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(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF THE LECT2 GENE

(57) Abstract: The invention relates to double-stranded ribonucleic acid (dsRNA) compositions targeting the LECT2 gene, and methods of using such dsRNA compositions to alter (*e.g.*, inhibit) expression of LECT2.

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COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF THE LECT2 GENE

Cross-Reference to Related Applications

The present application claims the benefit of U.S. Provisional Application No. 62/764,909 filed August 16, 2018, hereby incorporated by reference herein.

Field of the Invention

The invention relates to the specific inhibition of the expression of the LECT2 gene.

Background of the Invention

Amyloidosis is a group of diseases characterized by deposition of insoluble fibrous protein aggregates, called amyloids, in organs or tissues. Amyloids can form from mutant or wild type proteins. One system of nomenclature for amyloid diseases uses an abbreviation for the protein that forms amyloid deposits, preceded by the letter "A." Thus, for example, ALECT2 is the abbreviation for an amyloidosis involving deposit of amyloids formed from leukocyte cell derived chemotactic factor-2 (ALECT2).

LECT2 amyloidosis (ALECT2) is one of the most recently discovered types of amyloidosis. LECT2 amyloidosis has been observed in individuals with renal or hepatic amyloidosis. This form of amyloidosis can present with nephrotic syndrome or with liver involvement (*e.g.,* hepatitis, *e.g.,* chronic hepatitis). It may be particularly prevalent in Mexican Americans and/or individuals who are homozygous for the G allele encoding valine at position 40 in the mature LECT2 protein (or at position 58 in the unprocessed protein). Treatments for LECT2 amyloidosis are limited, and new treatments are needed.

Summary of the Invention

The present invention describes methods and iRNA compositions for modulating the expression of a LECT2 gene. In certain embodiments, expression of a LECT2 gene is reduced or

inhibited using a LECT2-specific iRNA. Such inhibition can be useful in treating disorders related to LECT2 expression, such as amyloidosis, *e.g.* a LECT2 amyloidosis (ALECT2).

Accordingly, described herein are compositions and methods that effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of the LECT2 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 a LECT2 gene, such as a LECT2 amyloidosis.

In some embodiments, the LECT2 amyloidosis is a renal amyloidosis.

In some embodiments, the LECT2 amyloidosis involves amyloid deposition in the kidney.

In some embodiments, LECT2 amyloidosis is associated with renal disease (*e.g.*, nephrotic syndrome). In some embodiments, the amyloidosis is associated with proteinuria. In some embodiments, proteinuria is absent.

In some embodiments, the LECT2 amyloidosis is a hepatic amyloidosis. In some embodiments, the the LECT2 amyloidosis involves amyloid deposition in the liver.

In some embodiments, the LECT2 amyloidosis is associated with inflammation in the liver (*e.g.*, hepatitis, *e.g.*, chronic hepatitis).

In some embodiments, the methods described herein are effective to inhibit amyloid deposition (*e.g.*, by preventing amyloid deposition or reducing amyloid deposition, *e.g.*, by reducing size, number, or extent of amyloid deposits) or symptoms associated with amyloid deposition.

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 embodiment, an iRNA as described herein inhibits LECT2 expression in a cell or mammal.

The iRNAs (*e.g.*, dsRNAs) included in the compositions featured herein include 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 a LECT2 gene (*e.g.*, a mouse or human LECT2 gene) (also referred to herein as a "LECT2-specific iRNA"). In embodiments, the LECT2 mRNA transcript is a human LECT2 mRNA transcript, *e.g.,* SEQ ID NO: 1. In embodiments, the LECT2 mRNA transcript has a A to G substitution at nucleotide position 373 of SEQ ID NO: 1. In embodiments, the mRNA transcript encodes valine at position 40 in the mature LECT2 protein (or amino acid 58 in the unprocessed protein). In embodiments, the mRNA transcript encodes isoleucine at position 40 in the mature LECT2 protein (or amino acid 58 in the unprocessed protein).

In embodiments, the iRNA (*e.g,* dsRNA) described herein comprises an antisense strand having a region that is substantially complementary to a region of a human LECT2 mRNA. In embodiments, the human LECT2 mRNA has the sequence of NM_002302.2 (SEQ ID NO: 1). In embodiments, the human LECT2 mRNA has a A to G substitution at nucleotide position 373 of SEQ ID NO: 1.

In other embodiments, an iRNA encompasses a dsRNA having an RNA strand (the antisense strand) having a region that is substantially complementary to a portion of a LECT2 mRNA. In one embodiment, the iRNA encompasses a dsRNA having an RNA strand (the antisense strand) having a region that is substantially complementary to a portion of a LECT2 mRNA, *e.g.*, a human LECT2 mRNA (*e.g.*, a human LECT2 mRNA as provided in NM_002302.2 (SEQ ID NO: 1) or having a A to G substitution at nucleotide position 373 of SEQ ID NO: 1).

In one embodiment, an iRNA for inhibiting expression of a LECT2 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 a LECT2 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 embodiments, the iRNA is 19-21 nucleotides in length. In some embodiments, 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 one embodiment, the iRNA targeting LECT2 is formulated in a stable nucleic acid lipid particle (SNALP).

In some embodiments, the iRNA is 21-23 nucleotides in length. In some embodiments, 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 embodiments the iRNA is from about 15 to about 25 nucleotides in length, and in other embodiments the iRNA is from about 25 to about 30 nucleotides in length. An iRNA targeting LECT2, upon contact with a cell expressing LECT2, inhibits the expression of a LECT2 gene (*e.g.,* by at least 10%, at least 20%, at least 25%, at least 30%, at least 35% or at least 40%, at least 50%, at least 60%, at least 70%, or at least 80%) when assayed by a method known in the art or as described herein.

In one embodiment, an iRNA (*e.g.,* a dsRNA) featured herein comprises or consists of a first sequence of a dsRNA that is selected from the group consisting of the sense sequences of Tables 2 and 3, and a second sequence that is selected from the group consisting of the corresponding antisense sequences of Tables 2 and 3.

In embodiments, an iRNA (*e.g.,* dsRNA) featured herein comprises or consists of a sense and/or antisense sequence selected from those provided in Table 2 and 3.

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 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 Tables 2 and 3, and a second sequence selected from the group consisting of the corresponding antisense sequences of Tables 2 and 3.

In one embodiment, an iRNA as described herein targets a wildtype LECT2 RNA transcript variant, and in another embodiment, the iRNA targets a mutant transcript (*e.g.*, a LECT2 RNA carrying an allelic variant). For example, an iRNA featured in the invention can target a polymorphic variant, such as a single nucleotide polymorphism (SNP), of LECT2.

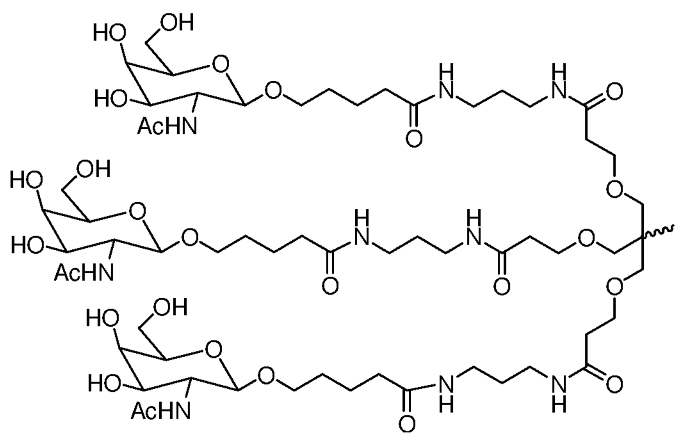
In some embodiments, the iRNA (*e.g.*, dsRNA) targets (*e.g.*, reduces) mRNA that encodes valine at position 40 in the mature LECT2 protein (or amino acid 58 in the unprocessed protein). In some embodiments, the iRNA (*e.g.*, dsRNA) targets (*e.g.*, reduces) mRNA that encodes isoleucine at position 40 in the mature LECT2 protein (or amino acid 58 in the unprocessed protein). In another embodiment, the iRNA (*e.g.*, dsRNA) targets (*e.g.*, reduces) both mRNA that encodes valine and mRNA that encodes isoleucine at position 40 in the mature LECT2 protein (or amino acid 58 in the unprocessed protein).

In another embodiment, the iRNA targets both a wildtype and a mutant LECT2 transcript. In yet another embodiment, the iRNA targets a particular transcript variant of LECT2. In yet another embodiment, the iRNA agent targets multiple transcript variants.

In one embodiment, an iRNA featured in the invention targets a non-coding region of a LECT2 RNA transcript, such as the 5' or 3' untranslated region of a transcript.

In some embodiments, 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 embodiment, the conjugate is attached to the 3' end of the sense strand of the dsRNA. In some embodiments, the conjugate is attached via a linker, *e.g.*, via a bivalent or trivalent branched linker.

In some embodiments, the conjugate comprises one or more N-acetylgalactosamine (GalNAc) derivatives. Such a conjugate is also referred to herein as a GalNAc conjugate. In some embodiments, the conjugate targets the RNAi agent (*e.g.*, dsRNA) 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 embodiments, the conjugate is



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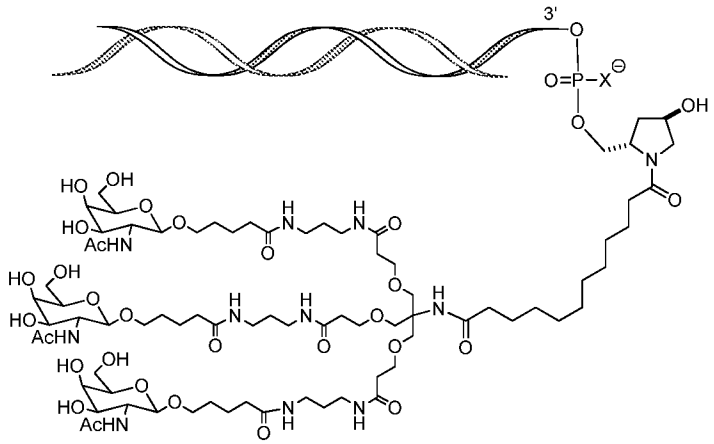
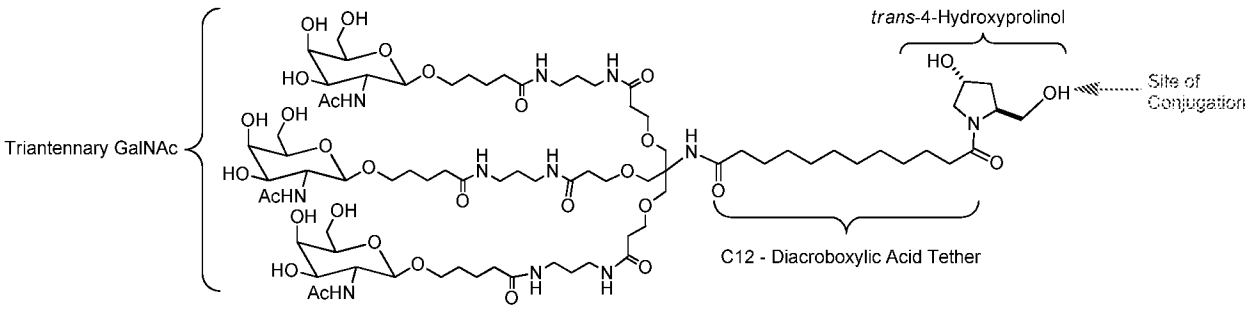
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate via a linker, *e.g.*, a linker as shown in the following schematic, wherein X is O or S



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

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



In some embodiments, the RNAi agent is conjugated to a ligand that targets the RNAi (*e.g.*, dsRNA) to a desired organ (*e.g.*, the liver) or to a particular cell type (*e.g.*, hepatocytes). In embodiments, the RNAi agent is conjugated to a ligand (*e.g.*, a GalNAc ligand, *e.g.*, L96) that targets the RNAi agent (*e.g.*, dsRNA) to the liver.

In an aspect provided herein is a pharmaceutical composition for inhibiting the expression of a LECT2 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. In one embodiment, the composition is used for treating a disorder

related to LECT2 expression, *e.g.*, amyloidosis, *e.g.*, LECT2 amyloidosis.

In one aspect, an iRNA provided herein is a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of LECT2, 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.

In a further aspect, 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 a LECT2 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 -N_a -(X X X)$_i$-N_b -Y Y Y -N_b -(Z Z Z)$_j$ -N_a - n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'- 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 embodiments, the sense strand is conjugated to at least one ligand.

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

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

In embodiments, XXX is complementary to X′X′X′, YYY is complementary to Y′Y′Y′, and ZZZ is complementary to Z′Z′Z′.

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

In embodiments, the Y′ is 2′-O-methyl.

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

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

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

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

In embodiments, the modifications on the nucleotides are 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.

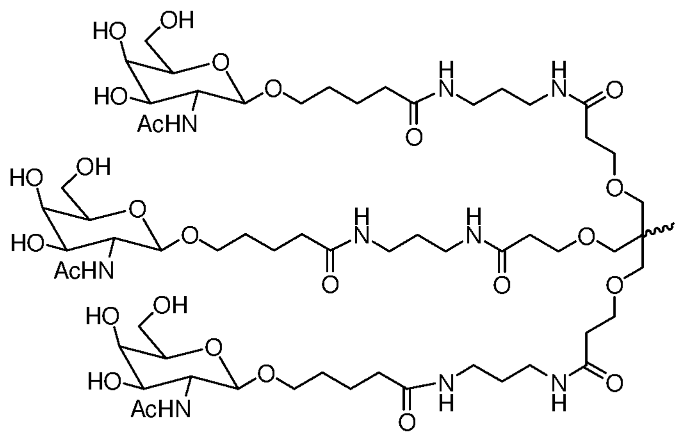
In embodiments, the modifications on the nucleotides are 2′-O-methyl, 2′-fluoro or both.

In embodiments, the ligand comprises a carbohydrate.

In embodiments, the ligand is attached via a linker.

In embodiments, the linker is a bivalent or trivalent branched linker.

In embodiments, the ligand is



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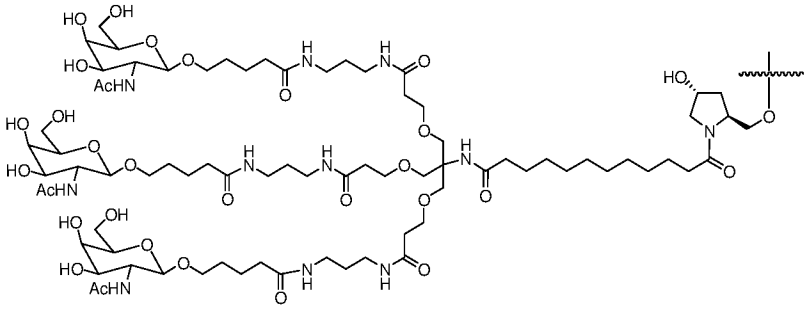
In embodiments, the ligand and linker are as shown in Formula XXIV:



In embodiments, the ligand is attached to the 3' end of the sense strand.

In embodiments, the dsRNA has (*e.g.,* comprises) a nucleotide sequence (*e.g.,* a sense and/or antisense sequence) selected from the group of sequences provided in Tables 2-5.

In a further aspect, an iRNA provided herein is a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of LECT2, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a LECT2 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 and 3.

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

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.

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 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 embodiments, the region of complementarity is at least 17 nucleotides in length.

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

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

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

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

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

In some embodiments, an iRNA (*e.g.*, a dsRNA) described herein further comprises a ligand.

In some embodiments, the ligand is a GalNAc ligand.

In some embodiments, the ligand targets the iRNA (*e.g.*, the dsRNA) to the liver (*e.g.*, to hepatocytes).

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

In some embodiments, the region of complementarity consists of an antisense sequence selected from the antisense sequences provided in Tables 2 and 3.

In embodiments, the region of complementarity consists of an antisense sequence selected from a duplex disclosed herein, wherein the duplex suppresses LECT2 mRNA or protein expression by at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85% or 90%.

In some embodiments, the dsRNA comprises a sense strand comprising or consisting of a sense strand sequence selected from Table 2, 3, 4, or 5, and an antisense strand comprising or consisting of an antisense sequence selected from Table 2, 3, 4, or 5. In embodiments, the dsRNA comprises or consists of a pair of corresponding sense and antisense sequences selected from those of the duplexes disclosed in Tables 2-5. In certain embodiments, the dsRNA comprises or consists of a pair of corresponding sense and antisense sequences selected from those of the duplexes disclosed in Table 2. In certain embodiments, the dsRNA comprises or consists of a pair of corresponding sense and antisense sequences selected from those of the duplexes disclosed in Table 3. In certain embodiments, the dsRNA comprises or consists of a pair of corresponding sense and antisense sequences selected from those of the duplexes

disclosed in Table 4. In certain embodiments, the dsRNA comprises or consists of a pair of corresponding sense and antisense sequences selected from those of the duplexes disclosed in Table 5.

In one aspect, the invention provides a cell containing at least one iRNA (*e.g.*, dsRNAs) disclosed herein. The cell is typically a mammalian cell, such as a human cell. In embodiments, the cell is a liver cell (*e.g.*, a hepatocyte).

In an aspect provided herein is a pharmaceutical composition for inhibiting expression of a LECT2 gene, the composition comprising an iRNA (*e.g.*, a dsRNA) described herein.

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

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

In embodiments of the pharmaceutical compositions described herein, the iRNA (*e.g.*, dsRNA) is targeted to the liver (*e.g.*, to hepatocytes).

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

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

In embodiments, 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 a liver cell, *e.g.*, a hepatocyte.

In embodiments, 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 embodiments, the ligand targets the iRNA (*e.g.*, dsRNA) to a liver cell, *e.g.,* a hepatocyte.

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

In another embodiment, 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 embodiment, 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 embodiment, a composition containing an iRNA featured in the invention, *e.g.*, a dsRNA targeting LECT2, is administered in conjunction with a second therapy for a disorder related to LECT2 expression (*e.g.*, a LECT2 amyloidosis). An iRNA or composition comprising an iRNA provided herein can be administered before, after, or concurrent with a second therapy. In embodiments, the iRNA is administered before the second therapy. In embodiments, the iRNA is administered after the second therapy. In embodiments, the iRNA is administered concurrent with the second therapy.

In some embodiments, the second therapy is a non-iRNA therapeutic agent that is effective to treat the disorder or symptoms of the disorder.

In some embodiments, the disorder to be treated by the compositions or methods disclosed herein is a LECT2 amyloidosis that affects kidney function, *e.g.*, through amyloid deposition in the kidney. In some such embodiments, the iRNA is administered in conjunction with a therapy that supports kidney function (*e.g.*, dialysis). In embodiments, the iRNA is administered in conjunction with a diuretic, an ACE (angiotensin converting enzyme) inhibitor, an angiotensin receptor blocker, and/or dialysis, *e.g.*, to support or manage kidney function.

In some embodiments, the disorder to be treated by the compositions or methods disclosed herein is a LECT2 amyloidosis involving amyloid deposits in the liver. In some such embodiments, the iRNA is administered in conjunction with a therapy that supports liver function.

In some embodiments, the disorder to be treated by the compositions or methods disclosed herein is a LECT2 amyloidosis, and the iRNA is administered in conjunction with removal of all or part of the organ(s) affected by the amyloidosis (*e.g.*, resection of all or part of kidney or liver tissue affected by the amyloidosis). The removal is optionally conducted in conjunction with a replacement of all or part of the organ removed (*e.g.*, in conjunction with a kidney or liver organ transplant).

In an aspect provided herein is a method of inhibiting LECT2 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 a LECT2 gene, thereby inhibiting expression of the LECT2 gene in the cell.

In an aspect provided herein is a method for reducing or inhibiting the expression of a LECT2 gene in a cell (*e.g.*, a liver cell, *e.g.*, a hepatocyte). The method includes contacting the cell with a dsRNA as described herein, thereby inhibiting expression of a LECT2 gene. "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.*, a liver cell) may be contacted when a composition comprising an RNAi is administered (*e.g.*, intravenously or subcutaneously) to the subject.

In embodiments, 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 LECT2, and where the region of complementarity is 30 nucleotides or less, *e.g.*, 15-30 nucleotides in length, and generally 19-24 nucleotides in length, and where the dsRNA upon contact with a cell expressing LECT2, inhibits expression of a LECT2 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 LECT2 gene, thereby reducing or inhibiting expression of a LECT2 gene in the cell.

In embodiments of the foregoing methods of inhibiting LECT2 expression in a cell, the cell is treated *ex vivo*, *in vitro*, or *in vivo*. In embodiments, the cell is a hepatocyte.

In embodiments, the cell is present in a subject in need of treatment, prevention and/or management of a disorder related to LECT2 expression.

In embodiments, the disorder is a LECT2 amyloidosis, as described herein.

In embodiments, the expression of LECT2 is inhibited by at least 30%.

In embodiments, the iRNA (*e.g.*, dsRNA) has an IC_{50} in the range of 0.0005-1 nM, *e.g.*, between 0.001 and 0.2 nM, between 0.002 and 0.1 nM, between 0.005 and 0.075 nM, or between 0.01 and 0.05 nM. In embodiments, the iRNA (*e.g.*, dsRNA) has an IC_{50} equal to or less than 0.02 nM, *e.g.*, between 0.0005 and 0.02 nM, between 0.001 and 0.02 nM, between 0.005 and 0.02 nM, or between 0.01 and 0.02 nM. In embodiments, the iRNA (*e.g.*, dsRNA) has an IC_{50} in the range of 0.01-1 nM.

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

In one embodiment, the subject is a mammal (*e.g.*, a human) having a LECT2 amyloidosis.

In one embodiment, the dsRNA introduced reduces or inhibits expression of a LECT2 gene in the cell.

In one embodiment, the dsRNA inhibits expression of a LECT2 gene, or inhibits amyloid deposition (*e.g.*, by preventing amyloid deposition or reducing amyloid deposition, *e.g.*, by reducing size, number, or extent of amyloid deposits). The inhibition optionally involves an inhibition of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% or more compared to a reference, (*e.g.*, a control that is untreated or treated with a non-targeting dsRNA (*e.g.*, a dsRNA that does not target LECT2)).

In other aspects, the disclosure provides methods for treating pathological processes related to LECT2 expression (*e.g.*, amyloid deposition). In one embodiment, the method

includes administering to a subject, *e.g.,* a patient in need of such treatment, an effective (*e.g.*, a therapeutically or prophylactically effective) amount of a dsRNA provided herein.

In an aspect provided herein is a method of treating and/or preventing a disorder related to LECT2 expression (*e.g.*, a LECT2 amyloidosis) 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.

In an aspect provided herein is a method of treating a disorder related to LECT2 expression (*e.g.*, LECT2 amyloidosis) 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 a LECT2 mRNA transcript, *e.g.*, a human LECT2 mRNA transcript, *e.g.*, SEQ ID NO: 1 or a nucleotide sequence having a A to G substitution at nucleotide position 373 of SEQ ID NO: 1. In one embodiment, the iRNA (*e.g.*, dsRNA) targets mRNA that encodes valine at position 40 in the mature LECT2 protein (or amino acid 58 in the unprocessed protein).

In one embodiment provided herein is a method of treating a subject having a LECT2 amyloidosis, 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 a LECT2 mRNA transcript, *e.g.*, a human LECT2 mRNA transcript, *e.g.*, SEQ ID NO: 1 or a nucleotide sequence having a A to G substitution at nucleotide position 373 of SEQ ID NO: 1. In one embodiment, the iRNA (*e.g.*, dsRNA) targets mRNA that encodes valine at position 40 in the mature LECT2 protein (or amino acid 58 in the unprocessed protein).

In some embodiments, administration of the iRNA targeting LECT2 alleviates or relieves the severity of at least one symptom of a disorder related to LECT2 expression in the patient.

In one embodiment, subject has a LECT2 amyloidosis. In another embodiment, the subject is at risk for developing a LECT2 amyloidosis.

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

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

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

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

In embodiments, the iRNA (*e.g.*, dsRNA) is formulated as an LNP formulation and is administered at a dose of 0.05-5 mg/kg. In embodiments, the iRNA (*e.g.*, dsRNA) is formulated as an LNP formulation and is administered at a dose of 0.1 to 0.5 mg/kg.

In embodiments, 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 embodiments, the iRNA (*e.g.*, dsRNA) is in the form of a GalNAc conjugate and is administered at a dose of 1 to 10 mg/kg.

In embodiments, the method inhibits expression of a LECT2 gene, or inhibits amyloid deposition (*e.g.*, by preventing amyloid deposition or reducing amyloid deposition, *e.g.*, by reducing size, number, or extent of amyloid deposits). The inhibition optionally involves an inhibition of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% compared to a reference (*e.g.*, a control that is untreated or treated with a non-targeting dsRNA (*e.g.,* a dsRNA that does not target LECT2)).

In embodiments, the iRNA (*e.g.*, dsRNA) has an IC_{50} in the range of 0.0005-1 nM, *e.g.*, between 0.001 and 0.2 nM, between 0.002 and 0.1 nM, between 0.005 and 0.075 nM, or between 0.01 and 0.05 nM. In embodiments, the iRNA (*e.g.*, dsRNA) has an IC_{50} equal to or less than 0.02 nM, *e.g.*, between 0.0005 and 0.02 nM, between 0.001 and 0.02 nM, between 0.005 and 0.02 nM, or between 0.01 and 0.02 nM. In embodiments, the iRNA (*e.g.*, dsRNA) has an IC_{50} in the range of 0.01-1 nM.

In embodiments, a method described herein ameliorates a symptom associated with a LECT2 related disorder (*e.g.*, a LECT2 amyloidosis).

In embodiments, a method described herein inhibits expression of a LECT2 gene in the subject.

In embodiments, a method described herein inhibits inhibits amyloid deposition (*e.g.*, by preventing amyloid deposition or reducing amyloid deposition, *e.g.*, by reducing size, number, or extent of amyloid deposits).

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

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

In embodiments, the iRNA (*e.g.*, dsRNA) or composition comprising the iRNA is administered subcutaneously. In embodiments, 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. In embodiments, the iRNA (*e.g.*, dsRNA) is in the form of a GalNAc conjugate and is administered at a dose of 1 to 10 mg/kg.

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

In an aspect provided 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 LECT2, wherein said dsRNA is 30 base pairs or less in length, and wherein said dsRNA targets said mRNA for cleavage.

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

In embodiments, the region of complementarity is 19 to 21 nucleotides in length.

In one aspect, a vector is provided for inhibiting the expression of a LECT2 gene in a cell. In one embodiment, the vector comprises an iRNA as described herein. In one embodiment, 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 embodiment the vector comprises at least one strand of a LECT2 iRNA.

In an aspect provided herein is a cell comprising a vector as described herein.

In an aspect provided herein is a cell containing a vector for inhibiting the expression of a LECT2 gene in a cell. The vector includes a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of an iRNA described herein.

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 a human LECT2 mRNA transcript sequence (Ref. Seq. NM_002302.2 GI:59806344, record dated April 17, 2013; SEQ ID NO: 1).

Detailed Description of the Invention

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 modulating (*e.g.*, inhibiting) the expression of a LECT2 gene. Also provided are compositions and methods for treatment of disorders related to LECT2 expression, such as amyloidosis (*e.g.*, LECT2 amyloidosis).

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 a LECT2 gene (also referred to herein as an "LECT2-specific iRNA"). The use of such an iRNA enables the targeted degradation of mRNAs of genes that are implicated in disorders related to LECT2 expression, as described herein. Very low dosages of LECT2-specific iRNAs can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of a LECT2 gene. iRNAs targeting LECT2 can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of a LECT2 gene, which can be assessed, *e.g.*, in cell based assays.

The following description discloses how to make and use compositions containing iRNAs to modulate (*e.g.*, inhibit) the expression of a LECT2 gene, as well as compositions and methods for treating disorders related to expression of a LECT2 gene.

Embodiments of the pharmaceutical compositions featured herein 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 a LECT2 gene.

In some aspects, pharmaceutical compositions containing a LECT2 iRNA and a pharmaceutically acceptable carrier, methods of using the compositions to inhibit expression of a LECT2 gene, and methods of using the pharmaceutical compositions to treat disorders related to expression of a LECT2 gene (*e.g.,* LECT2 amyloidosis) are featured herein.

I. Definitions

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.

As used herein, "LECT2" refers to leukocyte chemotactic factor 2 (also known as leukocyte cell-derived chemotaxin 2, chondromodulin-II, chm-II or chm2). *See, e.g.,* Yamagoe S *et al. Genomics*, 1998 Mar 15; 48(3):324-9. LECT2 was first identified as a novel neutrophil chemotactic protein and is identical with chondromodulin II, a growth stimulator for chondrocytes and osteoblasts. The human LECT2 gene was mapped to chromosome 5q31.1-q32. *Ibid.*

The sequence of a human LECT2 mRNA transcript can be found at NM_002302.2 (SEQ ID NO: 1). The sequence of a mouse LECT2 mRNA can be found at NM_010702.1 and at NM_010702.2, and the sequence of a rat LECT2 mRNA can be found at NM_001108405.1.

The human LECT2 protein is a secreted, 16 kDa protein. The LECT2 protein is secreted by the liver. It has high sequence similarity to the chondromodulin repeat regions of the chicken myb-induced myeloid 1 protein (http://www.genecards.org/cgi-bin/carddisp.pl?gene=LECT2; accessed August 29, 2013). Polymorphism in the LECT2 gene has been associated with rheumatoid arthritis. *Ibid.*

LECT2 is expressed in various tissues, including the brain and stomach as well as the liver. Koshimizu, Y & Ohtomi, M. (2010) *Brain Res.* 1311:1-11. In a study using indirect immunoperoxidase staining to investigate the expression of LECT2 in normal and diseased human organs and tissues other than liver, it was found that LECT2 was generally expressed in

vascular, endothelial and smooth muscle cells, adipocytes, cerebral nerve cells, apical squamous epithelia, parathyroid cells, sweat and sebaceous glandular epithelia, Hassall bodies and some mononuclear cells in immunohematopoeietic tissue. This protein was generally negative, although occasionally positively stained in osteoblasts, chondrocytes, cardiac and skeletal muscle cells, smooth mucle cells of the gastrointestinal tract, and the epithelial cells of some tissues. Nagai *et al.* (1998) *Pathol Int.* 48(11):882-6.

The human LECT2 gene codes for 151 amino acids including an 18 amino acid signal peptide. The secreted protein has 133 residues. A G/A polymorphism at nucleotide 172 in exon 3 of the gene (codon change GTC to ATC) has been identified and accounts for the presence of either valine or isoleucine at position 58 of the unprocessed protein (or position 40 of the mature protein). The G allele has an overall frequency of 0.477 and a frequency range of 0.6-0.7 in individuals of European descent. *See* Benson, M.D. *et al.* (2008) *Kidney International*, 74: 218-222; Murphy, C. L. *et al.* (2010) *Am J Kidney Dis*, 56(6):1100-1107. Patients with LECT2 amyloidosis typically are homozygous for the G allele. Without wishing to be bound by theory, it has been suggested that replacement of the buried isoleucine (A allele) side chain with valine (G allele) could destabilize the protein and possibly account for the amyloidogenic propensity of this LECT2 variant. Murphy, C. L. *et al.* (2010) *Am J Kidney Dis*, 56(6):1100-1107.

As used herein, a "LECT2 amyloidosis" or "ALECT2" includes an amyloidosis involving deposits of amyloid or amyloid fibrils that contain a LECT2 protein (*e.g.,* any polymorphic variant of a LECT2 protein) or a portion of a LECT2 protein. The LECT2 protein can be a variant (*e.g.,* a mutant) LECT2 protein. The amyloidosis can be systemic or local. In embodiments, the LECT2 amyloidosis involves amyloid deposits in the kidney and/or liver.

"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.

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. In one embodiment, an iRNA as described herein effects inhibition of LECT2 expression. Inhibition of ALECT2 expression may be assessed based on a reduction in the level of ALECT2 mRNA or a reduction in the level of the ALECT2 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 ALECT2 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.

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.

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.

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.

"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.

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.

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 ALECT2 protein). For example, a polynucleotide is complementary to at least a part of a LECT2 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding LECT2. As another example, a polynucleotide is complementary to at least a part of a LECT2 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding LECT2.

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; in some embodiments 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.

In another embodiment, 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 2-5) 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.

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, in the ribose 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 nucleoside, 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, an acyclic 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.

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. In certain embodiments, the RNA molecule comprises a percentage of deoxyribonucleoses of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95% or higher (but not 100%) deoxyribonucleosides, *e.g.*, in one or both strands. In other embodiments, the term "iRNA" does not encompass a double stranded DNA molecule (*e.g.*, a naturally-occurring double stranded DNA molecule or a 100% deoxynucleoside-containing DNA molecule).

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 invention relates to a single stranded RNA that promotes the formation of a RISC complex to effect silencing of the target gene.

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.

In one embodiment, the antisense strand of a dsRNA has a 1-10 nucleotide overhang at the 3' end and/or the 5' end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide overhang at the 3' end and/or the 5' end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

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

The term "antisense strand" or "guide strand" refers to the strand of an iRNA, *e.g.*, a dsRNA, which includes a region that is substantially complementary to a target sequence. 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.

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

As used herein, the term "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. 2006/0240093, 2007/0135372, and in International Application No. WO 2009/082817. These applications are incorporated herein by reference in their entirety.

"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, which are hereby incorporated by reference in their entirety. *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.

As used herein, the term "modulate the expression of," refers to at an least partial "inhibition" or partial "activation" of a LECT2 gene expression in a cell treated with an iRNA

composition as described herein compared to the expression of LECT2 in a control cell. A control cell includes an untreated cell, or a cell treated with a non-targeting control iRNA.

The terms "activate," "enhance," "up-regulate the expression of," "increase the expression of," and the like, in so far as they refer to a LECT2 gene, herein refer to the at least partial activation of the expression of a LECT2 gene, as manifested by an increase in the amount of LECT2 mRNA, which may be isolated from or detected in a first cell or group of cells in which a LECT2 gene is transcribed and which has or have been treated such that the expression of a LECT2 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).

In one embodiment, expression of a LECT2 gene is activated by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by administration of an iRNA as described herein. In some embodiments, a LECT2 gene is activated by at least about 60%, 70%, or 80% by administration of an iRNA featured in the invention. In some embodiments, expression of a LECT2 gene is activated by at least about 85%, 90%, or 95% or more by administration of an iRNA as described herein. In some embodiments, the LECT2 gene expression is 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 US2007/0111963 and US2005/226848, each of which is incorporated herein by reference.

The terms "silence," "inhibit expression of," "down-regulate expression of," "suppress expression of," and the like, in so far as they refer to a LECT2 gene, herein refer to the at least partial suppression of the expression of a LECT2 gene, as assessed, *e.g.*, based on on LECT2 mRNA expression, LECT2 protein expression, or another parameter functionally linked to LECT2 gene expression. For example, inhibition of LECT2 expression may be manifested by a reduction of the amount of LECT2 mRNA which may be isolated from or detected in a first cell or group of cells in which a LECT2 gene is transcribed and which has or have been treated such that the expression of a LECT2 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.*,

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

Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to LECT2 gene expression, *e.g.*, the amount of protein encoded by a LECT2 gene. The reduction of a parameter functionally linked to LECT2 gene expression may similarly be expressed as a percentage of a control level. In principle, LECT2 gene silencing may be determined in any cell expressing LECT2, either constitutively or by genomic engineering, and by any appropriate assay.

For example, in certain instances, expression of a LECT2 gene is suppressed by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by administration of an iRNA disclosed herein. In some embodiments, a LECT2 gene is suppressed by at least about 60%, 65%, 70%, 75%, or 80% by administration of an iRNA disclosed herein. In some embodiments, a LECT2 gene is suppressed by at least about 85%, 90%, 95%, 98%, 99%, or more by administration of an iRNA as described herein.

In the context of the present disclosure, the terms "treat," "treatment," and the like mean to prevent, relieve or alleviate at least one symptom associated with a disorder related to LECT2 expression, or to slow or reverse the progression or anticipated progression of such a disorder. For example, the methods featured herein, when employed to treat a LECT2 amyloidosis, may serve to inhibit amyloid deposition, to reduce or prevent one or more symptoms of the amyloidosis, or to reduce the risk or severity of associated conditions (*e.g.,* nephrotic syndrome or hepatitis). 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 LECT2 expression.

By "lower" in the context of a disease marker or symptom is meant any decrease, *e.g.*, 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%, at least 40%, at least 50%, at least 60%, at

least 70%, at least 80%, or at least 90%. The decrease can be down to a level accepted as within the range of normal for an individual without such disorder.

As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" and the like refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of any disorder or pathological process related to LECT2 expression. The specific amount that is therapeutically effective may vary depending on factors known in the art, such as, for example, the type of disorder or pathological process, the patient's history and age, the stage of the disorder or pathological process, and the administration of other therapies.

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 LECT2 expression (*e.g.*, a LECT2 amyloidosis), an effective amount includes an amount effective to reduce one or more symptoms associated with the LECT2 amyloidosis, an amount effective to inhibit amyloid deposition (*e.g.*, LECT2 amyloid deposition), or an amount effective to reduce the risk of developing conditions associated with LECT2 amyloidosis. 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 obtain at least a 10% reduction in that parameter. For example, a therapeutically effective amount of an iRNA targeting LECT2 can reduce a level of LECT2 mRNA or a level of LECT2 protein by any measurable amount, *e.g.*, by at least 10%, 20%, 30%, 40% or 50%.

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.

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. iRNA Agents

Described herein are iRNA agents that modulate (*e.g.*, inhibit) the expression of a LECT2 gene.

In some embodiments, the iRNA agent activates the expression of a LECT2 gene in a cell or mammal.

In some embodiments, the iRNA agent includes double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a LECT2 gene in a cell or in a subject (*e.g.*, in a mammal, *e.g.*, in a human), 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 a LECT2 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 LECT2 gene, inhibits the expression of the LECT2 gene, *e.g.*, by at least 10%, 20%, 30%, 40%, or 50%.

The modulation (*e.g.*, inhibition) of expression of the LECT2 gene can be assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by Western blot. Expression of a LECT2 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 LECT2 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.

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 a LECT2 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.

In some embodiments, the dsRNA is between 15 and 20 nucleotides in length, inclusive, and in other embodiments, the dsRNA is 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.

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, in one embodiment, to the extent that it becomes processed to a functional duplex of *e.g.*, 15-30 base pairs that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, then, an miRNA is a dsRNA. In another embodiment, a

dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target LECT2 expression is not generated in the target cell by cleavage of a larger dsRNA.

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.

In one embodiment, a LECT2 gene is a human LECT2 gene. In another embodiment the LECT2 gene is a mouse or a rat LECT2 gene.

In specific embodiments, the dsRNA comprises a sense strand that comprises or consists of a sense sequence selected from the sense sequences provided in Tables 2 and 3, and an antisense strand that comprises or consists of an antisense sequence selected from the antisense sequences provided in Tables 2 and 3.

In one aspect, a dsRNA will include at least sense and antisense nucleotide sequences, whereby the sense strand is selected from the sequences provided in Tables 2 and 3, and the corresponding antisense strand is selected from the sequences provided in Tables 2 and 3.

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 a LECT2 gene. As such, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand, and the second oligonucleotide is described as the corresponding antisense strand. 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.

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 and 3, dsRNAs described herein can include at least one strand of a length of minimally 19 nucleotides. It can be reasonably expected that shorter duplexes

having one of the sequences of Tables 2 and 3 minus only a few nucleotides on one or both ends will be similarly effective as compared to the dsRNAs described above.

In some embodiments, the dsRNA has a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Tables 2, 3, 4 or 5.

In some embodiments, the dsRNA has an antisense sequence that comprises at least 15, 16, 17, 18, or 19 contiguous nucleotides of an antisense sequence provided in Table 2 and a sense sequence that comprises at least 15, 16, 17, 18, or 19 contiguous nucleotides of a corresponding sense sequence provided in Table 2.

In some embodiments, the dsRNA comprises an antisense sequence that comprises at least 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleotides of an antisense sequence provided in Table 3 and a sense sequence that comprises at least 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides of a corresponding sense sequence provided in Table 3.

In some embodiments, the dsRNA comprises an antisense sequence that comprises at least 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleotides of an antisense sequence provided in Table 4 and a sense sequence that comprises at least 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides of a corresponding sense sequence provided in Table 4.

In some embodiments, the dsRNA comprises an antisense sequence that comprises at least 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleotides of an antisense sequence provided in Table 5 and a sense sequence that comprises at least 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides of a corresponding sense sequence provided in Table 5.

In some such embodiments, the dsRNA, although it comprises only a portion of the sequences provided in Table 2, 3, 5, 6, 9 or 10, is equally effective in inhibiting a level of LECT2 expression as is a dsRNA that comprises the full length sequences provided in Table 2, 3, 4, or 5. In some embodiments, the dsRNA differs in its inhibition of a level of expression of a LECT2 gene by not more than 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 % inhibition compared with a dsRNA comprising the full sequence disclosed herein.

The iRNAs provided in Tables 2-5 identify a site in a LECT2 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 2-5 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a LECT2 gene.

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 described herein or 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 and 3, represent effective target sequences, it is contemplated that further optimization of inhibition efficiency can be achieved by progressively "walking the window" one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

Further, it is contemplated that for any sequence identified, *e.g.*, in Tables 2 and 3, 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.

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 a LECT2 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 a LECT2 gene. Consideration of the efficacy of iRNAs with mismatches in inhibiting expression of a LECT2 gene is important, especially if the particular region of complementarity in a LECT2 gene is known to have polymorphic sequence variation within the population.

In one embodiment, at least one end of a dsRNA has 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. In yet another embodiment, the RNA of an iRNA (*e.g.*, a dsRNA) is 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, which is hereby incorporated herein by reference. 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 having an acyclic sugar) 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. In particular embodiments, the modified RNA will have a phosphorus atom in its internucleoside backbone.

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

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. 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, each of which is herein incorporated by reference.

Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or

heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

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, each of which is herein incorporated by reference.

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, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found, for example, in Nielsen *et al.*, Science, 1991, 254, 1497-1500.

Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2--NH--CH_2--, --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

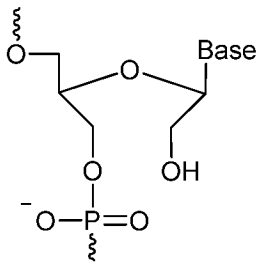
some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

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. In other embodiments, dsRNAs 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. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e.*, a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH_2--O--CH_2--$N(CH_2)_2$.

In other embodiments, an iRNA agent comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides (or nucleosides). In certain embodiments, the sense strand or the antisense strand, or both sense strand and antisense strand, include less than five acyclic nucleotides per strand (*e.g.*, four, three, two or one acyclic nucleotides per strand). The one or more acyclic nucleotides can be found, for example, in the double-stranded region, of the sense or antisense strand, or both strands; at the 5'-end, the 3'-end, both of the 5' and 3'-ends of the sense or antisense strand, or both strands, of the iRNA agent. In one embodiment, one or more acyclic nucleotides are present at positions 1 to 8 of the sense or antisense strand, or both. In one embodiment, one or more acyclic nucleotides are found in the antisense strand at positions 4 to

10 (*e.g.*, positions 6-8) from the 5'-end of the antisense strand. In another embodiment, the one or more acyclic nucleotides are found at one or both 3'-terminal overhangs of the iRNA agent.

The term "acyclic nucleotide" or "acyclic nucleoside" as used herein refers to any nucleotide or nucleoside having an acyclic sugar, *e.g.*, an acyclic ribose. An exemplary acyclic nucleotide or nucleoside can include a nucleobase, *e.g.*, a naturally-occurring or a modified nucleobase (*e.g.*, a nucleobase as described herein). In certain embodiments, a bond between any of the ribose carbons (C1, C2, C3, C4, or C5), is independently or in combination absent from the nucleotide. In one embodiment, the bond between C2-C3 carbons of the ribose ring is absent, *e.g.*, an acyclic 2'-3'-seco-nucleotide monomer. In other embodiments, the bond between C1-C2, C3-C4, or C4-C5 is absent (*e.g.*, a 1'-2', 3'-4' or 4'-5'-seco nucleotide monomer). Exemplary acyclic nucleotides are disclosed in US 8,314,227, incorporated herein by reference in its entirely. For example, an acyclic nucleotide can include any of monomers D-J in Figures 1-2 of US 8,314,227. In one embodiment, the acyclic nucleotide includes the following monomer:



wherein Base is a nucleobase, *e.g.*, a naturally-occurring or a modified nucleobase (*e.g.*, a nucleobase as described herein).

In certain embodiments, the acyclic nucleotide can be modified or derivatized, *e.g.*, by coupling the acyclic nucleotide to another moiety, *e.g.*, a ligand (*e.g.*, a GalNAc, a cholesterol ligand), an alkyl, a polyamine, a sugar, a polypeptide, among others.

In other embodiments, the iRNA agent includes one or more acyclic nucleotides and one or more LNAs (*e.g.*, an LNA as described herein). For example, one or more acyclic nucleotides and/or one or more LNAs can be present in the sense strand, the antisense strand, or both. The number of acyclic nucleotides in one strand can be the same or different from the number of LNAs in the opposing strand. In certain embodiments, the sense strand and/or the antisense

strand comprises less than five LNAs (*e.g.*, four, three, two or one LNAs) located in the double-stranded region or a 3'-overhang. In other embodiments, one or two LNAs are located in the double stranded region or the 3'-overhang of the sense strand. Alternatively, or in combination, the sense strand and/or antisense strand comprises less than five acyclic nucleotides (*e.g.*, four, three, two or one acyclic nucleotides) in the double-stranded region or a 3'-overhang. In one embodiment, the sense strand of the iRNA agent comprises one or two LNAs in the 3'-overhang of the sense strand, and one or two acyclic nucleotides in the double-standed region of the antisense strand (*e.g.*, at positions 4 to 10 (*e.g.*, positions 6-8) from the 5'-end of the antisense strand) of the iRNA agent.

In other embodiments, inclusion of one or more acyclic nucleotides (alone or in addition to one or more LNAs) in the iRNA agent results in one or more (or all) of: (i) a reduction in an off-target effect; (ii) a reduction in passenger strand participation in RNAi; (iii) an increase in specificity of the guide strand for its target mRNA; (iv) a reduction in a microRNA off-target effect; (v) an increase in stability; or (vi) an increase in resistance to degradation, of the iRNA molecule.

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, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference.

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.

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, each of which is herein incorporated by reference, and U.S. Pat. No. 5,750,692, also herein incorporated by reference.

The RNA of an iRNA can also be modified to include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) locked nucleic acids (LNA) (also referred to herein as "locked nucleotides"). In one embodiment, a locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting, *e.g.*, 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, increase thermal stability, 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).

Representative U.S. Patents that teach the preparation of locked nucleic acids 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; 7,399,845, and 8,314,227, each of which is herein incorporated by reference in its entirety. Exemplary LNAs include but are not limited to, a 2', 4'-C methylene bicyclo nucleotide (see for example Wengel *et al.,* International PCT Publication No. WO 00/66604 and WO 99/14226).

In other embodiments, the iRNA agents include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.,* 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in the iRNA molecules can result in enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands.

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"- phosphate,

inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

iRNA Motifs

In one embodiment, the sense strand sequence may be represented by formula (I):

5' n_p-N_a-(X X X)$_i$-N_b-Y Y Y -N_b-(Z Z Z)$_j$-N_a-n_q 3' (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.

In one embodiment, the N_a and/or N_b comprise modifications of alternating pattern.

In one embodiment, 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.

In one embodiment, 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-N_a-YYY-N_b-ZZZ-N_a-n_q 3' (Ib);

5' n_p-N_a-XXX-N_b-YYY-N_a-n_q 3' (Ic); or

5' n_p-N_a-XXX-N_b-YYY-N_b-ZZZ-N_a-n_q 3' (Id).

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.

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.

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.

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

In other embodiments, 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).

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.

In one embodiment, 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')$_l$-N'_a-n_p' 3' (II)

wherein:

k and l 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.

In one embodiment, the N_a' and/or N_b' comprise modifications of alternating pattern.

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 17-23 nucleotides in 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.

In one embodiment, Y′Y′Y′ motif is all 2'-OMe modified nucleotides.

In one embodiment, k is 1 and l is 0, or k is 0 and l is 1, or both k and l are 1.

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).

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.

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.

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.

In other embodiments, k is 0 and l 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).

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.

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

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.

In one embodiment, 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.

In one embodiment 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.

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.

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

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

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'-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 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.

In one embodiment, 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. In another embodiment, k is 0 and l is 0; or k is 1 and l is 0; k is 0 and l is 1; or both k and l are 0; or both k and l are 1.

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

5' n_p - N_a -Y Y Y -N_a-n_q 3'

3' n_p'-N_a'-Y′Y′Y′ -$N_a'$$n_q'$ 5'

(IIIa)

5' n_p -N_a -Y Y Y -N_b -Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-Y′Y′Y′-N_b'-Z′Z′Z′-$N_a'$$n_q'$ 5'

(IIIb)

5' n_p-N_a- X X X -N_b -Y Y Y - N_a-n_q 3'

3' n_p'-N_a'-X′X′X′-N_b'-Y′Y′Y′-N_a'-n_q' 5'

(IIIc)

5' n_p -N_a -X X X -N_b-Y Y Y -N_b- Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-X′X′X′-N_b'-Y′Y′Y′-N_b'-Z′Z′Z′-N_a-n_q' 5'

(IIId)

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.

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.

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.

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.

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

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.

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.

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.

In one embodiment, 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.

In one embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications. In another embodiment, 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. In yet another embodiment, 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. In another embodiment, 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.

In one embodiment, 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.

In one embodiment, 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.

In one embodiment, 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.

In one embodiment, 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

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.

In some embodiments, 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).

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

Ligands can include a naturally occurring substance, such as a protein (*e.g*., human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g*., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin 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.

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-acetyl-galactosamine, 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.

In some embodiments, the ligand is a GalNAc ligand that comprises one or more N-acetylgalactosamine (GalNAc) derivatives. In some embodiments, the GalNAc ligand is used to target the iRNA to the liver (*e.g*., to hepatocytes). Additional description of GalNAc ligands is provided in the section titled Carbohydrate Conjugates.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *e.g*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine)and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, $[MPEG]_2$, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a 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.

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

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins *etc*. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen,

vitamin E, biotin *etc*. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

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

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied 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.

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

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

conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

Lipid Conjugates

In one embodiment, the ligand is 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. 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.

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.

In one embodiment, the lipid based ligand binds 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.

In another embodiment, the lipid based ligand binds 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.

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

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.

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.

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: 323). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 324)) 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: 325)) and the *Drosophila Antennapedia* protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 326)) 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.

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.

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).

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).

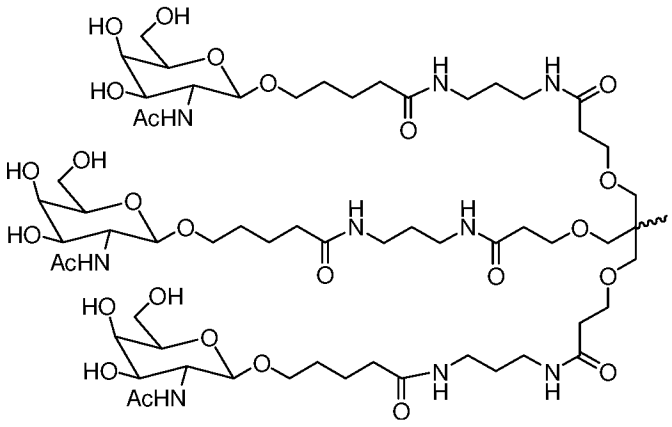
Carbohydrate Conjugates

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

In one embodiment, a carbohydrate conjugate comprises a monosaccharide. In one embodiment, the monosaccharide is an N-acetylgalactosamine (GalNAc). GalNAc conjugates, which comprise one or more N-acetylgalactosamine (GalNAc) derivatives, are described, for example, in U.S. Patent No. 8,106,022, the entire content of which is hereby incorporated herein by reference. In some embodiments, 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).

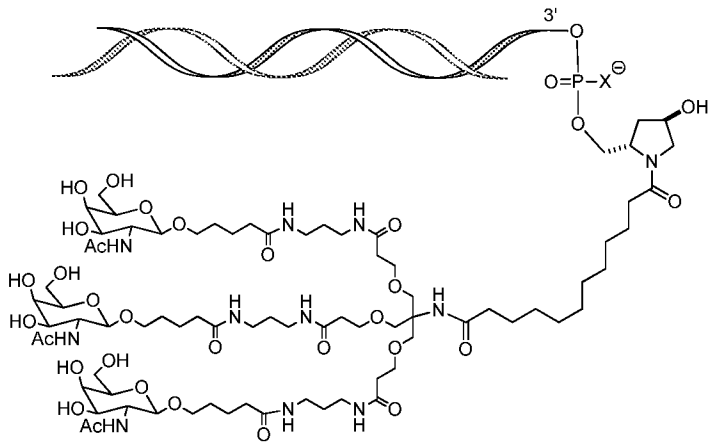
In some embodiments, the carbohydrate conjugate comprises one or more GalNAc derivatives. The GalNAc derivatives may be attached via a linker, *e.g.*, a bivalent or trivalent branched linker. In some embodiments the GalNAc conjugate is conjugated to the 3' end of the sense strand. In some embodiments, 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.

In some embodiments, the GalNAc conjugate is

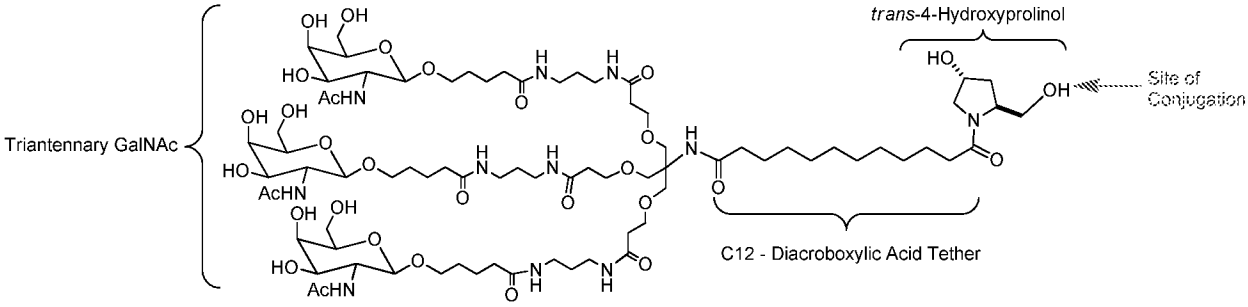


Formula II.

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

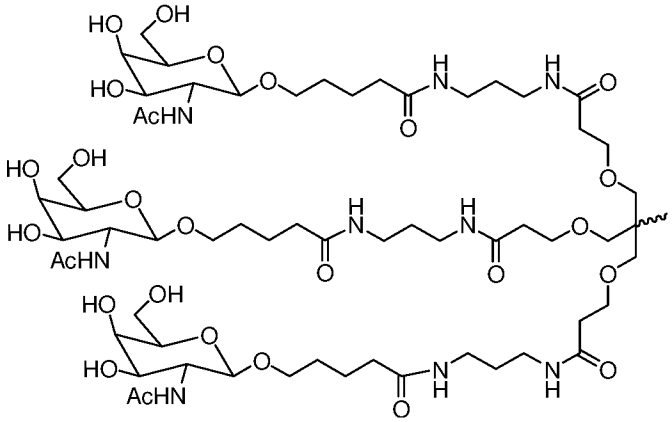


In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below

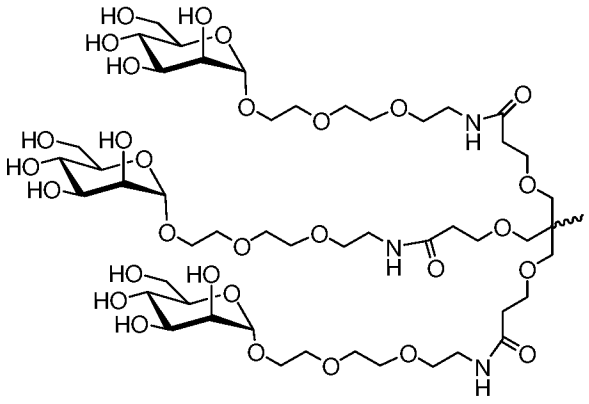


.

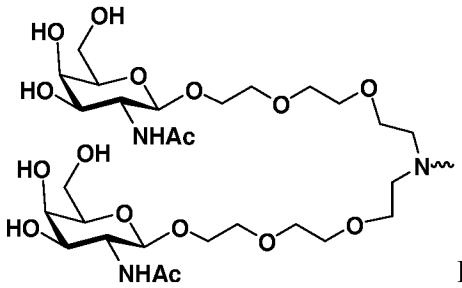
In some embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



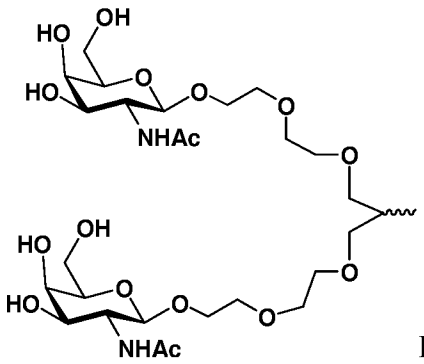
Formula II,



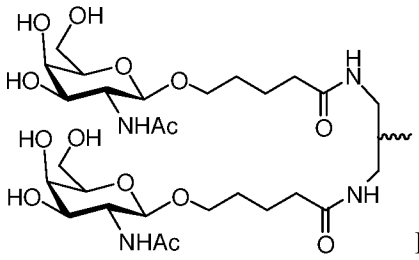
Formula III,



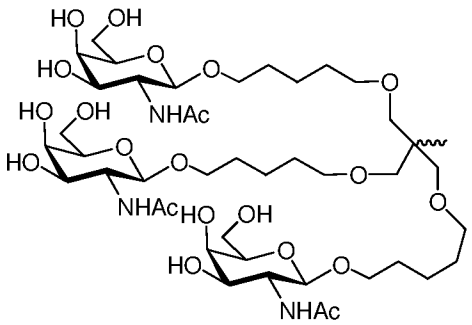
Formula IV,



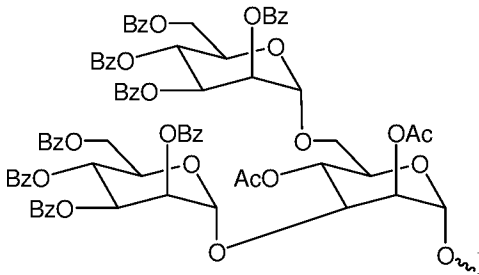
Formula V,



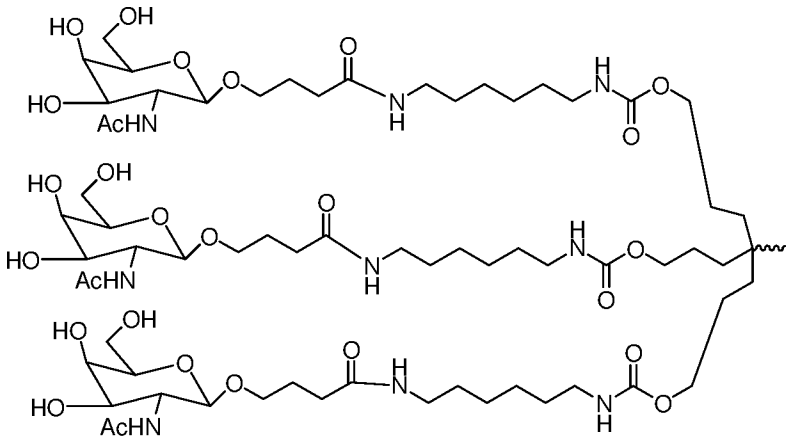
Formula VI,



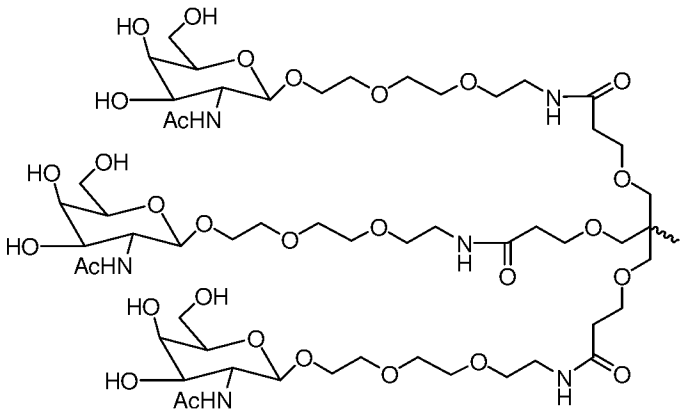
Formula VII,



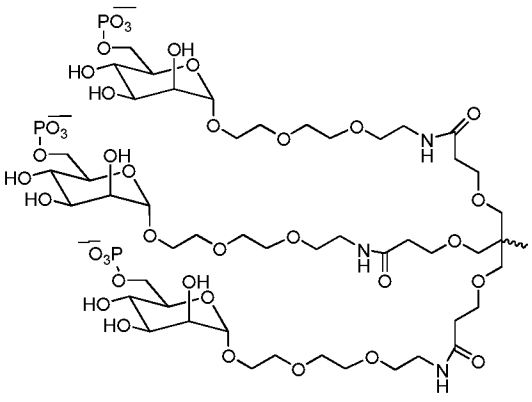
Formula VIII,



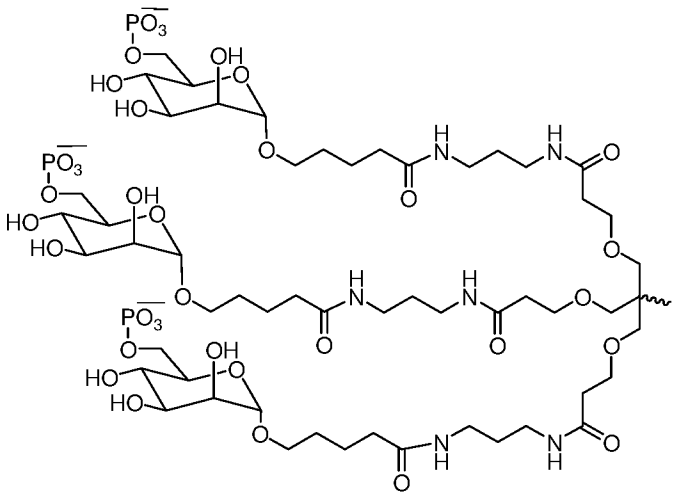
Formula IX,



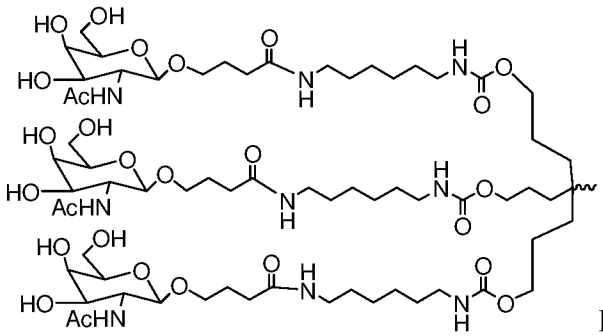
Formula X,



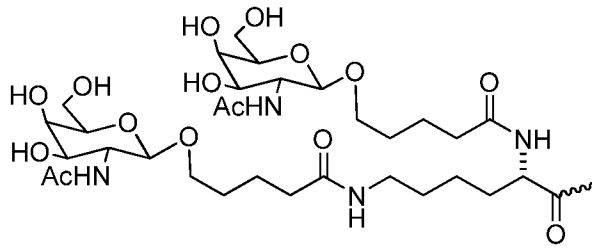
Formula XI,



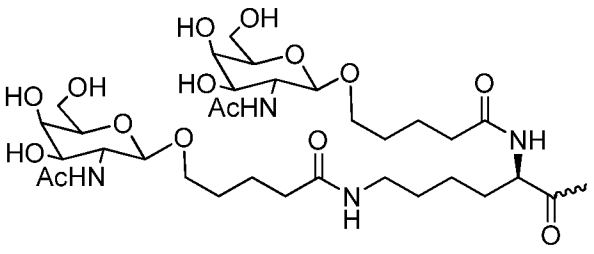
Formula XII,



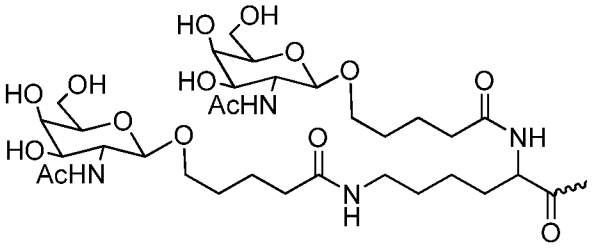
Formula XIII,



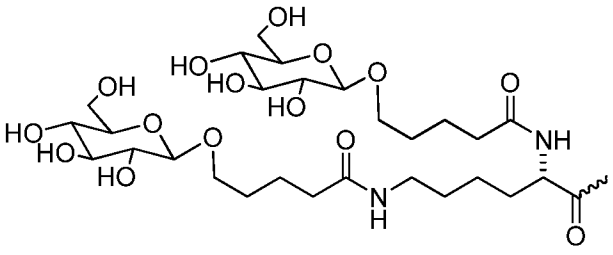
Formula XIV,



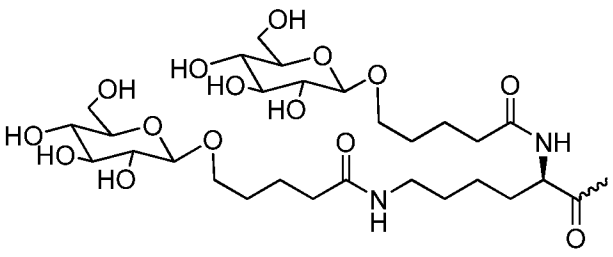
Formula XV,



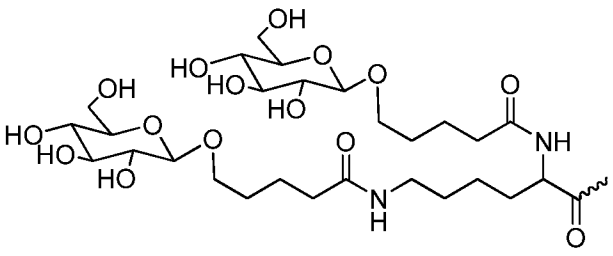
Formula XVI,



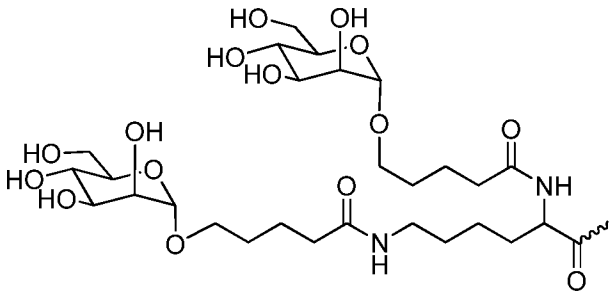
Formula XVII,



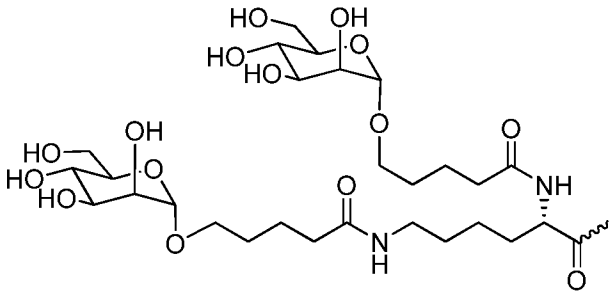
Formula XVIII,



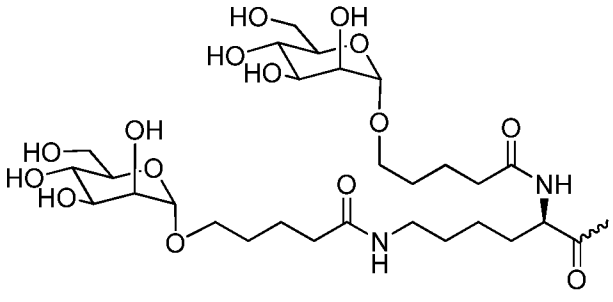
Formula XIX,



Formula XX,

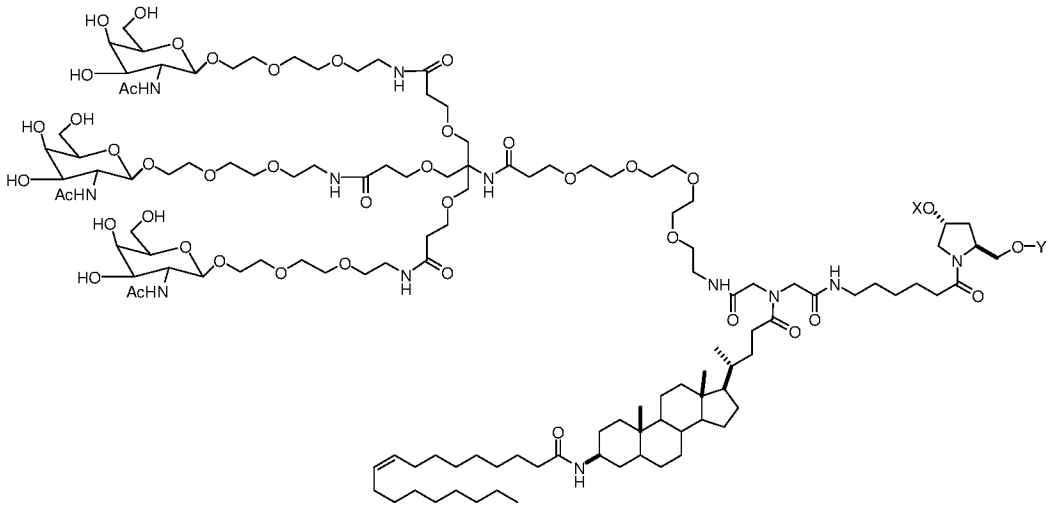


Formula XXI,



Formula XXII.

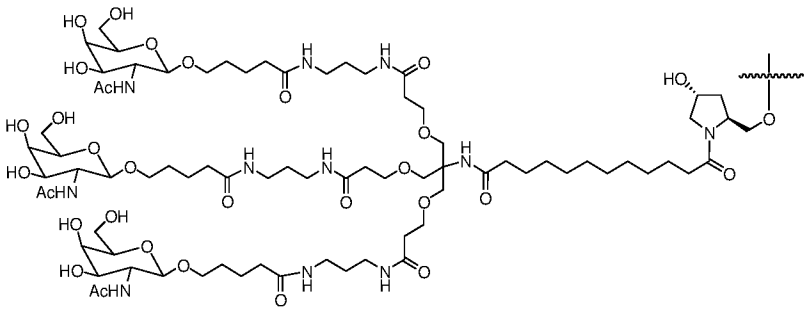
Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



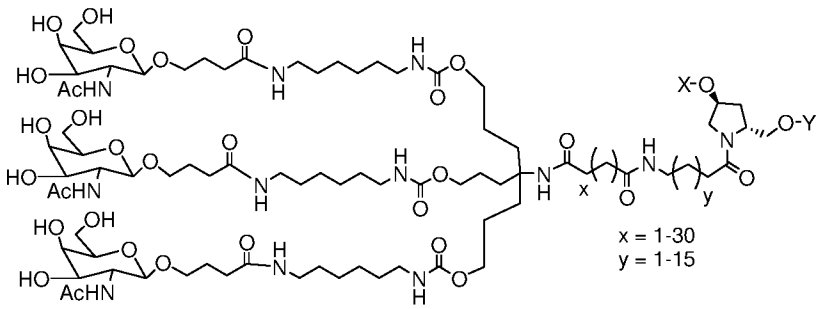
(Formula XXIII), when one of X or Y is an oligonucleotide, the other is a hydrogen.

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

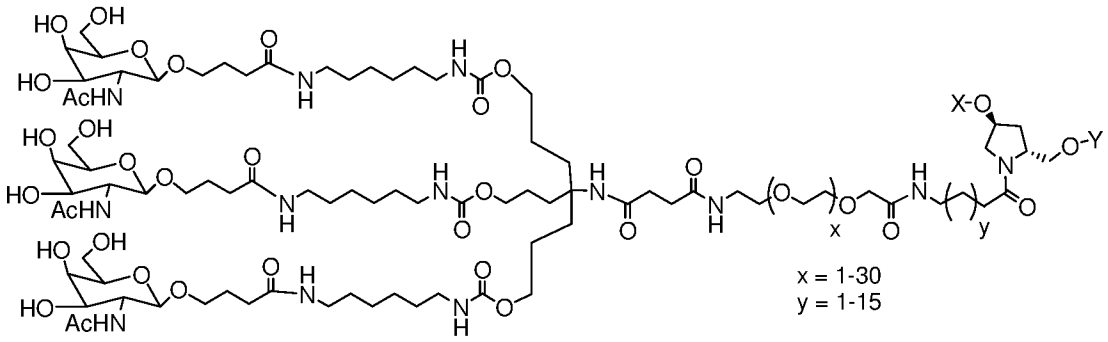
In one embodiment, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



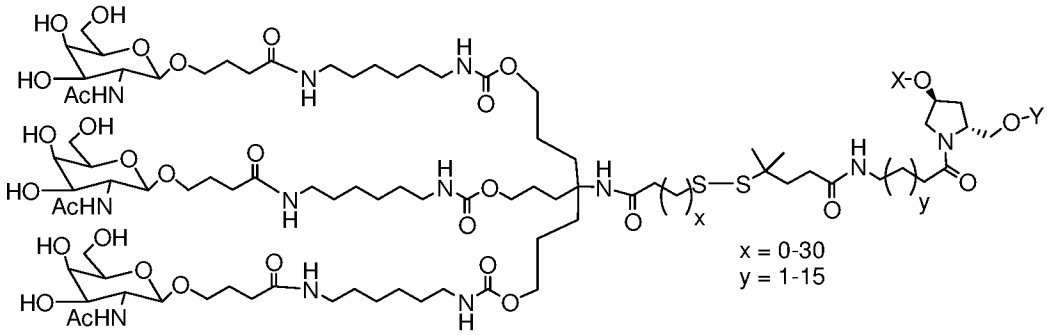
(Formula XXIV),



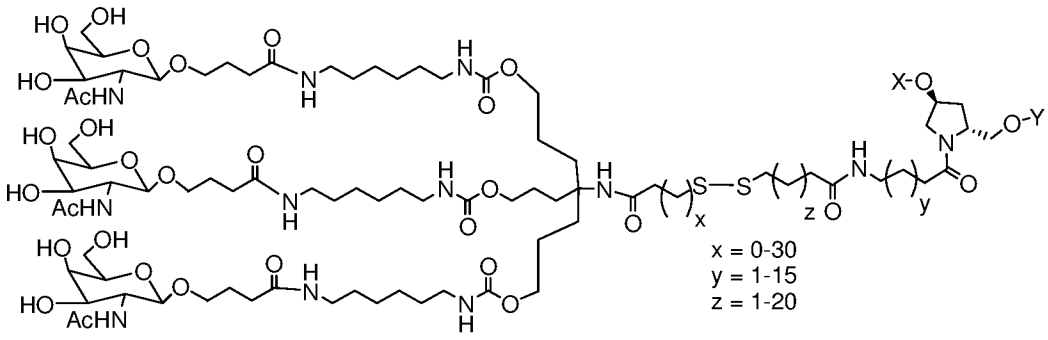
(Formula XXV),



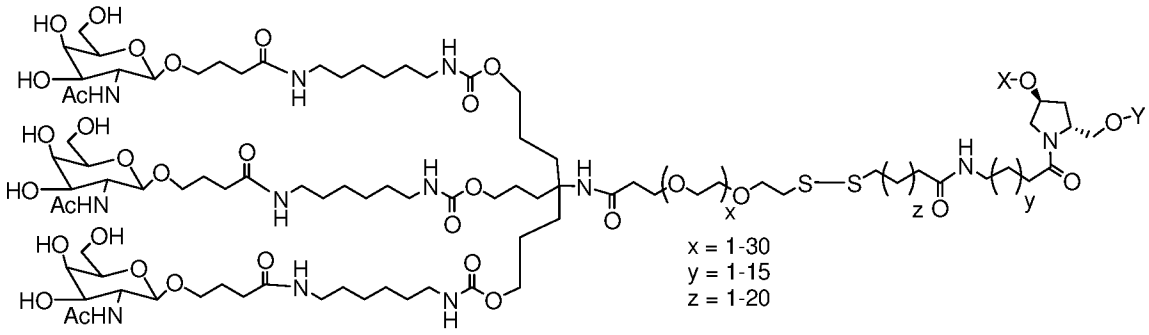
(Formula XXVI),



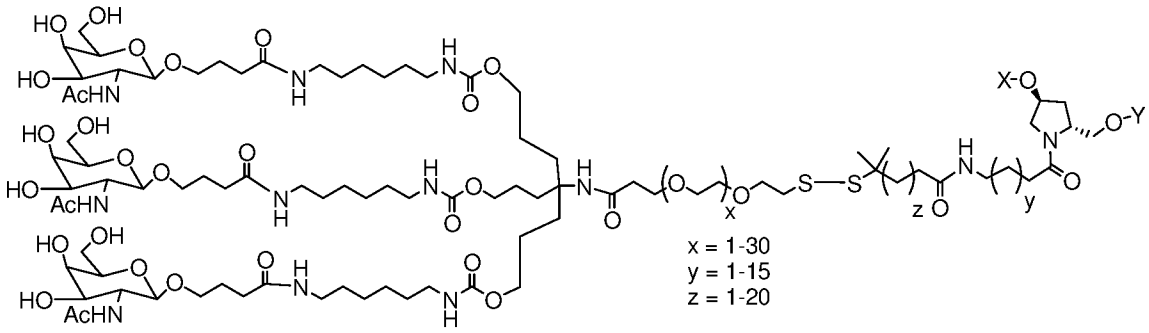
(Formula XXVII),



(Formula XXVIII),



(Formula XXIX), and



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

Linkers

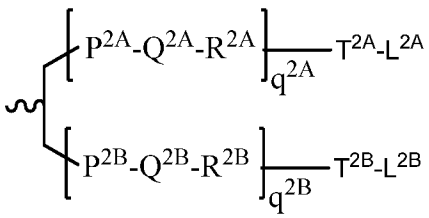
In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

The term "linker" or “linking group” means an organic moiety that connects two parts of a compound, *e.g.,* covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, 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, alkynylheterocyclylalkyl, 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. In one embodiment, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-16, or 8-16 atoms.

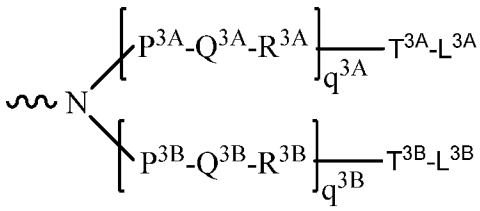
In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XXXI) – (XXXIV):

Formula XXXI



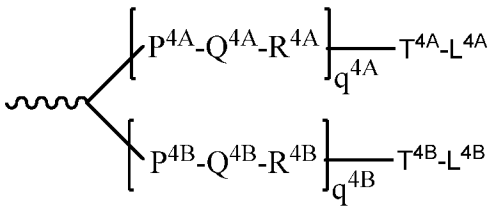
,

Formula XXXII

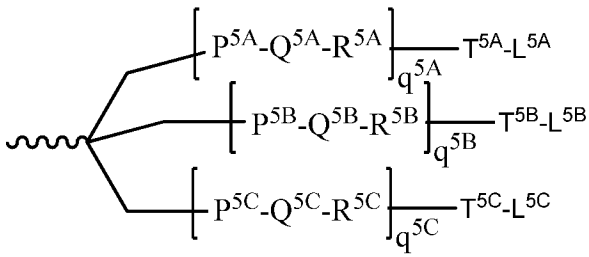


,

Formula XXXIII



Formula XXXIV



;

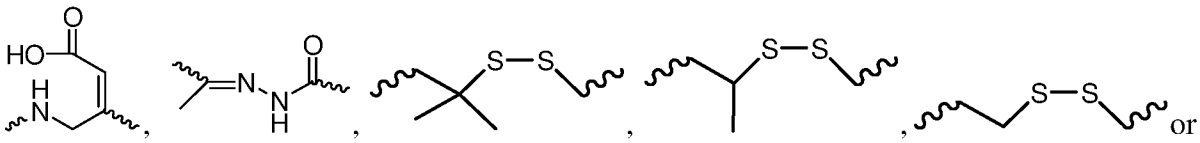
wherein:

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);

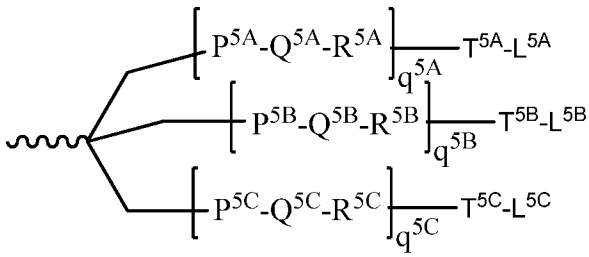
R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, -C(O)-$CH(R^a)$-NH-, CO, CH=N-O,



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; andR^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.

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.

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).

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.

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.

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.

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

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

In one embodiment, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents 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. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or

under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

Phosphate-based cleavable linking groups

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

Acid cleavable linking groups

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

Ester-based cleavable linking groups

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

Peptide-based cleavable linking groups

In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides *etc.*) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or 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)-, 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, the entire contents of each of which is herein incorporated by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications 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.

"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.

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

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

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, which are incorporated herein by reference in their entireties). 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 administration 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*.

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.

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. Bio*l 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). In some embodiments, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be found in U.S. Patent No. 7,427,605, which is herein incorporated by reference in its entirety.

Vector encoded iRNAs

In another aspect, iRNA targeting the LECT2 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).

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

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.

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.

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.

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.

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.

In a specific embodiment, 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, which are herein incorporated by reference.

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.

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, the entire disclosures of which are herein incorporated by reference.

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.

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, the entire disclosure of which is herein incorporated by reference.

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

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 a LECT2 gene (*e.g.*, a LECT2 amyloidosis). 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. In some embodiments, a composition provided herein (*e.g.*, an LNP formulation) is formulated for intravenous delivery. In some embodiments, a composition provided herein (*e.g.*, a composition comprising a GalNAc conjugate) is formulated for subcutaneous delivery.

The pharmaceutical compositions featured herein are administered in a dosage sufficient to inhibit expression of a LECT2 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.

The effect of a single dose on LECT2 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.

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 a suitable animal model.

A suitable animal model, *e.g.*, a mouse containing a transgene expressing human LECT2, can be used to determine the therapeutically effective dose and/or an effective dosage regimen administration of LECT2 siRNA.

The present disclosure also includes pharmaceutical compositions and formulations that include the iRNA compounds featured herein. 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.

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.

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, which is incorporated herein by reference.

Liposomal formulations

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.

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*.

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.

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.

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.

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.

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

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).

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).

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.

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).

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).

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).

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*).

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.

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.

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.

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

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

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.

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.

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

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

Nucleic acid lipid particles

In one embodiment, a LECT2 dsRNA featured in the invention is 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.

In one embodiment, the lipid to drug ratio (mass/mass ratio) (*e.g.*, lipid to dsRNA ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to 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.

The cationic lipid may be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I -(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I -(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), l,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), l,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.

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

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

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-l- 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.

The conjugated lipid that inhibits aggregation of particles may be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a

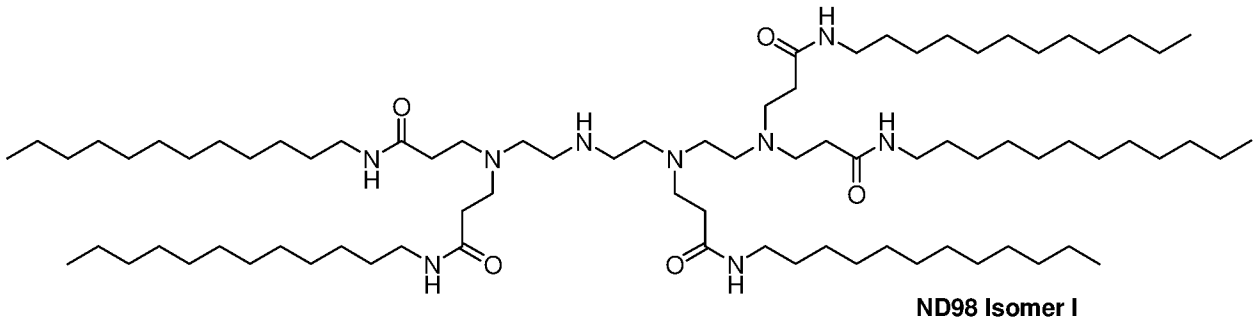
PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate may be, for example, a PEG-dilauryloxypropyl (C_{12}), a PEG-dimyristyloxypropyl (C_{14}), a PEG-dipalmityloxypropyl (C_{16}), or a PEG- distearyloxypropyl (C_{18}). The conjugated lipid 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.

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

In some embodiments, the iRNA is formulated in a lipid nanoparticle (LNP).

LNP01

In one embodiment, the lipidoid ND98·4HCl (MW 1487) (see U.S. Patent Application No. 12/056,230, filed 3/26/2008, which is herein incorporated by reference), 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

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

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

Table 6: Exemplary lipid formulations

	Cationic Lipid	**cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio**
SNALP	l,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 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1

LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (C12-200)	C12-200/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:siRNA: 11:1
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)

SNALP (l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed April 15, 2009, which is hereby incorporated by reference.

XTC comprising formulations are described, *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. 61/185,712, 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, which are hereby incorporated by reference.

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, which are hereby incorporated by reference.

ALNY-100 comprising formulations are described, *e.g.,* International patent application number PCT/US09/63933, filed on November 10, 2009, which is hereby incorporated by reference.

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, which are hereby incorporated by reference.

Synthesis of cationic lipids

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. All substituents are as defined below unless indicated otherwise.

"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.

"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.

"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-1 butynyl, and the like.

"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.

"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.

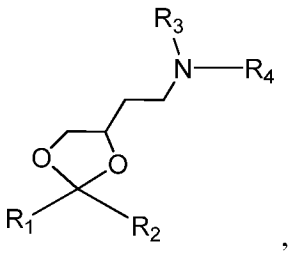
The terms "optionally substituted alkyl", "optionally substituted alkenyl", "optionally substituted alkynyl", "optionally substituted acyl", and "optionally substituted heterocycle" means that, when substituted, at least one hydrogen atom is replaced with a substituent. In the case of an oxo substituent (=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 -$SO_nNR^xR^y$.

"Halogen" means fluoro, chloro, bromo and iodo.

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

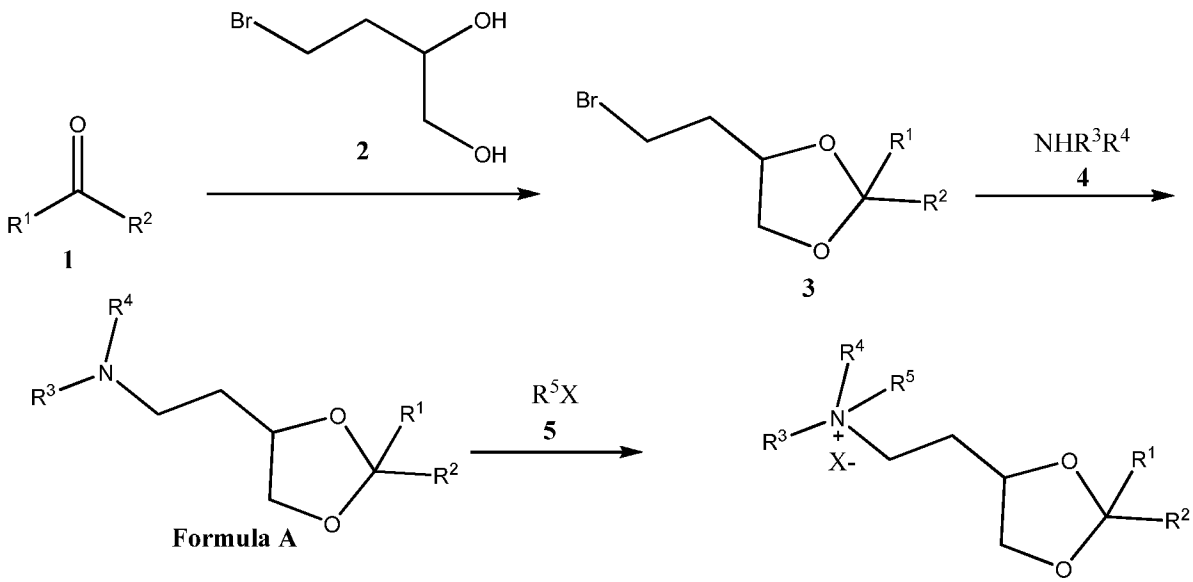
In one embodiments, nucleic acid-lipid particles featured in the invention are formulated using a cationic lipid of formula A:



,

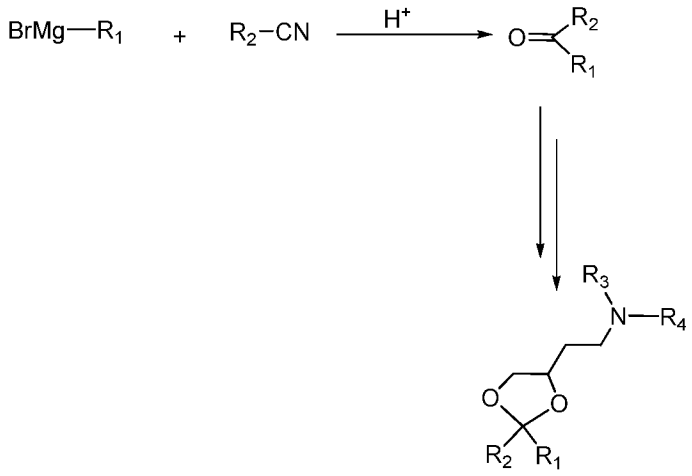
where R1 and R2 are independently alkyl, alkenyl or alkynyl, each can be optionally substituted, and R3 and R4 are independently lower alkyl or R3 and R4 can be taken together to form an optionally substituted heterocyclic ring. In some embodiments, the cationic lipid is XTC (2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane). 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



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



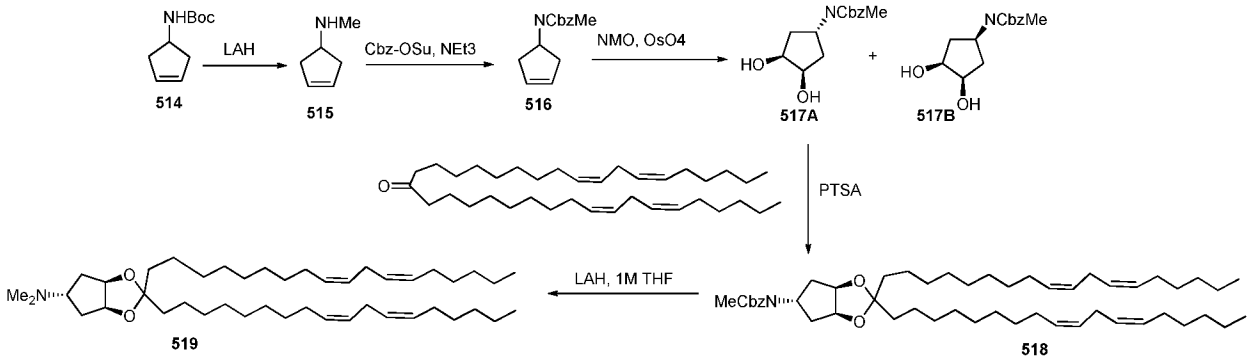
Alternatively, the ketone 1 starting material can be prepared according to Scheme 2. Grignard reagent 6 and cyanide 7 can be purchased or prepared according to methods known to those of ordinary skill in the art. Reaction of 6 and 7 yields ketone 1. Conversion of ketone 1 to the corresponding lipids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

Synthesis of MC3

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

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



Synthesis of 515:

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:

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 1N 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:

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 518:

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:

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.

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.

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, each of which is incorporated herein by reference.

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.

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.

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.

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

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.

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).

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).

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

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty

alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

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

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

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.

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).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (*see e.g.*, Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in *Pharmaceutical*

Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, 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.

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.

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

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly iRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs 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.

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.

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).

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).

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).

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).

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).

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.

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

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

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*).

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

Formulations for topical administration of nucleic acids 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.

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

The compositions of the present invention 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.

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.

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 LECT2 and at least one LECT2 binding partner.

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.

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.

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 LECT2 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 of treating disorders related to expression of a LECT2 gene

The present disclosure relates to the use of an iRNA targeting LECT2 to inhibit LECT2 expression and/or to treat a disease, disorder, or pathological process that is related to LECT2 expression.

In one aspect, a method of treatment of a disorder related to expression of LECT2 is provided, the method comprising administering an iRNA (*e.g.*, a dsRNA) disclosed herein to a subject in need thereof. In some embodiments, the iRNA inhibits (decreases) LECT2 expression. In some embodiments, the iRNA increases LECT2 expression.

As used herein, "a disorder related to LECT2 expression," a "disease related to LECT2 expression, a "pathological process related to LECT2 expression," or the like includes any condition, disorder, or disease in which LECT2 expression is altered (*e.g.,* decreased or increased relative to a normal level). In some embodiments, LECT2 expression is decreased. In some embodiments, LECT2 expression is increased. In embodiments, the decrease or increase in LECT2 expression is detectable in the blood (*e.g.*, in the plasma) of the subject. In embodiments, the decrease or increase in LECT2 expression is detectable in a tissue sample from the subject (*e.g.,* in a kidney sample or a liver sample). The decrease or increase may be assessed relative the level observed in the same individual prior to the development of the disorder or relative to other individual(s) who do not have the disorder. The decrease or increase may be limited to a particular organ, tissue, or region of the body (*e.g.,* the kidney or the liver).

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.

A "subject in need thereof" includes a subject having, suspected of having, or at risk of developing a disorder related to LECT2 expression. In some embodiments, the subject has, or is suspected of having, a disorder related to LECT2 expression. In some embodiments, the subject is at risk of developing a disorder related to LECT2 expression.

In some embodiments, the subject is an animal that serves as a model for a disorder related to LECT2 expression, *e.g.*, a LECT2 amyloidosis.

LECT2 Amyloidosis

In embodiments, the disorder related to LECT2 expression is an amyloidosis, *e.g.*, a LECT2 amyloidosis. LECT2 amyloidosis has been described in several clinical studies. *See, e.g.,* Benson, M.D. *et al* (2008) *Kidney International*, 74: 218-222; Murphy, C. L. *et al.* (2010) *Am J Kidney Dis*, 56(6):1100-1107; Larsen, C.P. *et al.* (2010) *Kidney Int.*, 77(9):816-819; Holanda, D.G. *et al.* (20011) *Nephrol. Dial. Transplant.*, 26 (1): 373-376; and Sethi, S. *et al.* (2012) *Kidney International* 82, 226–234 (hereinafter Sethi *et al.*).

Clinical and pathological features of LECT2 amyloidosis mimic those of amyloid light chain (AL) amyloidosis. These symptoms include, *e.g.,* symptoms of kidney disease and renal failure, *e.g.,* fluid retention, swelling, and shortness of breath. Amyloidosis may affect the heart, peripheral nervous system, gastrointestinal tract, blood, lungs and skin. Heart complications include, *e.g.,* heart failure and irregular heart beat. Other symptoms include, *e.g.,* stroke, gastrointestinal disorders, enlarged liver, diminished spleen function, diminished function of the adrenal and other endocrine glands, skin color change or growths, lung problems, bleeding and bruising problems, fatigue and weight loss. In embodiments, the methods described herein are associated with improvement in one or more symptoms described herein.

Methods for diagnosis of amyloidosis, *e.g.*, LECT2 amyloidosis, are described, *e.g.,* in Leung, N. *et al.* (2010) *Blood*, published online September 4, 2012; DOI 10.1182/blood-2012-03-413682; Shiller, S.M. *et al.* (2011). Laboratory Methods for the Diagnosis of Hereditary Amyloidoses, Amyloidosis - Mechanisms and Prospects for Therapy, Dr. Svetlana Sarantseva (Ed.), ISBN: 978-953-307-253-1; Sethi *et al.* (*see* above) and in U.S. Patent Application Publication No. 20100323381.

Based on the results provided by Sethi *et al.*, LECT2 amyloidosis accounts for a significant percentage of cases of renal amyloidosis. *See* Table 1 of Sethi *et al.*, which shows that 26 out of 127 cases of renal amyloidosis studied by laser microdissection and mass spectrometry of renal biopsy and/or nephrectomy specimens were determined to be of the LECT2 amyloid type. Sethi *et al.* further report that apolipoprotein E protein and serum amyloid P component (SAP) were also present in all cases of LECT2 amyloidosis.

In embodiments, the amyloidosis, *e.g.*, the LECT2 amyloidosis, involves systemic amyloid deposition. In embodiments, the amyloidosis, *e.g.,* the LECT2 amyloidosis, is localized entirely or predominately to a particular tissue or organ (*e.g.,* to the kidney or liver).

In embodiments, the amyloidosis, *e.g.*, the LECT2 amyloidosis, is hereditary.

In embodiments, a LECT2 amyoidosis is diagnosed using analysis of a sample from the subject (*e.g.*, a biopsy sample). In embodiments, the biopsy sample is a renal biopsy. In embodiments, the sample is a nephrectomy sample. In embodiments, the sample is from a liver biopsy or from other resected liver tissue. In embodiments, the sample is analyzed using methods selected from one or more of immunohistochemistry, LECT2 immunoassay, electron microscopy, laser microdissection, and mass spectrometry. In embodiments, the LECT2 amyloidosis is diagnosed using laser microdissection and mass spectrometry.

In embodiments, the amyloidosis, *e.g.*, the LECT2 amyloidosis, affects the kidney, *e.g.*, involves amyloid deposition in the kidney. In embodiments, kidney function is compromised as a result of the amyloidosis. In embodiments, the subject suffers from one or more of fluid retention, swelling, and shortness of breath. In embodiments, the subject has nephrotic syndrome. In embodiments, the subject suffers from proteinuria. In embodiments, the subject has renal failure.

In embodiments, the amyloidosis, *e.g.*, the LECT2 amyloidosis, affects the liver, *e.g.,* involves amyloid deposition in the liver. In embodiments, liver function is compromised as a result of the amyloidosis. In embodiments, the subject has hepatitis, *e.g.,* chronic hepatitis. In embodiments, the hepatitis is a viral hepatitis.

LECT2 amyloidosis has been found to be particularly prevalent in Mexican Americans and has also been associated with homozygosity for the G allele of the LECT2 gene that encodes valine at position 40 in the mature protein (amino acid 58 in the unprocessed protein). *See, e.g.*,

Benson, M.D. *et al.* (2008) *Kidney International*, 74: 218-222; Murphy, C. L. *et al.* (2010) *Am J Kidney Dis*, 56(6):1100-1107.

In some embodiments, the subject is of Mexican descent. In some embodiments, the subject is a Mexican American.

In embodiments, the subject carries the G allele of the LECT2 gene that encodes valine at position 40 in the mature protein (amino acid 58 in the unprocessed protein). In embodiments, the subject is homozygous for the G allele (G/G genotype). In embodiments, a LECT2 protein expressed in the subject has valine at position 40 in the mature protein (or at amino acid 58 in the unprocessed protein).

In some embodiments, the method decreases LECT2 expression. In embodiments, the decrease in LECT2 expression is assessed relative to the level in the same individual prior to the treatment. In some embodiments, the method is shown to decrease LECT2 expression by comparing the levels of LECT2 expression in a treated subject (or group of subjects) with the levels in a control subject (or group of subjects), *e.g.,* an untreated subject (or group of subjects) or a subject (or group of subjects) treated with a control treatment (*e.g.,* an iRNA (*e.g.,* a dsRNA) that does not target LECT2).

In embodiments, the method reduces amyloid deposition, *e.g.*, deposition of amyloid comprising a LECT2 protein or a portion thereof. In embodiments, the protein is a wild type protein. In embodiments, the protein is a human LECT2 protein, or a portion thereof, that includes valine at position 40 (position 40 of the mature, secreted protein, or at amino acid 58 in the unprocessed protein, as described herein). In embodiments, the method decreases the size, number, and/or extent of amyloid deposits.

In embodiments, the method decreases one or more symptoms associated with amyloid deposition.

In some embodiments, the dsRNA is administered in a form that targets the dsRNA to a particular organ or tissue to inhibit amyloid deposition in the organ or tissue.

In some embodiments, the dsRNA is targeted to the liver. In some embodiments, the dsRNA is conjugated to a ligand, *e.g.,* a GalNAc ligand (*e.g.,* a GalNAc ligand as described herein) that targets the dsRNA to the liver (*e.g.,* to hepatocytes).

Also provided herein is a method of reducing amyloid deposition, the method comprising administering a dsRNA as disclosed herein to a subject in need thereof (*e.g.*, a subject having, suspected of having, or at risk for developing a LECT2 amyloidosis). In embodiments, the method decreases (*e.g.,* prevents or diminishes) the size, number, and/or extent of amyloid deposits. The size, number, and/or extent of amyloid deposits may be assessed using any method known in the art (*e.g.*, immunoassay, immunohistochemistry, mass spectrometry). The reduction of amyloid deposition may involve a decrease in amyloid deposition (*e.g.*, size, number, and/or extent of amyloid deposits) of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% or more.

In the methods provided herein, the iRNA (*e.g.*, dsRNA) and compositions thereof are administered in a therapeutically effective amount. Therapeutic effects of administration of a LECT2 siRNA can be established, for example, by comparison with an appropriate control. For example, inhibition of amyloid deposition may be established, for example, in a group of patients with amyloidosis (*e.g.*, LECT2 amyloidosis) by comparison of any appropriate parameter (*e.g.,* a parameter assessing the size, number, or extent of amyloid deposition) with the same parameter in 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; a population that has been treated with placebo or a non-targeting iRNA; and the like.

Rheumatoid Arthritis

Rheumatoid arthritis is also a disorder related to LECT2 expression. In particular, in a Japanese population, it was found that possession of one A allele of the LECT2 gene that encodes isoleucine at position 40 in the mature protein (or amino acid 58 in the unprocessed protein) was found to increase the overall risk of developing rheumatoid arthritis. Possessing two A alleles was strongly associated with disease severity. *See* Kameoka, Y. *et al.* (2000) *Arth Rheum*, 43(6):1419-20.

In one embodiment of the methods provided herein, the disorder related to LECT2 expression is rheumatoid arthritis. In one embodiment, the dsRNA inhibits LECT2 expression in a subject having rheumatoid arthritis. In some such embodiments, the dsRNA inhibits LECT2

expression in synovial tissue and/or in synovial fluid–derived cells (*e.g.,* mononuclear cells and fibroblasts). In some embodiments, the dsRNA targets an mRNA that encodes isoleucine at position 40 in the mature protein (amino acid 58 in the unprocessed protein).

Liver Injury

LECT2 expression can increase during acute liver injury.

In one embodiment of the methods provided herein, the disorder related to LECT2 expression is acute liver injury. In embodiments, the iRNA (*e.g.,* dsRNA) modulates (*e.g.,* increases or decreases) LECT2 expression. In embodiments, the iRNA modulates LECT2 expression in the liver. In embodiments, the iRNA decreases LECT2 expression in the liver. In embodiments, the iRNA increases LECT2 expression in the liver.

Combination Therapies

In embodiments, an iRNA (*e.g.*, a dsRNA) disclosed herein is administered in combination with a second therapy (*e.g.*, one or more additional therapies) known to be effective in treating a disorder related to LECT2 expression (*e.g.*, a LECT2 amyloidosis) or a symptom of such a disorder. The iRNA may be administered before, after, or concurrent with the second therapy. In embodiments, the iRNA is administered before the second therapy. In embodiments, the iRNA is administered after the second therapy. In embodiments, the iRNA is administered concurrent with the second therapy.

The second therapy may be an additional therapeutic agent. The iRNA and the additional therapeutic agent can be administered in combination in the same composition or the additional therapeutic agent can be administered as part of a separate composition.

In some embodiments, the second therapy is a non-iRNA therapeutic agent that is effective to treat the disorder or symptoms of the disorder.

In some embodiments, the disorder to be treated by the compositions or methods disclosed herein is a LECT2 amyloidosis that affects kidney function, *e.g.*, through amyloid deposition in the kidney. In some such embodiments, the iRNA is administered in conjunction with a therapy that supports kidney function (*e.g.*, dialysis, a diuretic, an angiotensin converting enzyme (ACE) inhibitor, an angiotensin receptor blocker (ARB), or dialysis).

In some embodiments, the disorder to be treated by the compositions or methods disclosed herein is a LECT2 amyloidosis involving amyloid deposits in the liver. In some such embodiments, the iRNA is administered in conjunction with a therapy that supports liver function.

In some embodiments, the disorder to be treated by the compositions or methods disclosed herein is a LECT2 amyloidosis, and the iRNA is administered in conjunction with removal of all or part of the organ(s) affected by the amyloidosis (*e.g.*, resection of all or part of kidney or liver tissue affected by the amyloidosis). The removal is optionally conducted in conjunction with a replacement of all or part of the organ removed (*e.g.*, in conjunction with a kidney or liver organ transplant).

Administration dosages, routes, and timing

A subject (*e.g.*, a human subject, *e.g.,* a patient) 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.

In some embodiments, the iRNA is formulated for delivery to a target organ, *e.g.,* to the liver.

In some embodiments, the iRNA is formulated as a lipid formulation, *e.g.,* an LNP formulation as described herein. In some such embodiments, 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. In some embodiments, the lipid formulation, *e.g.*, LNP formulation, is administered intravenously. In embodiments, the iRNA (*e.g.,* dsRNA) is formulated as an LNP formulation and is administered (*e.g.*, intravenously administered) at a dose of 0.1 to 0.5 mg/kg.

In some embodiments, 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.
In some embodiments, the iRNA is in the form of a GalNAc conjugate as described herein. In some such embodiments, 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. In some embodiments, the GalNAc conjugate is administered subcutaneously. In embodiments,

the iRNA (*e.g.*, dsRNA) is in the form of a GalNAc conjugate and is administered (*e.g.*, subcutaneously administered) at a dose of 1 to 10 mg/kg.

In some embodiments, 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.

In some embodiments, the iRNA agent is administered in two or more doses. In some embodiments, the number or amount of subsequent doses is dependent on the achievement of a desired effect, *e.g.*, inhibition of amyloid deposition, or the achievement of a therapeutic or prophylactic effect, *e.g.*, reduction or prevention of one or more symptoms associated with the disorder.

In some embodiments, 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. In some embodiments, 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. In embodiments, the iRNA agent is administered at the frequency required to achieve a desired effect.

In embodiments, 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. In one embodiment, the iRNA agent is initially administered hourly and is later administered at a longer interval (*e.g.*, daily, weekly, biweekly, or monthly). In another embodiment, the iRNA agent is initially administered daily and is later administered at a longer interval (*e.g.*, weekly, biweekly, or monthly). In certain embodiments,

the longer interval increases over time or is determined based on the achievement of a desired effect.

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 a LECT2 gene

In yet another aspect, the invention provides a method for modulating (*e.g.*, inhibiting or activating) the expression of LECT2 gene, *e.g.*, in a cell or in a subject. In some embodiments, the cell is *ex vivo*, *in vitro*, or *in vivo*. In some embodiments, the cell is in the liver (*e.g.*, a hepatocyte). In some embodiments, the cell is in a subject (*e.g.*, a mammal, such as, for example, a human). In some embodiments, the subject (*e.g.*, the human) is at risk, or is diagnosed with a disorder related to expression of LECT2 expression, as described herein.

In one embodiment, the method includes contacting the cell with an iRNA as described herein, in an amount effective to decrease the expression of a LECT2 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 may be contacted when a composition comprising an iRNA is administered (*e.g.*, intravenously or subcutaneously) to the subject.

The expression of a LECT2 gene may be assessed based on the level of expression of a LECT2 mRNA, a LECT2 protein, or the level of another parameter functionally linked to the level of expression of a LECT2 gene. In some embodiments, the expression of LECT2 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%. In some embodiments, 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.

In some embodiments, 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 a LECT2 gene, thereby inhibiting the expression of the LECT2 gene in the cell.

In one embodiment, the method includes administering a composition described herein, *e.g.*, a composition comprising an iRNA that targets LECT2, to the mammal such that expression of the target LECT2 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 LECT2 is detectable within 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, or 24 hours of the first administration.

In another embodiment, the method includes administering a composition as described herein to a mammal such that expression of the target LECT2 gene is increased by *e.g.*, at least 10% compared to an untreated animal. In some embodiments, the activation of LECT2 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 LECT2 expression by stabilizing the LECT2 mRNA transcript, interacting with a promoter in the genome, and/or inhibiting an inhibitor of LECT2 expression.

The iRNAs useful for the methods and compositions featured in the invention specifically target RNAs (primary or processed) of a LECT2 gene. Compositions and methods for inhibiting the expression of a LECT2 gene using iRNAs can be prepared and performed as described elsewhere herein.

In one embodiment, 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 LECT2 gene of the subject, *e.g.*, the mammal, *e.g.*, the human, to be treated. The composition may be administered by any appropriate means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration.

In certain embodiments, the composition is administered by intravenous infusion or injection. In some such embodiments, the composition comprises a lipid formulated siRNA (*e.g.*, an LNP formulation, such as an LNP11 formulation) for intravenous infusion.

In other embodiments, the composition is administered subcutaneously. In some such embodiments, the composition comprises an iRNA conjugated to a GalNAc ligand. In some such embodiments, the ligand targets the iRNA to the liver (*e.g.*, to hepatocytes).

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

EXAMPLES

Example 1. LECT2 siRNA

Nucleic acid sequences provided herein are represented using standard nomenclature. *See* the abbreviations of Table 1.

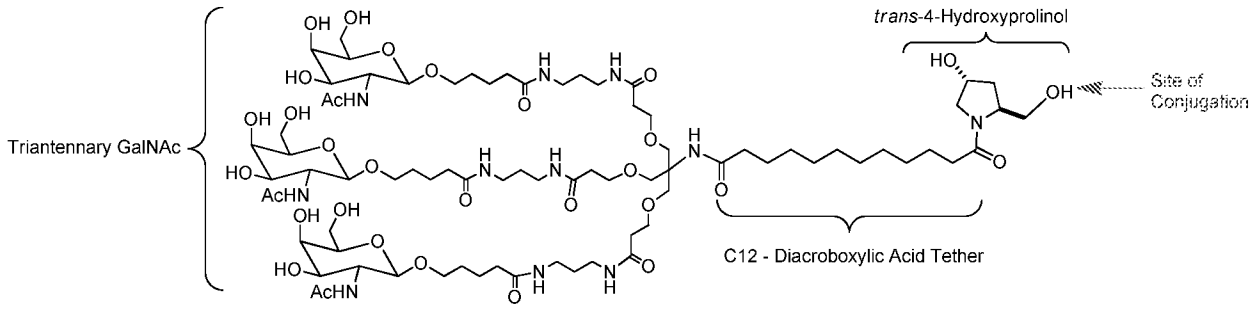
Table 1: Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation	Nucleotide(s)
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3`-phosphate
Abs	beta-L-adenosine-3`-phosphorothioate
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
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

[1]The chemical structure of L96 is as follows:



Experimental Methods

Bioinformatics

Transcripts

A set of siRNAs targeting the human LECT2, "leukocyte cell derived chemotaxin 2" (human: NCBI refseqID NM_002302; NCBI GeneID: 3950), as well as toxicology-species LECT2 orthologs (cynomolgus monkey: XM_005557840; mouse: NM_010702; rat: NM_001108405) were designed using custom R and Python scripts. The human NM_002302REFSEQ mRNA, version 2, has a length of 1077 bases. The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 10 through position 1077 was determined with a linear model derived the direct measure of mRNA knockdown from more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. Subsets of the LECT2 siRNAs were designed with perfect or near-perfect matches between human and cynomolgus monkey. A further subset was designed with perfect or near-perfect matches to human, cynomolgus monkey and mouse LECT2 orthologs. A further subset was designed with perfect or near-perfect matches to human, cynomolgus monkey, mouse and rat LECT2 orthologs. For each strand of the siRNA, a custom Python script was used in a brute force search to measure the number and positions of mismatches between the siRNA and all potential alignments in the target species transcriptome. Extra weight was given to mismatches in the seed region, defined here as positions 2-9 of the antisense oligonucleotide, as well the cleavage site of the siRNA, defined here as positions 10-11 of the antisense oligonucleotide. The relative weight of the mismatches was 2.8; 1.2: 1 for seed mismatches, cleavage site, and other positions up through antisense position 19. Mismatches in the first position were ignored. A specificity score was calculated for each strand by summing the value of each weighted mismatch. Preference was given to siRNAs whose antisense score in human was >= 2.2 and predicted efficacy was >= 50% knockdown of the LECT2 transcript. Pairs of exemplay oligos are evidenced in Table 2 and Table 3, modified and unmodified sequences respectively.

Table 2. LECT2-Modified sequences

Duplex Name	Seq ID No: (sense)	Sense oligoSeq	Seq ID No: (anti sense)	Antisense oligoSeq	Seq ID No: mRNA target	mRNA target sequence
AD-75396	2	ususguguCfuCfAfCfacuaaagaaaL96	3	VPusUfsucuUfuAfGfugugAfgAfcacaasasa	4	UUUUGUGUCUCACACUAAAGAAA
AD-75385	5	gsusgucuCfaCfAfCfuaaagaaauaL96	6	VPusAfsuuuCfuUfUfagugUfgAfgacacsasa	7	UUGUGUCUCACACUAAAGAAAUG
AD-75397	8	asasuucuAfaGfGfCfuaaauagcuaL96	9	VPusAfsgcuAfuUfUfagccUfuAfgaauuscsu	10	AGAAUUCUAAGGCUAAAUAGCUA
AD-75386	11	gsgsaaguAfuUfCfAfuucaaacuuaL96	12	VPusAfsaguUfuGfAfaugaAfuAfcuuccsusa	13	UAGGAAGUAUUCAUUCAAACUUG
AD-75374	14	ususcauuCfaAfAfCfuugaauauuaL96	15	VPusAfsauaUfuCfAfaguuUfgAfaugaasusa	16	UAUUCAUUCAAACUUGAAUAUUC
AD-75391	17	uscsauucAfaAfCfUfugaauauucaL96	18	VPusGfsaauAfuUfCfaaguUfuGfaaugasasu	19	AUUCAUUCAAACUUGAAUAUUCU
AD-75393	20	csasuucaAfaCfUfUfgaauauucuaL96	21	VPusAfsgaaUfaUfUfcaagUfuUfgaaugsasa	22	UUCAUUCAAACUUGAAUAUUCUU
AD-75381	23	uscsaaacUfuGfAfAfuauucuucaaL96	24	VPusUfsgaaGfaAfUfauucAfaGfuuugasasu	25	AUUCAAACUUGAAUAUUCUUCAA
AD-75383	26	csasaacuUfgAfAfUfauucuucaaaL96	27	VPusUfsugaAfgAfAfuauuCfaAfguuugsasa	28	UUCAAACUUGAAUAUUCUUCAAA
AD-75372	29	asasacuuGfaAfUfAfuucuucaaaaL96	30	VPusUfsuugAfaGfAfauauUfcAfaguuusgsa	31	UCAAACUUGAAUAUUCUUCAAAG
AD-75371	32	csusugaaUfaUfUfCfuucaaagagaL96	33	VPusCfsucuUfuGfAfagaaUfaUfucaagsusu	34	AACUUGAAUAUUCUUCAAAGAGA
AD-75365	35	ascsucuaAfuCfAfGfaggaagaaaaL96	36	VPusUfsuucUfuCfCfucugAfuUfagagususg	37	CAACUCUAAUCAGAGGAAGAAAC
AD-75384	38	gsasaguaAfaAfCfCfagauguuuuaL96	39	VPusAfsaaaCfaUfCfugguUfuUfacuucscsu	40	AGGAAGUAAAACCAGAUGUUUUC
AD-75377	41	gsusaaaaCfcAfGfAfuguuuuccaaL96	42	VPusUfsggaAfaAfCfaucuGfgUfuuuacsusu	43	AAGUAAAACCAGAUGUUUUCCAC
AD-75380	44	asasaaccAfgAfUfGfuuuuccaccaL96	45	VPusGfsgugGfaAfAfacauCfuGfguuuusasc	46	GUAAAACCAGAUGUUUUCCACCA
AD-75375	47	asasaccaGfaUfGfUfuuuccaccaaL96	48	VPusUfsgguGfgAfAfaacaUfcUfgguuususa	49	UAAAACCAGAUGUUUUCCACCAA
AD-75376	50	asasccagAfuGfUfUfuuccaccaaaL96	51	VPusUfsuggUfgGfAfaaacAfuCfugguususu	52	AAAACCAGAUGUUUUCCACCAAA
AD-75400	53	ascscagaUfgUfUfUfuccaccaaaaL96	54	VPusUfsuugGfuGfGfaaaaCfaUfcuggususu	55	AAACCAGAUGUUUUCCACCAAAG
AD-75366	56	asusggcuGfuGfGfAfcaguacucuaL96	57	VPusAfsgagUfaCfUfguccAfcAfgccausgsg	58	CCAUGGCUGUGGACAGUACUCUG
AD-75379	59	csasggagAfaAfCfCfuuaucaaaaaL96	60	VPusUfsuuuGfaUfAfagguUfuCfuccugsgsc	61	GCCAGGAGAAACCUUAUCAAAAC
AD-75394	62	gsgsagaaAfcCfUfUfaucaaaacaaL96	63	VPusUfsguuUfuGfAfuaagGfuUfucuccsusg	64	CAGGAGAAACCUUAUCAAAACAA
AD-75388	65	ususguguCfaAfAfAfuguucuacaaL96	66	VPusUfsguaGfaAfCfauuuUfgAfcacaasasa	67	UUUUGUGUCAAAAUGUUCUACAU
AD-75390	68	usgsugucAfaAfAfUfguucuacauaL96	69	VPusAfsuguAfgAfAfcauuUfuGfacacasasa	70	UUUGUGUCAAAAUGUUCUACAUU

AD-75378	71	gsusgucaAfaAfUfGfuucuacauuaL96	72	VPusAfsaugUfaGfAfacauUfuUfgacacsasa	73	UUGUGUCAAAAUGUUCUACAUUA
AD-75357	74	usgsucaaAfaUfGfUfucuacauuaaL96	75	VPusUfsaauGfuAfGfaacaUfuUfugacascsa	76	UGUGUCAAAAUGUUCUACAUUAA
AD-75354	77	asasuguuCfuAfCfAfuuaagccaaaL96	78	VPusUfsuggCfuUfAfauguAfgAfacauususu	79	AAAAUGUUCUACAUUAAGCCAAU
AD-75356	80	asusguucUfaCfAfUfuaagccaauaL96	81	VPusAfsuugGfcUfUfaaugUfaGfaacaususu	82	AAAUGUUCUACAUUAAGCCAAUU
AD-75360	83	ususcuacAfuUfAfAfgccaauuaaaL96	84	VPusUfsuaaUfuGfGfcuuaAfuGfuagaascsa	85	UGUUCUACAUUAAGCCAAUUAAG
AD-75362	86	uscsuacaUfuAfAfGfccaauuaagaL96	87	VPusCfsuuaAfuUfGfgcuuAfaUfguagasasc	88	GUUCUACAUUAAGCCAAUUAAGU
AD-75364	89	csusacauUfaAfGfCfcaauuaaguaL96	90	VPusAfscuuAfaUfUfggcuUfaAfuguagsasa	91	UUCUACAUUAAGCCAAUUAAGUA
AD-75358	92	usascauuAfaGfCfCfaauuaaguaaL96	93	VPusUfsacuUfaAfUfuggcUfuAfauguasgsa	94	UCUACAUUAAGCCAAUUAAGUAU
AD-75355	95	csasuuaaGfcCfAfAfuuaaguauaaL96	96	VPusUfsauaCfuUfAfauugGfcUfuaaugsusa	97	UACAUUAAGCCAAUUAAGUAUAA
AD-75359	98	asusuaagCfcAfAfUfuaaguauaaaL96	99	VPusUfsuauAfcUfUfaauuGfgCfuuaausgsu	100	ACAUUAAGCCAAUUAAGUAUAAA
AD-75367	101	ususaagcCfaAfUfUfaaguauaaaaL96	102	VPusUfsuuaUfaCfUfuaauUfgGfcuuaasusg	103	CAUUAAGCCAAUUAAGUAUAAAG
AD-75369	104	usasagccAfaUfUfAfaguauaaagaL96	105	VPusCfsuuuAfuAfCfuuaaUfuGfgcuuasasu	106	AUUAAGCCAAUUAAGUAUAAAGG
AD-75370	107	asasgccaAfuUfAfAfguauaaaggaL96	108	VPusCfscuuUfaUfAfcuuaAfuUfggcuusasa	109	UUAAGCCAAUUAAGUAUAAAGGU
AD-75368	110	asgsccaaUfuAfAfGfuauaaagguaL96	111	VPusAfsccuUfuAfUfacuuAfaUfuggcususa	112	UAAGCCAAUUAAGUAUAAAGGUC
AD-75399	113	ascsuuggAfaCfUfCfuauugcccuaL96	114	VPusAfsgggCfaAfUfagagUfuCfcaagususu	115	AAACUUGGAACUCUAUUGCCCUU
AD-75398	116	ususggaaCfuCfUfAfuugcccuugaL96	117	VPusCfsaagGfgCfAfauagAfgUfuccaasgsu	118	ACUUGGAACUCUAUUGCCCUUGC
AD-75395	119	gscsccuuGfcAfGfAfaaguuuaucaL96	120	VPusGfsauaAfaCfUfuucuGfcAfagggcsasa	121	UUGCCCUUGCAGAAAGUUUAUCC
AD-75387	122	uscsgcauGfuGfCfAfcauugaaaaaL96	123	VPusUfsuuuCfaAfUfgugcAfcAfugcgasusu	124	AAUCGCAUGUGCACAUUGAAAAC
AD-75373	125	gscsauguGfcAfCfAfuugaaaacuaL96	126	VPusAfsguuUfuCfAfauguGfcAfcaugcsgsa	127	UCGCAUGUGCACAUUGAAAACUG
AD-75361	128	csasugugCfaCfAfUfugaaaacugaL96	129	VPusCfsaguUfuUfCfaaugUfgCfacaugscsg	130	CGCAUGUGCACAUUGAAAACUGU
AD-75363	131	asusgugcAfcAfUfUfgaaaacuguaL96	132	VPusAfscagUfuUfUfcaauGfuGfcacausgsc	133	GCAUGUGCACAUUGAAAACUGUG
AD-75382	134	asusggucAfgAfUfCfuucaaaauaaL96	135	VPusUfsauuUfuGfAfagauCfuGfaccaususg	136	CAAUGGUCAGAUCUUCAAAAUAA
AD-75389	137	asasauuuGfuGfUfUfcacaaaggaaL96	138	VPusUfsccuUfuGfUfgaacAfcAfaauuuscsu	139	AGAAAUUUGUGUUCACAAAGGAA
AD-75392	140	asusuuguGfuUfCfAfcaaaggaaaaL96	141	VPusUfsuucCfuUfUfgugaAfcAfcaaaususu	142	AAAUUUGUGUUCACAAAGGAAAA
AD-75396	236	ususguguCfuCfAfCfacuaaagaaa	237	usUfsucuUfuAfGfugugAfgAfcacaasasa	4	UUUUGUGUCUCACACUAAAGAAA
AD-75385	238	gsusgucuCfaCfAfCfuaaagaaaua	239	usAfsuuuCfuUfUfagugUfgAfgacacsasa	7	UUGUGUCUCACACUAAAGAAAUG
AD-75397	240	asasuucuAfaGfGfCfuaaauagcua	241	usAfsgcuAfuUfUfagccUfuAfgaauuscsu	10	AGAAUUCUAAGGCUAAAUAGCUA

AD-75386	242	gsgsaaguAfuUfCfAfuucaaacuua	243	usAfsaguUfuGfAfaugaAfuAfcuuccsusa	13	UAGGAAGUAUUCAUUCAAACUUG
AD-75374	244	ususcauuCfaAfAfCfuugaauauua	245	usAfsauaUfuCfAfaguuUfgAfaugaasusa	16	UAUUCAUUCAAACUUGAAUAUUC
AD-75391	246	uscsauucAfaAfCfUfugaauauuca	247	usGfsaauAfuUfCfaaguUfuGfaaugasasu	19	AUUCAUUCAAACUUGAAUAUUCU
AD-75393	248	csasuucaAfaCfUfUfgaauauucua	249	usAfsgaaUfaUfUfcaagUfuUfgaaugsasa	22	UUCAUUCAAACUUGAAUAUUCUU
AD-75381	250	uscsaaacUfuGfAfAfuauucuucaa	251	usUfsgaaGfaAfUfauucAfaGfuuugasasu	25	AUUCAAACUUGAAUAUUCUUCAA
AD-75383	252	csasaacuUfgAfAfUfauucuucaaa	253	usUfsugaAfgAfAfuauuCfaAfguuugsasa	28	UUCAAACUUGAAUAUUCUUCAAA
AD-75372	254	asasacuuGfaAfUfAfuucuucaaaa	255	usUfsuugAfaGfAfauauUfcAfaguuusgsa	31	UCAAACUUGAAUAUUCUUCAAAG
AD-75371	256	csusugaaUfaUfUfCfuucaaagaga	257	usCfsucuUfuGfAfagaaUfaUfucaagsusu	34	AACUUGAAUAUUCUUCAAAGAGA
AD-75365	258	ascsucuaAfuCfAfGfaggaagaaaa	259	usUfsuucUfuCfCfucugAfuUfagagususg	37	CAACUCUAAUCAGAGGAAGAAAC
AD-75384	260	gsasaguaAfaAfCfCfagauguuuua	261	usAfsaaaCfaUfCfugguUfuUfacuucscsu	40	AGGAAGUAAAACCAGAUGUUUUC
AD-75377	262	gsusaaaaCfcAfGfAfuguuuuccaa	263	usUfsggaAfaAfCfaucuGfgUfuuuacsusu	43	AAGUAAAACCAGAUGUUUUCCAC
AD-75380	264	asasaaccAfgAfUfGfuuuuccacca	265	usGfsgugGfaAfAfacauCfuGfguuuusasc	46	GUAAAACCAGAUGUUUUCCACCA
AD-75375	266	asasaccaGfaUfGfUfuuuccaccaa	267	usUfsgguGfgAfAfaacaUfcUfgguuususa	49	UAAAACCAGAUGUUUUCCACCAA
AD-75376	268	asasccagAfuGfUfUfuuccaccaaa	269	usUfsuggUfgGfAfaaacAfuCfugguususu	52	AAAACCAGAUGUUUUCCACCAAA
AD-75400	328	ascscagaUfgUfUfUfuccaccaaaa	329	usUfsuugGfuGfGfaaaaCfaUfcuggususu	55	AAACCAGAUGUUUUCCACCAAAG
AD-75366	330	asusggcuGfuGfGfAfcaguacucua	331	usAfsgagUfaCfUfguccAfcAfgccausgsg	58	CCAUGGCUGUGGACAGUACUCUG
AD-75379	332	csasggagAfaAfCfCfuuaucaaaaa	333	usUfsuuuGfaUfAfagguUfuCfuccugsgsc	61	GCCAGGAGAAACCUUAUCAAAAC
AD-75394	334	gsgsagaaAfcCfUfUfaucaaaacaa	335	usUfsguuUfuGfAfuaagGfuUfucuccsusg	64	CAGGAGAAACCUUAUCAAAACAA
AD-75388	270	ususguguCfaAfAfAfuguucuacaa	271	usUfsguaGfaAfCfauuuUfgAfcacaasasa	67	UUUUGUGUCAAAAUGUUCUACAU
AD-75390	272	usgsugucAfaAfAfUfguucuacaua	273	usAfsuguAfgAfAfcauuUfuGfacacasasa	70	UUUGUGUCAAAAUGUUCUACAUU
AD-75378	274	gsusgucaAfaAfUfGfuucuacauua	275	usAfsaugUfaGfAfacauUfuUfgacacsasa	73	UUGUGUCAAAAUGUUCUACAUUA
AD-75357	276	usgsucaaAfaUfGfUfucuacauuaa	278	usUfsaauGfuAfGfaacaUfuUfugacascsa	76	UGUGUCAAAAUGUUCUACAUUAA
AD-75354	279	asasuguuCfuAfCfAfuuaagccaaa	280	usUfsuggCfuUfAfauguAfgAfacauususu	79	AAAAUGUUCUACAUUAAGCCAAU
AD-75356	281	asusguucUfaCfAfUfuaagccaaua	282	usAfsuugGfcUfUfaaugUfaGfaacaususu	82	AAAUGUUCUACAUUAAGCCAAUU
AD-75360	283	ususcuacAfuUfAfAfgccaauuaaa	284	usUfsuaaUfuGfGfcuuaAfuGfuagaascsa	85	UGUUCUACAUUAAGCCAAUUAAG
AD-75362	285	uscsuacaUfuAfAfGfccaauuaaga	286	usCfsuuaAfuUfGfgcuuAfaUfguagasasc	88	GUUCUACAUUAAGCCAAUUAAGU
AD-75364	287	csusacauUfaAfGfCfcaauuaagua	288	usAfscuuAfaUfUfggcuUfaAfuguagsasa	91	UUCUACAUUAAGCCAAUUAAGUA
AD-75358	289	usascauuAfaGfCfCfaauuaaguaa	290	usUfsacuUfaAfUfuggcUfuAfauguasgsa	94	UCUACAUUAAGCCAAUUAAGUAU

AD-75355	291	csasuuaaGfcCfAfAfuuaaguauaa	292	usUfsauaCfuUfAfauugGfcUfuaaugsusa	97	UACAUUAAGCCAAUUAAGUAUAA
AD-75359	293	asusuaagCfcAfAfUfuaaguauaaa	294	usUfsuauAfcUfUfaauuGfgCfuuaausgsu	100	ACAUUAAGCCAAUUAAGUAUAAA
AD-75367	295	ususaagcCfaAfUfUfaaguauaaaa	296	usUfsuuaUfaCfUfuaauUfgGfcuuaasusg	103	CAUUAAGCCAAUUAAGUAUAAAG
AD-75369	297	usasagccAfaUfUfAfaguauaaaga	298	usCfsuuuAfuAfCfuuaaUfuGfgcuuasasu	106	AUUAAGCCAAUUAAGUAUAAAGG
AD-75370	299	asasgccaAfuUfAfAfguauaaagga	300	usCfscuuUfaUfAfcuuaAfuUfggcuusasa	109	UUAAGCCAAUUAAGUAUAAAGGU
AD-75368	301	asgsccaaUfuAfAfGfuauaaaggua	302	usAfsccuUfuAfUfacuuAfaUfuggcususa	112	UAAGCCAAUUAAGUAUAAAGGUC
AD-75399	303	ascsuuggAfaCfUfCfuauugcccua	304	usAfsgggCfaAfUfagagUfuCfcaagususu	115	AAACUUGGAACUCUAUUGCCCUU
AD-75398	305	ususggaaCfuCfUfAfuugcccuuga	306	usCfsaagGfgCfAfauagAfgUfuccaasgsu	118	ACUUGGAACUCUAUUGCCCUUGC
AD-75395	307	gscsccuuGfcAfGfAfaaguuuauca	308	usGfsauaAfaCfUfuucuGfcAfagggcsasa	121	UUGCCCUUGCAGAAAGUUUAUCC
AD-75387	309	uscsgcauGfuGfCfAfcauugaaaaa	310	usUfsuuuCfaAfUfgugcAfcAfugcgasusu	124	AAUCGCAUGUGCACAUUGAAAAC
AD-75373	311	gscsauguGfcAfCfAfuugaaaacua	312	usAfsguuUfuCfAfauguGfcAfcaugcsgsa	127	UCGCAUGUGCACAUUGAAAACUG
AD-75361	313	csasugugCfaCfAfUfugaaaacuga	314	usCfsaguUfuUfCfaaugUfgCfacaugscsg	130	CGCAUGUGCACAUUGAAAACUGU
AD-75363	315	asusgugcAfcAfUfUfgaaaacugua	316	usAfscagUfuUfUfcaauGfuGfcacausgsc	133	GCAUGUGCACAUUGAAAACUGUG
AD-75382	317	asusggucAfgAfUfCfuucaaaauaa	318	usUfsauuUfuGfAfagauCfuGfaccaususg	136	CAAUGGUCAGAUCUUCAAAAUAA
AD-75389	319	asasauuuGfuGfUfUfcacaaaggaa	320	usUfsccuUfuGfUfgaacAfcAfaauuuscsu	139	AGAAAUUUGUGUUCACAAAGGAA
AD-75392	321	asusuuguGfuUfCfAfcaaaggaaaa	322	usUfsuucCfuUfUfgugaAfcAfcaaaususu	142	AAAUUUGUGUUCACAAAGGAAAA

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Table 3. LECT2-Unmodified sequences

Duplex Name	Seq ID No: (sense)	transSeq	Seq ID No: (anti sense)	transSeq	mRna Target Range
AD-75396	143	UUGUGUCUCACACUA AAGAAA	144	UUUCUUUAGUGUGAG ACACAAAA	58-80
AD-75385	145	GUGUCUCACACUAAA GAAAUA	146	UAUUUCUUUAGUGUG AGACACAA	60-82
AD-75397	147	AAUUCUAAGGCUAAA UAGCUA	148	UAGCUAUUUAGCCUUA GAAUUCU	91-113
AD-75386	149	GGAAGUAUUCAUUCA AACUUA	150	UAAGUUUGAAUGAAUA CUUCCUA	112-134
AD-75374	151	UUCAUUCAAACUUGA AUAUUA	152	UAAUAUUCAAGUUUGA AUGAAUA	119-141
AD-75391	153	UCAUUCAAACUUGAA UAUUCU	154	AGAAUAUUCAAGUUUG AAUGAAU	120-142
AD-75393	155	CAUUCAAACUUGAAU AUUCUU	156	AAGAAUAUUCAAGUUU GAAUGAA	121-143
AD-75381	157	UCAAACUUGAAUAUU CUUCAA	158	UUGAAGAAUAUUCAAG UUUGAAU	124-146
AD-75383	159	CAAACUUGAAUAUUC UUCAAA	160	UUUGAAGAAUAUUCAA GUUUGAA	125-147
AD-75372	161	AAACUUGAAUAUUCU UCAAAA	162	UUUUGAAGAAUAUUCA AGUUUGA	126-148
AD-75371	163	CUUGAAUAUUCUUCA AAGAGA	164	UCUCUUUGAAGAAUAU UCAAGUU	129-151
AD-75365	165	ACUCUAAUCAGAGGA AGAAAA	166	UUUUCUUCCUCUGAUU AGAGUUG	161-183
AD-75384	167	GAAGUAAAACCAGAU GUUUUA	168	UAAAACAUCUGGUUUU ACUUCCU	187-209
AD-75377	169	GUAAAACCAGAUGUU UUCCAA	170	UUGGAAAACAUCUGGU UUUACUU	190-212
AD-75380	171	AAAACCAGAUGUUUU CCACCA	172	UGGUGGAAAACAUCUG GUUUUAC	192-214
AD-75375	173	AAACCAGAUGUUUUC CACCAA	174	UUGGUGGAAAACAUCU GGUUUUA	193-215
AD-75376	175	AACCAGAUGUUUUCC ACCAAA	176	UUUGGUGGAAAACAUC UGGUUUU	194-216
AD-75400	177	ACCAGAUGUUUUCCA CCAAAA	178	UUUUGGUGGAAAACAU CUGGUUU	195-217
AD-75366	179	AUGGCUGUGGACAGU	180	UAGAGUACUGUCCACA	315-337

		ACUCUA		GCCAUGG	
AD-75379	181	CAGGAGAAACCUUAUCAAAAA	182	UUUUUGAUAAGGUUUCUCCUGGC	428-450
AD-75394	183	GGAGAAACCUUAUCAAAACAA	184	UUGUUUUGAUAAGGUUUCUCCUG	430-452
AD-75388	185	UUGUGUCAAAAUGUUCUACAU	186	AUGUAGAACAUUUUGACACAAAA	493-515
AD-75390	187	UGUGUCAAAAUGUUCUACAUU	188	AAUGUAGAACAUUUUGACACAAA	494-516
AD-75378	189	GUGUCAAAAUGUUCUACAUUA	190	UAAUGUAGAACAUUUUGACACAA	495-517
AD-75357	191	UGUCAAAAUGUUCUACAUUAA	192	UUAAUGUAGAACAUUUUGACACA	496-518
AD-75354	193	AAUGUUCUACAUUAAGCCAAU	194	AUUGGCUUAAUGUAGAACAUUUU	502-524
AD-75356	195	AUGUUCUACAUUAAGCCAAUU	196	AAUUGGCUUAAUGUAGAACAUUU	503-525
AD-75360	197	UUCUACAUUAAGCCAAUUAAA	198	UUUAAUUGGCUUAAUGUAGAACA	506-528
AD-75362	199	UCUACAUUAAGCCAAUUAAGU	200	ACUUAAUUGGCUUAAUGUAGAAC	507-529
AD-75364	201	CUACAUUAAGCCAAUUAAGUA	202	UACUUAAUUGGCUUAAUGUAGAA	508-530
AD-75358	203	UACAUUAAGCCAAUUAAGUAU	204	AUACUUAAUUGGCUUAAUGUAGA	509-531
AD-75355	205	CAUUAAGCCAAUUAAGUAUAA	206	UUAUACUUAAUUGGCUUAAUGUA	511-533
AD-75359	207	AUUAAGCCAAUUAAGUAUAAA	208	UUUAUACUUAAUUGGCUUAAUGU	512-534
AD-75367	209	UUAAGCCAAUUAAGUAUAAAA	210	UUUUAUACUUAAUUGGCUUAAUG	513-535
AD-75369	211	UAAGCCAAUUAAGUAUAAAGA	212	UCUUUAUACUUAAUUGGCUUAAU	514-536
AD-75370	213	AAGCCAAUUAAGUAUAAAGGU	214	ACCUUUAUACUUAAUUGGCUUAA	515-537
AD-75368	215	AGCCAAUUAAGUAUAAAGGUA	216	UACCUUUAUACUUAAUUGGCUUA	516-538
AD-75399	217	ACUUGGAACUCUAUUGCCCUU	218	AAGGGCAAUAGAGUUCCAAGUUU	556-578
AD-75398	219	UUGGAACUCUAUUGCCCUUGA	220	UCAAGGGCAAUAGAGUUCCAAGU	558-580
AD-75395	221	GCCCUUGCAGAAAGUUUAUCA	222	UGAUAAACUUUCUGCAAGGGCAA	571-593

AD-75387	223	UCGCAUGUGCACAUUGAAAAA	224	UUUUUCAAUGUGCACAUGCGAUU	602-624
AD-75373	225	GCAUGUGCACAUUGAAAACUA	226	UAGUUUUCAAUGUGCACAUGCGA	604-626
AD-75361	227	CAUGUGCACAUUGAAAACUGU	228	ACAGUUUUCAAUGUGCACAUGCG	605-627
AD-75363	229	AUGUGCACAUUGAAAACUGUA	230	UACAGUUUUCAAUGUGCACAUGC	606-628
AD-75382	231	AUGGUCAGAUCUUCAAAAUAA	232	UUAUUUUGAAGAUCUGACCAUUG	667-689
AD-75389	233	AAAUUUGUGUUCACAAAGGAA	234	UUCCUUUGUGAACACAAAUUUCU	730-752
AD-75392	235	AUUUGUGUUCACAAAGGAAAA	327	UUUUCCUUUGUGAACACAAAUUU	732-754

Example 2. *In Vitro* Screening of LECT2 siRNA

Experimental Methods

Cell culture and transfections:

Cynomolgus monkey primary hepatocytes and mouse primary hepatocytes were transfected independently by adding 4.9µl of Opti-MEM plus 0.1µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of siRNA duplexes per well into a 384-well plate and incubated at room temperature for 15 minutes. 40µl of William's media containing 5 x10^3 primary mouse hepatocytes or primary cynomolgus monkey hepatocytes were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Two dose experiments were performed at 10nM and 0.1nM final duplex concentrations.

RNA isolation:

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

cDNA synthesis:

cDNA was synthesized using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813). 10µl of a master mix containing 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl 10x Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H_2O per reaction was added to RNA isolated above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2h 37°C.

Real time PCR:

2µl of cDNA were added to a master mix containing 0.5µl of GAPDH TaqMan Probe (Hs99999905), 0.5µl LECT2 probe and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Mouse primary hepatocytes qPCR was probed with Mouse GAPDH TaqMan Probe (Mm03302249_g1) and Mouse LECT2 Taqman Probe (Mm00521920_m1). Cynomolgus monkey primary hepatocytes qPCR was probed with custom Cynomolgus GAPDH probe and Cynomolgus LECT2 probe (Mf02803673_m1). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche) using the ΔΔCt(RQ) assay. Each duplex was tested in four independent transfections. To calculate relative fold change, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 20nM AD-1955, or mock transfected cells.

Results

The results of single dose screen in primary monkey hepatocytes are shown in Table 4. The single dose experiments were performed at 10nM and 0.1nM final duplex concentration and the data are expressed as percent message remaining relative to AD-1955 non-targeting control.

Table 4. Cynomolgus monkey LECT2 endogenous *in vitro* 10nM and 0.1 nM screen

Duplex Name	AVG_10nM	AVG_0.1nM
AD-75396	15.47	44.29
AD-75385	17.03	31.17
AD-75397	10.31	47.42
AD-75386	6.75	23.04
AD-75374	7.65	20.61
AD-75391	7.93	23.75
AD-75393	11.51	35.91
AD-75381	6.31	25.34
AD-75383	11.16	33.70
AD-75372	6.04	29.32
AD-75371	8.39	45.17
AD-75365	20.79	36.92
AD-75384	22.52	69.74
AD-75377	16.34	55.19
AD-75380	19.45	53.80
AD-75375	19.25	60.03

AD-75376	11.60	46.16
AD-75400	12.85	51.18
AD-75366	7.01	31.16
AD-75379	8.45	22.32
AD-75394	10.88	32.93
AD-75388	11.73	29.19
AD-75390	18.65	40.18
AD-75378	11.86	22.91
AD-75357	11.97	15.71
AD-75354	12.23	18.92
AD-75356	12.53	24.12
AD-75360	19.08	29.89
AD-75362	30.82	73.33
AD-75364	16.05	27.52
AD-75358	14.87	26.10
AD-75355	15.07	25.39
AD-75359	14.05	38.31
AD-75367	21.75	41.54
AD-75369	27.29	47.39
AD-75370	37.54	90.36
AD-75368	15.78	54.40
AD-75399	16.04	49.48
AD-75398	14.60	43.63
AD-75395	14.41	42.82
AD-75387	22.55	55.61
AD-75373	31.91	63.76
AD-75361	12.51	30.63
AD-75363	41.36	74.88
AD-75382	8.75	19.63
AD-75389	70.65	89.12
AD-75392	54.14	85.42

The results of single dose screen in primary murine hepatocytes are shown in Table 5. The single dose experiments were performed at 10nM and 0.1nM final duplex concentration and the data are expressed as percent message remaining relative to AD-1955 non-targeting control.

Table 5. Mouse LECT2 endogenous *in vitro* 10nM and 0.1 nM screen

Duplex Name	10nM_AVG	0.1nM_AVG_mouse
AD-75396	116.89	104.83
AD-75385	92.20	89.51
AD-75397	110.39	104.50
AD-75386	100.18	95.89

AD-75374	91.10	84.70
AD-75391	104.66	96.55
AD-75393	90.86	95.22
AD-75381	85.25	88.23
AD-75383	92.12	94.47
AD-75372	88.04	94.84
AD-75371	87.88	91.73
AD-75365	88.21	90.64
AD-75384	97.27	101.56
AD-75377	85.21	85.01
AD-75380	98.64	93.75
AD-75375	91.78	92.58
AD-75376	92.63	73.95
AD-75400	117.41	116.65
AD-75366	6.03	30.27
AD-75379	63.13	71.73
AD-75394	89.65	88.30
AD-75388	27.12	78.75
AD-75390	32.03	70.21
AD-75378	32.41	75.13
AD-75357	35.43	78.18
AD-75354	12.91	42.26
AD-75356	10.18	40.33
AD-75360	24.41	47.51
AD-75362	43.38	72.64
AD-75364	13.02	47.30
AD-75358	4.82	31.20
AD-75355	5.97	33.68
AD-75359	11.47	47.57
AD-75367	8.17	39.39
AD-75369	20.82	56.91
AD-75370	39.52	77.19
AD-75368	27.10	64.16
AD-75399	102.64	93.97
AD-75398	105.67	99.44
AD-75395	96.54	91.07
AD-75387	95.88	93.36
AD-75373	87.42	93.42
AD-75361	34.51	71.94
AD-75363	85.75	71.92
AD-75382	40.63	66.40
AD-75389	85.63	83.01
AD-75392	94.42	87.01

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

5

We claim:

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of LECT2, wherein said dsRNA comprises a sense strand that is 15-30 base pairs in length and an antisense strand that is 15-30 base pairs in length and the antisense strand is complementary to at least 15 contiguous nucleotides of SEQ ID NO: 1.

2. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of LECT2, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a LECT2 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 Tables 2 and 3.

3. The dsRNA of claim 1 or 2, wherein said dsRNA comprises at least one modified nucleotide.

4. The dsRNA of claim of any of the preceding claims, wherein the duplex region is 15-30 nucleotide pairs in length.

5. The dsRNA of claim of any of the preceding claims, wherein the duplex region is 17-23 nucleotide pairs in length.

6. The dsRNA of claim of any of the preceding claims, wherein the duplex region is 19-21 nucleotide pairs in length.

7. The dsRNA of claim of any of the preceding claims, wherein the duplex region is 21-23 nucleotide pairs in length.

8. The dsRNA of any of the preceding claims, wherein the region of complementarity is at least 17 nucleotides in length.

9. The dsRNA of of any of the preceding claims, wherein the region of complementarity is 19 nucleotides in length.

10. The dsRNA of any of the preceding claims, wherein the region of complementarity is between 19 and 21 nucleotides in length.

11. The dsRNA of any one of the preceding claims, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

12. The dsRNA of claim 4, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

13. The dsRNA of claim 3, wherein at least one of said 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.

14. The dsRNA of claim 3, wherein at least one of said modified nucleotides is chosen from the group consisting of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleic acid (LNA), 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.

15. The dsRNA of claim 3, wherein the modifications on the nucleotides are selected from the group consisting of locked nucleic acid (LNA), an acyclic nucleotide, hexitol or hexose nucleic acid (HNA), cyclohexene nucleic acid (CeNA), 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-O-methyl, 2′-deoxy, 2'-hydroxyl, and combinations thereof.

16. The dsRNA of claim 3, wherein the modifications on the nucleotides are 2′-O-methyl, 2′-fluoro or both.

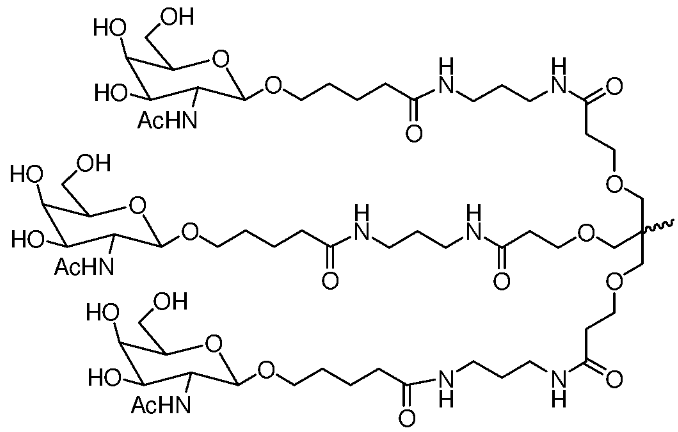
17. The dsRNA of any of the preceding claims wherein the sense strand is conjugated to at least one ligand.

18. The dsRNA of claim 17, wherein the ligand is attached to the 3' end of the sense strand.

19. The dsRNA of claim 17, wherein the ligand comprises a carbohydrate.

20. The dsRNA of claim 17, wherein the ligand is a GalNAc ligand.

21. The dsRNA of claim 17, wherein the ligand is



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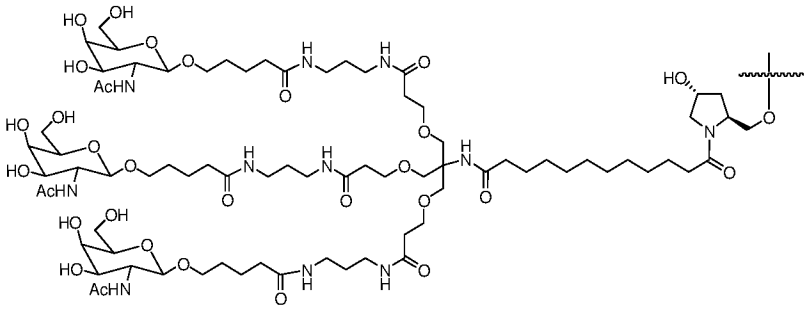
22. The dsRNA of any one of claims 17 to 21, wherein the ligand is attached via a linker.

23. The dsRNA of claim 22, wherein the linker is a bivalent or trivalent branched linker.

24. The dsRNA of claim 22, wherein the ligand and linker are as shown in Formula XXIV:



25. The dsRNA of any one of claims 17 to 24, wherein the ligand targets the dsRNA to hepatocytes.

26. The dsRNA of any one of the preceding claims, wherein the region of complementarity consists of an antisense sequence selected from the antisense sequences disclosed in Tables 2 and 3.

27. The dsRNA of any one of the preceding claims, wherein the dsRNA comprises a sense strand consisting of a sense sequence selected from the sense sequences disclosed in Tables 2 and 3, and an antisense strand consisting of an antisense sequence selected from the antisense sequences disclosed in Tables 2 and 3.

28. A cell containing the dsRNA of any one of the preceding claims.

29. A pharmaceutical composition for inhibiting expression of a LECT2 gene, the composition comprising the dsRNA of any one of claims 1 to 27.

30. The pharmaceutical composition of claim 29, wherein dsRNA is administered in an unbuffered solution.

31. The pharmaceutical composition of claim 30, wherein said unbuffered solution is saline or water.

32. The pharmaceutical composition of claim 29, wherein said dsRNA is administered with a buffer solution.

33. The pharmaceutical composition of claim 32, wherein said buffer solution comprises acetate, citrate, prolamine, carbonate, phosphate or any combination thereof.

34. The pharmaceutical composition of claim 32, wherein said buffer solution is phosphate buffered saline (PBS).

35. The pharmaceutical composition of claim 29, wherein said composition comprises a lipid formulation.

36. The pharmaceutical composition of claim 35, wherein the lipid formulaton is a LNP formulation.

37. The pharmaceutical composition of claim 36, wherein the lipid formulation is a LNP11 formulation.

38. The pharmaceutical composition of any one of claims 29-37, wherein the dsRNA is targeted to a liver cell or a hepatocyte.

39. The pharmaceutical composition of any one of claims 29-38, wherein said composition is administered intravenously.

40. The pharmaceutical composition of any one of claims 29-38, wherein said composition is administered subcutaneously.

41. The pharmaceutical composition of claim 39, wherein said composition comprises a lipid formulation and is administered intravenously.

42. The pharmaceutical composition of claim 40, wherein said composition comprises a dsRNA that is conjugated to a ligand chosen from a carbohydrate ligand or a GalNAc ligand.

43. A composition for use in a method of inhibiting LECT2 expression in a cell, the method comprising:

(a) introducing into the cell the dsRNA of any one of claims 1-27, and

(b) maintaining the cell of step (a) for a time sufficient to obtain degradation of the mRNA transcript of a LECT2 gene, thereby inhibiting expression of the LECT2 gene in the cell.

44. The composition for use of claim 43, wherein the cell is treated *ex vivo*, *in vitro*, or *in vivo*.

45. The composition for use of claim 43, wherein the cell is present in a subject in need of treatment, prevention and/or management of a disorder related to LECT2 expression.

46. The composition for use of claim 45, wherein said disorder is amyloidosis.

47. The composition for use of claim 46, wherein the amyloidosis is a LECT2 amyloidosis.

48. The composition for use of any one of claims 43-47, wherein the cell is a liver cell or a hepatocyte.

49. The composition for use of any one of claims 43-48, wherein wherein the expression of LECT2 is inhibited by at least 20%.

50. The composition for use of any one of claims 43-48, wherein the expression of LECT2 is inhibited by at least 30%.

51. A composition for use in a method of treating a disorder related to LECT2 expression comprising administering to a subject in need of such treatment a therapeutically effective amount of

(i) the dsRNA of any one of claims 1-27 or

(ii) the composition of any one of claims 29-42.

52. A composition for use in a method of treating a LECT2 amyloidosis comprising administering to a subject in need of such treatment a double-stranded ribonucleic acid (dsRNA), wherein said dsRNA comprises a sense strand that is 15-30 base pairs in length and an antisense strand that is 15-30 base pairs in length and the antisense strand is complementary to at least 15 contiguous nucleotides of SEQ ID NO: 1 or a nucleotide sequence having a A to G substitution at nucleotide position 373 of SEQ ID NO: 1.

53. The composition for use of claim 51 or 52, wherein the subject has amyloidosis or is at risk for developing amyloidosis.

54. The composition for use of claim 51 or 52, wherein the amyloidosis is a LECT2 amyloidosis.

55. The composition for use of any one of claims 43-54, wherein the dsRNA or composition comprising the dsRNA is administered according to a dosing regimen.

56. The composition for use of claim 55, wherein the dosing regimen is weekly, biweekly, or monthly.

57. The composition for use of any one of claims 43-56, wherein the treating reduces LECT2 amyloid deposition.

58. A composition for use in a method of reducing LECT2 amyloid deposition in a subject having a LECT2 amyloidosis, the method comprising administering to the subject

(i) the dsRNA of any one of claims 1-27 or

(ii) the composition of any one of claims 29-42.

59. The composition for use of any one of claims 51-58, wherein the dsRNA is administered at a dose of 0.05-50 mg/kg.

60. The composition for use of any one of claims 51-58, wherein the dsRNA is administered at a concentration of 0.01 mg/kg to 5 mg/kg bodyweight of the subject.

61. The composition for use of claim 59, wherein the dsRNA is formulated as an LNP formulation and is administered at a dose of 0.1 mg/kg to 0.5 mg/kg.

62. The composition for use of claim 59, wherein the dsRNA is conjugated to a GalNAc ligand.

63. The composition for use of claim 59, wherein the dsRNA is conjugated to a GalNAc ligand and is administered at a dose of 1 mg/kg to 10 mg/kg.

64. 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 LECT2, wherein said dsRNA is 30 base pairs or less in length, and wherein said dsRNA targets said mRNA for cleavage.

65. The vector of claim 64, wherein the region of complementarity is at least 15 nucleotides in length.

66. The vector of claim 64, wherein said dsRNA comprises an antisense sequence and/or a sense sequence selected from a sequence disclosed in Table 2, 3, 4, or 5.

67. The vector of any one of claims 64 to 66, wherein the region of complementarity is 19 to 21 nucleotides in length.

68. A cell comprising the vector of any one of claims 64-67.

FIG. 1

```
   1 aaatcaaata gctatccatg gaatattaga acttgacttg ctccatcctc ttaaactttt
  61 tgtgtctcac actaaagaaa tgagagatgc agaattctaa ggctaaatag ctaggaagta
 121 ttcattcaaa cttgaatatt cttcaaagag agtgtggggg caactctaat cagaggaaga
 181 aactaaagga agtaaaacca gatgttttcc accaaagccc tccttttggc tggtctgatt
 241 tctaccgcac tggcagggcc atgggctaat atatgtgctg gcaagtcttc caatgagatc
 301 cggacgtgtg accgccatgg ctgtggacag tactctgctc aaagaagtca gaggcctcac
 361 cagggtgtgg acatcttgtg ctctgctgga tctactgtgt acgcaccatt cactggaatg
 421 attgtgggcc aggagaaacc ttatcaaaac aagaatgcta tcaataatgg tgttcgaata
 481 tctggaagag gtttttgtgt caaaatgttc tacattaagc caattaagta taaaggtcct
 541 attaagaagg gagaaaaact tggaactcta ttgcccttgc agaaagttta tcctggcata
 601 caatcgcatg tgcacattga aaactgtgac tcgagtgacc ctactgcata cctgtaaatc
 661 gaaggccaat ggtcagatct tcaaaataaa aagtcatctt aaaaacctgg atgcataccc
 721 ttctcttcaa gaaatttgtg ttcacaaagg aaaaatgcat gaagggatgg ataccccatt
 781 ttccatgaca tgattattac acattgcatg cctgtatcaa aacatctcac gtacctcata
 841 aacatataca cctatgtacc cacaaaaatt ttttaattaa aaaaaggaaa tttgagttta
 901 aatagaaaca tgataaatgc aagaaagaaa acattttgat tttaactcat tgtcactctg
 961 atgttcatgt gaactggttg cttcgggctc tttgatctgt cacctatgga atctgagtgg
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SEQ ID NO:1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/046657

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 C12N15/11 A61K31/713
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/050990 A1 (ALNYLAM PHARMACEUTICALS INC [US]) 9 April 2015 (2015-04-09)	1-26, 28-65, 67,68
A	page 131; claims 1-74; examples 1-3; tables 2,5,6,9,10; sequence 27	27,66

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

☒ See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 November 2019	23/01/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Romano, Alper

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/046657

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

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

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

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

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

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

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

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

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

1-68(partially)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-68(partially)

a dsRNA for inhibiting expression of LECT2 wherein the antisense strand comprises at least 15 nucleotides differing by no more the 3 nucleotides from the first antisense sequence listed in tables 2 and 3 and comprised within the first largely overlapping target region according to table 3, namely 58-82, i.e. SEQ ID NOs:3/237/144 targeting mRNA region SEQ ID NO:4 and SEQ ID NOs:6/239/146 targeting mRNA region SEQ ID NO:7

2-10. claims: 1-68(partially)

a dsRNA for inhibiting expression of LECT2 wherein the antisense strand comprises at least 15 nucleotides differing by no more the 3 nucleotides from one of the remaining antisense sequences listed in tables 2 and 3 and comprised within the largely overlapping target regions according to table 3, namely namely 91-151,161-217, 315-337, 428-452, 493-538, 556-593, 602-628, 667-689, 730-754

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2019/046657

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015050990 A1	09-04-2015	CA 2925107 A1	09-04-2015
		CN 105793423 A	20-07-2016
		CN 109536493 A	29-03-2019
		EP 3052626 A1	10-08-2016
		TW 201606078 A	16-02-2016
		US 2016264966 A1	15-09-2016
		US 2019119674 A1	25-04-2019
		WO 2015050990 A1	09-04-2015