Extrahepatic Delivery

[0001] This application claims benefit of priority to U.S. Provisional Application No. 62/668,072 filed May 7, 2018; U.S. Provisional Application No. 62/738,747 filed September 28, 2018; and U.S. Provisional Application No. 62/773,082 filed November 29, 2018, all of which are herein incorporated by reference in their entirety.

BACKGROUND

[0002] Efficient delivery of an iRNA agent to cells *in vivo* requires specific targeting and substantial protection from the extracellular environment, particularly serum proteins. RNAi-based therapeutics show promising clinical data for treatment of liver-associated disorders. However, siRNA delivery into extra-hepatic tissues remains an obstacle, limiting the use of siRNA-based therapies.

[0003] One of the factors that limit the experimental and therapeutic application of iRNA agents *in vivo* is the ability to deliver intact siRNA efficiently. Particular difficulties have been associated with non-viral gene transfer into the retina *in vivo*. One of the challenges is to overcome the inner limiting membrane, which impedes the transfection of the retina. Additionally, negatively charged sugars of the vitreous have been shown to interact with positive DNA-transfection reagent complexes, promoting their aggregation, which impedes diffusion and cellular uptake.

[0004] Delivery of oligonucleotides to the central nervous system (CNS) poses particular problems due to the blood brain barrier (BBB) that free oligonucleotides cannot cross. One means to deliver oligonucleotides into the CNS is by intrathecal delivery. However, the oligonucleotides need also to be efficiently internalized into target cells of the CNS to achieve the desired therapeutic effect. Previous work has typically used delivery reagents such as liposomes, cationic lipids, and nanoparticles forming complexes to aid the intracellular internalization of oligonucleotides into cells of neuronal origin.

[0005] Thus, there is a continuing need for new and improved methods for delivering siRNA molecules *in vivo*, without the use of tissue delivery reagents, to achieve and enhance the therapeutic potential of iRNA agents.

SUMMARY

[0006] One aspect of the disclosure provides a double-stranded iRNA agent comprising:

an antisense strand which is complementary to a target gene; a sense strand which is complementary to said antisense strand; and one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier.

[0007] In some examples, the lipophilicity of the lipophilic moiety, measured by octanol-water partition coefficient, $\log K_{ow}$, exceeds 0. The lipophilic moiety may possess a $\log K_{ow}$ exceeding 1, exceeding 1.5, exceeding 2, exceeding 3, exceeding 4, exceeding 5, or exceeding 10.

[0008] In some examples, the hydrophobicity of the double-stranded iRNA agent, measured by the unbound fraction in the plasma protein binding assay of the double-stranded iRNA agent, exceeds 0.2. In one example, the plasma protein binding assay determined is an electrophoretic mobility shift assay (EMSA) using human serum albumin protein. The hydrophobicity of the double-stranded iRNA agent, measured by fraction of unbound siRNA in the binding assay, exceeds 0.15, exceeds 0.2, exceeds 0.25, exceeds 0.3, exceeds 0.35, exceeds 0.4, exceeds 0.45, or exceeds 0.5 for an enhanced *in vivo* delivery of siRNA.

[0009] In some examples, the lipophilic moiety is an aliphatic, cylic such as alicyclic, or polycyclic such as polyalicyclic compound, such as a steroid (e.g., sterol) or a linear or branched aliphatic hydrocarbon. Exemplary lipophilic moieties are lipid, cholesterol, retinoic acid, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-bis-O(hexadecyl)glycerol, geranyloxyhexyanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, ibuprofen, naproxen, dimethoxytrityl, or phenoxazine.

[0010] Suitable lipophilic moieties also include those containing a saturated or unsaturated C_4-C_{30} hydrocarbon chain (e.g., C_4-C_{30} alkyl or alkenyl), and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne. The functional groups are useful to attach the lipophilic moiety to the iRNA agent. In some examples, the lipophilic moiety contains a saturated or unsaturated C_6-C_{18} hydrocarbon chain (e.g., a linear C_6-C_{18} alkyl or alkenyl). In one example, the lipophilic moiety contains a saturated or unsaturated C_{16} hydrocarbon chain (e.g., a linear C_{16} alkyl or alkenyl).

[0011] In some examples, the lipophilic moiety is a C_6-C_{30} acid (e.g., hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, undecanoic acid, dodcanoic acid, tridecanoic acid, tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, heptadecanoic acid, octadecanoic acid, oleic acid, linoleic acid, arachidonic acid, cis-4,7,10,13,16,19-

docosahexanoic acid, vitamin A, vitamin E, cholesterol etc.) or a C_6-C_{30} alcohol (e.g., hexanol, heptanol, octanol, nonanol, decanol, undecanol, dodcanol, tridecanol, tetradecanol, pentadecanol, hexadecanol, heptadecanol, octadecanol, oleyl alcohol, linoleyl alcohol, arachidonic alcohol, cis-4,7,10,13,16,19-docosahexanol, retinol, vitamin E, cholesterol etc.).

[0012] The lipophilic moiety may be conjugated to the iRNA agent via a direct attachment to the ribosugar of the iRNA agent. Alternatively, the lipophilic moiety may be conjugated to the iRNA agent via a linker or a carrier.

[0013] In certain examples, the lipophilic moiety are conjugated to the iRNA agent via one or more linkers (tethers).

[0014] In some examples, the lipophilic moiety is conjugated to the double-stranded iRNA agent via a linker a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction (e.g., a triazole from the azide-alkyne cycloaddition), or carbamate.

[0015] In some examples, at least one of the linkers (tethers) is a redox cleavable linker (such as a reductively cleavable linker; e.g., a disulfide group), an acid cleavable linker (e.g., a hydrazone group, an ester group, an acetal group, or a ketal group), an esterase cleavable linker (e.g., an ester group), a phosphatase cleavable linker (e.g., a phosphate group), or a peptidase cleavable linker (e.g., a peptide bond).

[0016] In other examples, at least one of the linkers (tethers) is a bio-clevable linker selected from the group consisting of DNA, RNA, disulfide, amide, functionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, mannose, and combinations thereof.

[0017] In certain examples, the lipophilic moiety is conjugated to the double-stranded iRNA agent via a carrier that replaces one or more nucleotide(s). The carrier can be a cyclic group or an acyclic group. In one example, the cyclic group is selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl, and decalin. In one example, the acyclic group is a moiety based on a serinol backbone or a diethanolamine backbone.

[0018] In some examples, the carrier replaces one or more nucleotide(s) in the internal position(s) of the double-stranded iRNA agent.

[0019] In other examples, the carrier replaces the nucleotides at the terminal end of the sense strand or antisense strand. In one example, the carrier replaces the terminal nucleotide on the 3' end of the sense strand, thereby functioning as an end cap protecting the 3' end of the sense strand. In one example, the carrier is a cyclic group having an amine, for instance, the carrier may be pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, or decalinyl.

[0020] In one example, the lipophilic moiety is conjugated to one or more internal positions on at least one strand, which include all positions except the terminal two positions from each end of the strand. In one example, the lipophilic moiety is conjugated to one or more internal positions on at least one strand, which include all positions except the terminal three positions from each end of the strand.

[0021] In one example, at least one lipophilic moiety is conjugated to one or more positions of at least one end of the duplex region, which include all positions within the duplex region, but not include the overhang region or the carrier that replaces the terminal nucleotide on the 3' end of the sense strand.

[0022] In one example, at least one lipophilic moiety is conjugated on the sense strand within the first five base pairs at the 5'-end of the antisense strand of the duplex region.

[0023] In one example, at least one lipophilic moiety is conjugated on the sense strand within the first four base pairs at the 5'-end of the antisense strand of the duplex region.

[0024] In one example, at least one lipophilic moiety is conjugated on the sense strand within the first three base pairs at the 5'-end of the antisense strand of the duplex region.

[0025] In one example, at least one lipophilic moiety is conjugated on the sense strand within the first two base pairs at the 5'-end of the antisense strand of the duplex region.

[0026] In one example, at least one lipophilic moiety is conjugated on the sense strand on the first base pair at the 5'-end of the antisense strand of the duplex region.

[0027] In one example, the lipophilic moiety is conjugated to one or more internal positions on at least one strand, which exclude the cleavage site region of the sense strand. For instance, the internal positions exclude positions 9-12 counting from the 5'-end of the

sense strand. For example, the internal positions exclude positions 9-11 counting from the 5'-end of the sense strand. Alternatively, the internal positions exclude positions 11-13 counting from the 3'-end of the sense strand.

[0028] In one example, the lipophilic moiety is conjugated to one or more internal positions on at least one strand, which exclude the cleavage site region of the antisense strand. For instance, the internal positions exclude positions 12-14 counting from the 5'-end of the antisense strand.

[0029] In one example, the lipophilic moiety is conjugated to one or more internal positions on at least one strand, which exclude positions 11-13 on the sense strand, counting from the 3'-end, and positions 12-14 on the antisense strand, counting from the 5'-end.

[0030] In one example, one or more lipophilic moieties are conjugated to one or more of the following internal positions: positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand.

[0031] In one example, one or more lipophilic moieties are conjugated to one or more of the following internal positions: positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'end of each strand.

[0032] In some examples, the sense and antisense strands of the double-stranded iRNA agent are each 15 to 30 nucleotides in length.

[0033] In one example, the sense and antisense strands of a double-stranded iRNA agent are each 19 to 25 nucleotides in length.

[0034] In one example, the sense and antisense strands of the double-stranded iRNA agent are each 21 to 23 nucleotides in length.

[0035] In some examples, the double-stranded iRNA agent comprises a single-stranded overhang on at least one of the termini, e.g., 3' and/or 5' overhang(s) of 1-10 nucleotides in length, for instance, an overhang of 1, 2, 3, 4, 5, or 6 nucleotides. In some examples, both strands have at least one stretch of 1-5 (e.g., 1, 2, 3, 4, or 5) single-stranded nucleotides in the double stranded region. In one example, the single-stranded overhang is 1, 2, or 3 nucleotides in length. In some examples, the double-stranded iRNA agent may also have a blunt end, located at the 5'-end of the antisense strand (or the 3'-end of the sense strand), or vice versa. In one example, the double-stranded iRNA agent comprises a 3' overhang at the 3'-end of the antisense strand, and optionally a blunt end at the 5'-end of the antisense strand. In one example, the double-stranded iRNA agent has a 5' overhang at the 5'-end of the sense strand, and optionally a blunt end at the 5'-

end of the antisense strand. In one example, the double-stranded iRNA agent has two blunt ends at both ends of the iRNA duplex.

[0036] In one example, the sense strand of the double-stranded iRNA agent is 21-nucleotides in length, and the antisense strand is 23-nucleotides in length, wherein the strands form a double-stranded region of 21 consecutive base pairs having a 2-nucleotide long single-stranded overhangs at the 3'-end.

[0037] In some examples, the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage of the double-stranded iRNA agent.

[0038] In some examples, the double-stranded iRNA agent further comprises a phosphate or phosphate mimic at the 5'-end of the antisense strand. In one example, the phosphate mimic is a 5'-vinyl phosphonate (VP).

[0039] In some examples, the 5'-end of the antisense strand of the double-stranded iRNA agent does not contain a 5'-vinyl phosphonate (VP).

[0040] In some examples, the double-stranded iRNA agent further comprises at least one terminal, chiral phosphorus atom.

[0041] A site specific, chiral modification to the internucleotide linkage may occur at the 5' end, 3' end, or both the 5' end and 3' end of a strand. This is being referred to herein as a "terminal" chiral modification. The terminal modification may occur at a 3' or 5' terminal position in a terminal region, *e.g.,* at a position on a terminal nucleotide or within the last 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides of a strand. A chiral modification may occur on the sense strand, antisense strand, or both the sense strand and antisense strand. Each of the chiral pure phosphorus atoms may be in either Rp configuration or Sp configuration, and combination thereof. More details regarding chiral modifications and chirally-modified dsRNA agents can be found in PCT/US18/67103, entitled "Chirally-Modified Double-Stranded RNA Agents," filed December 21, 2018, which is incorporated herein by reference in its entirety.

[0042] In some examples, the double-stranded iRNA agent further comprises a terminal, chiral modification occuring at the first internucleotide linkage at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration; a terminal, chiral modification occuring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration; and a terminal, chiral modification occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp configuration or Sp configuration.

[0043] In one example, the double-stranded iRNA agent further comprises a terminal, chiral modification occuring at the first and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration; a terminal, chiral modification occuring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration; and a terminal, chiral modification occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

[0044] In one example, the double-stranded iRNA agent further comprises a terminal, chiral modification occuring at the first, second, and third internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration; a terminal, chiral modification occuring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration; and a terminal, chiral modification occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

[0045] In one example, the double-stranded iRNA agent further comprises a terminal, chiral modification occuring at the first and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration; a terminal, chiral modification occuring at the third internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Rp configuration; a terminal, chiral modification occuring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration; and a terminal, chiral modification occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

[0046] In one example, the double-stranded iRNA agent further comprises a terminal, chiral modification occuring at the first and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration; a terminal, chiral modification occuring at the first, and second internucleotide linkages at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration; and a terminal, chiral modification occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

[0047] In some examples, the double-stranded iRNA agent has at least two phosphorothioate internucleotide linkages at the first five nucleotides on the antisense strand (counting from the 5' end).

[0048] In some examples, the antisense strand comprises two blocks of one, two, or three phosphorothioate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 phosphate internucleotide linkages.

[0049] In some examples, the double-stranded iRNA agent further comprises a targeting ligand that targets a receptor which mediates delivery to a specific CNS tissue. In one example, the targeting ligand is selected from the group consisting of Angiopep-2, lipoprotein receptor related protein (LRP) ligand, bEnd.3 cell binding ligand, transferrin receptor (TfR) ligand, manose receptor ligand, glucose transporter protein, and LDL receptor ligand.

[0050] In some examples, the double-stranded iRNA agent further comprises a targeting ligand that targets a receptor which mediates delivery to an ocular tissue. In one example, the targeting ligand is selected from the group consisting of trans-retinol, RGD peptide, LDL receptor ligand, and carbohydrate-based ligands. In one example, the targeting ligand is a RGD peptide, such as H-Gly-Arg-Gly-Asp-Ser-Pro-Lys-Cys-OH or Cyclo(-Arg-Gly-Asp-D-Phe-Cys).

[0051] In some examples, the double-stranded iRNA agent further comprises a targeting ligand that targets a liver tissue. In some examples, the targeting ligand is a carbohydrate-based ligand. In one example, the targeting ligand is a GalNAc conjugate.

[0052] All the above examples and examples would be applicable to an oligonucleotide having one or more lipophilic moieties conjugated to one or more internal positions on the oligonucleotide. In some examples, 100%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35% or 30% of the oligonucleotide is modified. For example, when 50% of the oligonucleotide is modified, 50% of all nucleotides present in the oligonucleotide contain a modification as described herein.

[0053] In one example, the oligonucleotide is a double-stranded dsRNA agent, and at least 50% of the nucleotides of the double-stranded dsRNA agent is independently modified with 2'- O-methyl, 2'-O-allyl, 2'-deoxy, or 2'-fluoro.

[0054] In one example, the oligonucleotide is an antisense, and at least 50% of the nucleotides of the antisense is independently modified with LNA, CeNA, 2'-methoxyethyl, or 2'-deoxy.

[0055] In some examples, the double-stranded iRNA agent has less than 12, less than 10, less than 8, less than 6, less than 4, less than 2, or no 2'-F modifications on the sense strand. In some examples, the double-stranded iRNA agent has less than 12, less than 10,

less than 8, less than 6, less than 4, less than 2, or no 2'-F modifications on the antisense strand.

[0056] In some examples, the double-stranded iRNA agent has one or more 2'-F modifications on any position of the sense strand or antisense strand.

[0057] In some examples, the double-stranded iRNA agent has less than 20%, less than 15%, less than 10%, less than 5% non-natural nucleotide, or substantially no non-natural nucleotide. Examples of non-natural nucleotide include acyclic nucleotides, LNA, HNA, CeNA, 2'-O-methoxyalkyl (*e.g.*, 2'-O-methoxymethyl, 2'-O-methoxyethyl, or 2'-O-2-methoxypropanyl), 2'-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-O-N-methylacetamido (2'-O-NMA), a 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), 2'-O-aminopropyl (2'-O-AP), 2'-ara-F, L-nucleoside modification (such as 2'-modified L-nucleoside, e.g., 2'-deoxy-L-nucleoside), BNA abasic sugar, abasic cyclic and open-chain alkyl.

[0058] In some examples, the double-stranded iRNA agent has greater than 80%, greater than 85%, greater than 90%, greater than 95%, or virtually 100% natural nucleotides. For the purpose of these examples, natural nucleotides can include those having 2'-OH, 2'-deoxy, and 2'-OMe.

[0059] In one example, the double-stranded iRNA agent comprises a sense strand and antisense strand each having a length of 15-30 nucleotides; at least two phosphorothioate internucleotide linkages at the first five nucleotides on the antisense strand (counting from the 5' end); wherein the duplex region is between 19 to 25 base pairs (preferably 19, 20, 21 or 22); wherein the double-stranded iRNA agent has less than 20%, less than 15%, less than 10%, less than 5% non-natural nucleotide, or substantially no non-natural nucleotide.

[0060] In one example, the double-stranded iRNA agent comprises a sense strand and antisense strand each having a length of 15-30 nucleotides; at least two phosphorothioate internucleotide linkages at the first five nucleotides on the antisense strand (counting from the 5' end); wherein the duplex region is between 19 to 25 base pairs (preferably 19, 20, 21 or 22); wherein the double-stranded iRNA agent has greater than 80%, greater than 85%, greater than 95%, or virtually 100% natural nucleotides, such as those having 2'-OH, 2'-deoxy, or 2'-OMe.

[0061] Another aspect of the disclosure relates to a method of reducing the expression of a target gene in a cell, comprising contacting said cell with a double-stranded iRNA agent comprising an antisense strand which is complementary to a target gene; a sense strand which

is complementary to said antisense strand; and one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier.

[0062] All the above examples relating to the lipophilic moieties and their conjugation to the double-stranded iRNA agent in the first example of the disclosure relating to the double-stranded iRNA agent are suitable in this example of the disclosure relating to a method of reducing the expression of a target gene in a cell.

[0063] In one example, the cell is an extraheptic cell.

[0064] Another aspect of the disclosure relates to a use of a double-stranded iRNA agent comprising: an antisense strand which is complementary to a target gene; a sense strand which is complementary to said antisense strand; and one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier, in the manufacture of a medicament for reducing the expression of a target gene in a subject. Another example of the disclosure relates to a method of reducing the expression of a target gene in a subject, comprising administering to the subject a double-stranded iRNA agent comprising contacting said cell with a double-stranded iRNA agent comprising an antisense strand which is complementary to a target gene; a sense strand which is complementary to said antisense strand; and one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier.

[0065] All the above examples relating to the lipophilic moieties and their conjugation to the double-stranded iRNA agent in the first example of the disclosure relating to the double-stranded iRNA agent are suitable in this example of the disclosure relating to a method of reducing the expression of a target gene in a subject.

[0066] In some examples, the double-stranded iRNA agent is administered extrahepatically.

[0067] In one example, the double-stranded iRNA agent is administered intrathecally. By intrathecal administration of the double-stranded iRNA agent, the method can reduce the expression of a target gene in a brain or spine tissue, for instance, cortex, cerebellum, cervical spine, lumbar spine, and thoracic spine.

[0068] In some examples, exemplary target genes are APP, ATXN2, C9orf72, TARDBP, MAPT(Tau), HTT, SNCA, FUS, ATXN3, ATXN1, SCA1, SCA7, SCA8, MeCP2, PRNP, SOD1, DMPK, and TTR. To reduce the expression of these target genes in the subject, the double-stranded iRNA agent can be administered intravitreally. By intravitreal administration of the double-stranded iRNA agent, the method can reduce the expression of the target gene in an ocular tissue.

[0069] Another aspect of the disclosure relates to a use the double-stranded RNAi agent as disclosed herein in the manufacture of a medicament for treating a subject having a CNS disorder. Another example of the disclosure relates to a method of treating a subject having a CNS disorder, comprising administering to the subject a therapeutically effective amount of a double-stranded RNAi agent, thereby treating the subject. The double-stranded RNAi agent comprises an antisense strand which is complementary to a target gene; a sense strand which

is complementary to said antisense strand; and one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier.

[0070] All the above examples relating to the lipophilic moieties and their conjugation to the double-stranded iRNA agent in the first example of the disclosure relating to the double-stranded iRNA agent are suitable in this example of the disclosure relating to a method of treating a subject having a CNS disorder. Exemplary CNS disorders that can be treated by the method of the disclosure include alzheimer, amyotrophic lateral schlerosis (ALS), frontotemporal dementia, huntington, Parkinson, spinocerebellar, prion, and lafora.

BRIEF DESCRIPTION OF THE DRAWINGS

[0071] Figure 1 is a scheme showing ligands, such as lipophilic moieties, that are conjugated to siRNAs at internal positions of the sense or antisense strand (*i.e.,* somewhere within the siRNA sequence).

[0072] Figure 2 is a scheme showing ligands, such as lipophilic moieties, that are conjugated to siRNAs through linkers or carriers at the 3'- and/or 5'-ends of the sense or antisense strand.

[0073] Figure 3 is a scheme showing ligands, such as lipophilic moieties, that are conjugated to siRNAs via bio-cleavable linkers.

[0074] Figure 4 is a graph showing the results of beta catenin gene (ocular CTNNB1) silencing by an intravitreal injection of various exemplary siRNA conjugates in mice.

[0075] Figure 5 is a graph showing the results of SOD1 mRNA silencing by a single intrathecal injection of various exemplary siRNA conjugates in Cortex of Sprague Dawley Rats.

[0076] Figure 6 is a graph showing the results of SOD1 mRNA silencing by a single intrathecal injection of various exemplary siRNA conjugates in Cerebellum of Sprague Dawley Rats.

[0077] Figure 7 is a graph showing the results of SOD1 mRNA silencing by a single intrathecal injection of various exemplary siRNA conjugates in Cervical Spine of Sprague Dawley Rats.

[0078] Figure 8 is a graph showing the results of SOD1 mRNA silencing by a single intrathecal injection of various exemplary siRNA conjugates in Lumbar Spine of Sprague Dawley Rats.

We claim:

1. A double-stranded iRNA agent comprising:

   an antisense strand which is complementary to a target gene;

   a sense strand which is complementary to said antisense strand; and

   one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier.

2. The double-stranded iRNA agent of claim 1, wherein the lipophilicity of the lipophilic moiety, measured by $\log K_{ow}$, exceeds 0.

3. The double-stranded iRNA agent of claim 1, wherein the hydrophobicity of the double-stranded iRNA agent, measured by the unbound fraction in the plasma protein binding assay of the double-stranded iRNA agent, exceeds 0.2.

4. The double-stranded iRNA agent of claim 3, wherein the plasma protein binding assay is an electrophoretic mobility shift assay using human serum albumin protein.

5. The double-stranded iRNA agent of claim 1, wherein the internal positions include all positions except terminal two positions from each end of the strand.

6. The double-stranded iRNA agent of claim 5, wherein the internal positions include all positions except terminal three positions from each end of the strand.

7. The double-stranded iRNA agent of claim 5 or 6, wherein the internal positions exclude the cleavage site region of the sense strand.

8. The double-stranded iRNA agent of claim 7, wherein the internal positions exclude positions 9-12, counting from the 5'-end of the sense strand.

9. The double-stranded iRNA agent of claim 7, wherein the internal positions exclude positions 11-13, counting from the 3'-end of the sense strand.

10. The double-stranded iRNA agent of claim 5 or 6, wherein the internal positions exclude the cleavage site region of the antisense strand.

11. The double-stranded iRNA agent of claim 10, wherein the internal positions exclude positions 12-14, counting from the 5'-end of the antisense strand.

12. The double-stranded iRNA agent of claim 5 or 6, wherein the internal positions excluding positions 11-13 on the sense strand, counting from the 3'-end, and positions 12-14 on the antisense strand, counting from the 5'-end.

13. The double-stranded iRNA agent of claim 1, wherein one or more lipophilic moieties are conjugated to one or more of the following internal positions: positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand.

14. The double-stranded iRNA agent of claim 13, wherein one or more lipophilic moieties are conjugated to one or more of the following internal positions: positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'-end of each strand.

15. The double-stranded iRNA agent of any one of claims 1-14, wherein said sense and antisense strands are each 15 to 30 nucleotides in length.

16. The double-stranded iRNA agent of any one of claims 1-14, wherein said sense and antisense strands are each 19 to 25 nucleotides in length.

17. The double-stranded iRNA agent of any one of claims 1-14, wherein said sense and antisense strands are each 21 to 23 nucleotides in length.

18. The double-stranded iRNA agent of claim 17, wherein the sense strand is 21-nucleotides in length, and the antisense strands are 23-nucleotides in length, wherein the strands form a double-stranded region of 21 consecutive base pairs having a 2-nucleotide long single-stranded overhangs at the 3'-end.

19. The double-stranded iRNA agent of claim 1, wherein the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound.

20. The double-stranded iRNA agent of claim 19, wherein the lipophilic moiety is lipid, cholesterol, retinoic acid, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-bis-O(hexadecyl)glycerol, geranyloxyhexyanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine.

21. The double-stranded iRNA agent of claim 19, wherein the lipophilic moiety contains a saturated or unsaturated C_4-C_{30} hydrocarbon chain, and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

22. The double-stranded iRNA agent of claim 21, wherein the lipophilic moiety contains a saturated or unsaturated C_6-C_{18} hydrocarbon chain.

23. The double-stranded iRNA agent of claim 22, wherein the lipophilic moiety contains a saturated or unsaturated C_{16} hydrocarbon chain.

24. The double-stranded iRNA agent of any one of claims 1-23, wherein the lipophilic moiety is conjugated via a carrier that replaces one or more nucleotide(s) in the internal position(s).

25. The double-stranded iRNA agent of claim 24, wherein the carrier is a cyclic group selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl; or is an acyclic moiety based on a serinol backbone or a diethanolamine backbone.

26. The double-stranded iRNA agent of any one of claims 1-25, wherein the lipophilic moiety is conjugated to the double-stranded iRNA agent via a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction, or carbamate.

27. The double-stranded iRNA agent of any one of claims 1-26, wherein said iRNA agent comprises a single-stranded overhang on at least one of the termini.

28. The double-stranded iRNA agent of claim 27, wherein said single-stranded overhang is 1, 2 or 3 nucleotides in length.

29. The double-stranded iRNA agent of any one of claims 1-28, wherein the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

30. The double-stranded iRNA agent of any one of claims 1-29, further comprising a phosphate or phosphate mimic at the 5'-end of the antisense strand.

31. The double-stranded iRNA agent of claim 30, wherein the phosphate mimic is a 5'-vinyl phosphonate (VP).

32. The double-stranded iRNA agent of any one of claims 1-31, further comprising a targeting ligand that targets a receptor which mediates delivery to a CNS tissue.

33. The double-stranded iRNA agent of claim 32, wherein the targeting ligand is selected from the group consisting of Angiopep-2, lipoprotein receptor related protein (LRP) ligand, bEnd.3 cell binding ligand, transferrin receptor (TfR) ligand, manose receptor ligand, glucose transporter protein, and LDL receptor ligand.

34. The double-stranded iRNA agent of any one of claims 1-31, further comprising a targeting ligand that targets a receptor which mediates delivery to an ocular tissue.

35. The double-stranded iRNA agent of claim 34, wherein the targeting ligand is selected from the group consisting of trans-retinol, RGD peptide, LDL receptor ligand, and carbohydrate based ligands.

36. The double-stranded iRNA agent of claim 35, wherein the RGD peptide is H-Gly-Arg-Gly-Asp-Ser-Pro-Lys-Cys-OH or Cyclo(-Arg-Gly-Asp-D-Phe-Cys).

37. The double-stranded iRNA agent of any one of claims 1-36, further comprising a targeting ligand that targets a liver tissue.

38. The double-stranded iRNA agent of claim 37, wherein the targeting ligand is a GalNAc conjugate.

39. The double-stranded iRNA agent of any one of claims 1-38, wherein the lipophilic moeity or targeting ligand is conjugated via a bio-clevable linker selected from the group consisting of DNA, RNA, disulfide, amide, funtionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, mannose, and combinations thereof.

40. The double stranded iRNA agent of any one of claims 1-39, wherein the 3' end of the sense strand is protected via an end cap which is a cyclic group having an amine, said cyclic group being selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl.

41. A method of reducing the expression of a target gene in a cell, comprising contacting said cell with a double-stranded iRNA agent comprising:

an antisense strand which is complementary to a target gene;

a sense strand which is complementary to said antisense strand; and

one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier.

42. The method of claim 41, wherein the cell is an extraheptic cell.

43. The method of claim 41, wherein the lipophilicity of the lipophilic moiety, measured by $\log K_{ow}$, exceeds 0.

44. The method of claim 41, wherein the hydrophobicity of the double-stranded iRNA agent, measured by the unbound fraction in the plasma protein binding assay of the double-stranded iRNA agent, exceeds 0.2.

45. The method of claim 44, wherein the plasma protein binding assay is an electrophoretic mobility shift assay using human serum albumin protein.

46. The method of claim 41, wherein the lipophilic moiety contains a saturated or unsaturated C_{16} hydrocarbon chain.

47. Use of a double-stranded iRNA agent comprising:
an antisense strand which is complementary to a target gene;
a sense strand which is complementary to said antisense strand; and
one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier,
in the manufacture of a medicament for reducing the expression of a target gene in a subject.

48. The use of claim 47, wherein the double-stranded iRNA agent is to be administered extrahepatically.

49. The use of claim 48, wherein the double-stranded iRNA agent is to be administered intrathecally.

50. The use of claim 49, wherein the method reduces the expression of a target gene in a brain or spine tissue.

51. The use of claim 50, wherein the brain or spine tissue is selected from the group consisting of cortex, cerebellum, cervical spine, lumbar spine, and thoracic spine.

52. The use of claim 48, wherein the target gene is selected from the group consisiting of APP, ATXN2, C9orf72, TARDBP, MAPT(Tau), HTT, SNCA, FUS, ATXN3, ATXN1, SCA1, SCA7, SCA8, MeCP2, PRNP, SOD1, DMPK, and TTR.

53. The use of claim 52, wherein the double-stranded iRNA agent is to be administered intravitreally.

54. The use of claim 53, wherein the method reduces the expression of a target gene in an ocular tissue.

55. Use of the double-stranded RNAi agent of any one of claims 1-40 in the manufacture of a medicament for treating a subject having a CNS disorder.

56. The use of claim 55, wherein the CNS disorder is selected from the group of alzheimer, amyotrophic lateral schlerosis (ALS), frontotemporal dementia, huntington, Parkinson, spinocerebellar, prion, and lafora.