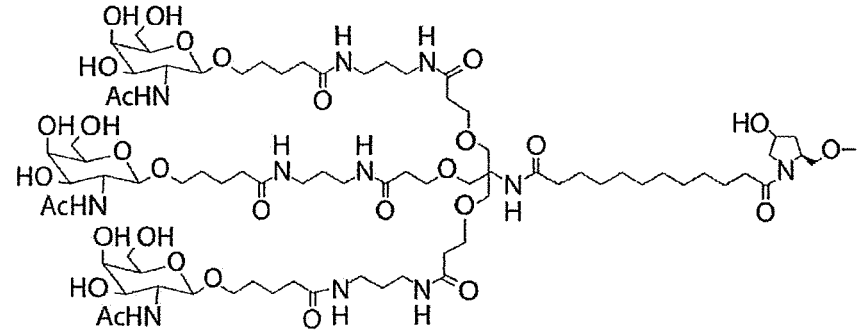


, or a pharmaceutically acceptable salt thereof,

wherein Af, Cf, Gf, Uf = 2′F ribonucleosides; Am, Cm, Gm, Um = 2′-OMe ribonucleosides;

 = phosphorthioate;  = phosphordiester, and

wherein

L96 = 

14. A cell comprising the dsRNA of any one of claims 1 to 13.

15. A pharmaceutical composition comprising the dsRNA of any one of claims 1 to 13.

16. The pharmaceutical composition of claim 15, wherein the composition comprises water for injection.

17. The pharmaceutical composition of claim 15, wherein the composition is suitable for subcutaneous administration.

18. The pharmaceutical composition of claim 15, wherein the composition comprises about 200 mg/mL of the dsRNA, and/or has a pH of 6.0-7.5, or about 7.0.

19. Use of the dsRNA of any one of claims 1 to 13, or the composition of any one of claims 15 to 18, in the manufacture of a medicament for treating a porphyria in a subject.

20. The use of claim 19, wherein the porphyria is hepatic porphyria or an acute intermittent porphyria (AIP).

21. The use of claim 20, wherein the porphyria is acute hepatic porphyria.

22. The use of any one of claims 19 to 21, wherein the use has one or more of the following:
(a) the subject is at risk for developing, or is diagnosed with, a porphyria;
(b) the porphyria is acute intermittent porphyria or ALA-dehydratase deficiency porphyria;
(c) (i) the dsRNA or composition comprising dsRNA is to be administered after an acute attack of porphyria; (ii) the dsRNA or composition is to be administered during an acute attack of porphyria, or (iii) the dsRNA or composition is to be administered prophylactically to prevent an acute attack of porphyria;
(d) the dsRNA is to be administered at a dose of 0.05 to 50 mg/kg bodyweight of the subject, or at a dose of 0.01 mg/kg to 5 mg/kg bodyweight of the subject;
(e) the use (i) decreases a level of a porphyrin or a porphyrin precursor in the subject, or wherein the level is decreased by at least 30%; or wherein the level is decreased by at least 40%, and/or (ii) inhibits ALAS1 expression in the subject;
(f) said use (i) ameliorates a symptom associated with an ALAS1 related disorder,
(ii) decreases frequency of acute attacks of symptoms associated with a porphyria in the subject, and/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;
(g) the dsRNA or composition comprising the dsRNA is to be administered according to a dosing regimen; or wherein the dsRNA is to be administered monthly at a dose of 2.5 mg/kg bodyweight of the subject;
(h) the dsRNA is to be administered before an acute attack of porphyria;
(i) the subject has an elevated level of ALA and/or PBG or wherein the subject suffers from chronic pain;
(j) the use decreases the elevated level of ALA and/or PBG;
(k) the use decreases or prevents pain, neuropathy, and/or nerve damage;
(l) the method prevents acute attacks of porphyria; or

(m) the dsRNA or composition comprising the dsRNA is to be administered repeatedly.

23. A dsRNA of any one of claims 1 to 13, or a pharmaceutical composition of any one of claims 15 to 18 for use in therapy.

5