cell's own RNAi machinery that has both high biological activity and *in vivo* stability, and that can effectively inhibit expression of a target Huntingtin gene.

SUMMARY

The present disclosure provides RNAi agent compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a huntingin (HTT) gene. The HTT gene may be within a cell, *e.g.,* a cell within a subject, such as a human. The present disclosure also provides methods of using the RNAi agent compositions of the disclosure for inhibiting the expression of an HTT gene or for treating a subject who would benefit from inhibiting or reducing the expression of an HTT gene, *e.g.,* a subject suffering or prone to suffering from an HTT-associated disease.

In one aspect, the present disclosure refers to a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Huntingtin (HTT), wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the complement of nucleotides 142-195 of SEQ ID NO:1, and wherein one or more lipophilic moieties are conjugated to one or more internal positions on at least one of the sense strand or the antisense strand.

In another aspect, the present disclosure refers to a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Huntingtin (HTT), wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO: 1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO: 6, and wherein one or more lipophilic moieties are conjugated to one or more internal positions on at least one of the sense strand or the antisense strand.

In another aspect, the present disclosure refers to a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Huntingtin (HTT) in a cell, wherein the dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding HTT, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21, 24, 25, 27-30, 32 or 33.

In yet another aspect, the present disclosure refers to a cell containing the dsRNA agent as disclosed herein.

In yet another aspect, the present disclosure refers to a pharmaceutical composition for inhibiting expression of a gene encoding HTT, comprising the dsRNA agent as disclosed herein.

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the dsRNA agent as disclosed herein and a lipid formulation.

In yet another aspect, the present disclosure refers to an *in vitro* method of inhibiting expression of a huntingtin (HTT) gene in a cell, the method comprising: (a) contacting the cell with the dsRNA agent as disclosed herein, or the pharmaceutical composition as disclosed herein; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the HTT gene, thereby inhibiting expression of the HTT gene in the cell.

In yet another aspect, the present disclosure refers to the use of the dsRNA agent as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a subject diagnosed with an HTT-associated disease.

In one example, the present disclosure provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Huntingtin (HTT), wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides differing by no more than 3, 2, 1, or 0 nucleotides from the nucleotide sequence of SEQ ID NO: 1 and the antisense strand comprises at least 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides differing by no more than 3, 2, 1, or 0 nucleotides from the nucleotide sequence of SEQ ID NO: 6, and wherein one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand.

In some examples, the nucleotide sequence of the sense strand comprises any one of the sense strand nucleotide sequences in any one of Tables 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21, 24, 25, 27-30, 32 and 33.

In some examples, the sense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 618-640, 1215-1237, 1248-1270, 1403-1425, 4051-4073, 4393-4415, 4398-4420, 4403-4425, 4441-4463, 4518-4540, 4548-4570, 5105-5127, 5215-5237, 5217-5239, 5221-5243, 5222-5244, 5366-5388, 5372-5394, 5450-5472, 5509-5531, 5883-5905, 6009-6031, 6010-6032, 6011-6033, 6012-6034, 6013-6035, 6014-6036, 6015-6037, 6347-6369, 6512-6534, 7523-7545, 7525-7547, 7526-7548, 9127-9149, 9531-9553, or 9538-9560 of SEQ ID NO:1.

In some examples, the antisense strand comprises at least 15 contiguous nucleotides differing by nor more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-953769.1, AD-953778.1, AD-953784.1, AD-953786.1, AD-953849.1, AD-953854.1, AD-953855.1, AD-953857.1, AD-953862.1, AD-953866.1, AD-953867.1, AD-953880.1, AD-953883.1, AD-953884.1, AD-953885.1, AD-953886.1, AD-953887.1, AD-953888.1, AD-953889.1, AD-953891.1, AD-953896.1, AD-953898.1, AD-953899.1, AD-953900.1, AD-953901.1, AD-953902.1, AD-953903.1, AD-953904.1, AD-953907.1, AD-953911.1, AD-953921.1, AD-953923.1, AD-953924.1, AD-953932.1, and AD-953933.1, AD-953937.1.

In some examples, the lipophilic moiety is conjugated *via* a linker or a carrier.

In another example, the present disclosure provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Huntingtin (HTT) in a cell, wherein the dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding HTT, and wherein the region of complementarity comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 3, 2, 1, or 0 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21, 24, 25, 27-30, 32 and 33.

The sense strand, the antisense strand, or both the sense strand and the antisense strand may be conjugated to one or more lipophilic moieties. In some examples, the lipophilic moiety is conjugated to one or more internal positions in the double stranded region of the dsRNA agent, *e.g.,* the one or more lipophilic moieties may be conjugated to one or more internal positions on the antisense strand. In some examples, the one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand *via* a linker or carrier.

In some examples, lipophilicity of the lipophilic moiety, measured by logKow, exceeds 0.

In some examples, the hydrophobicity of the dsRNA agent, measured by the unbound fraction in a plasma protein binding assay of the dsRNA agent, exceeds 0.2. In some examples, the plasma protein binding assay is an electrophoretic mobility shift assay using human serum albumin protein.

In some examples, the internal positions include all positions except the terminal two positions from each end of the sense strand or the antisense strand. In other examples, the internal positions include all positions except the terminal three positions from each end of the sense strand or the antisense strand.

In some examples, the internal positions exclude a cleavage site region of the sense strand, such as the internal positions include all positions except positions 9-12, counting from the 5'-end of the sense strand or the internal positions include all positions except positions 11-13, counting from the 3'-end of the sense strand.

In some examples, the internal positions exclude a cleavage site region of the antisense strand. In other examples, the internal positions include all positions except positions 12-14, counting from the 5'-end of the antisense strand. In some examples, the internal positions include all positions except 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.

In some examples, the one or more lipophilic moieties are conjugated to one or more of the internal positions selected from the group consisting of 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.

In some examples, the one or more lipophilic moieties are conjugated to one or more of the internal positions selected from the group consisting of 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.

In some examples, the positions in the double stranded region exclude a cleavage site region of the sense strand.

In some examples, the sense strand is 21 nucleotides in length, the antisense strand is 23 nucleotides in length, and the lipophilic moiety is conjugated to position 20, position 15, position 1, position 7, position 6, or position 2 of the sense strand or position 16 of the antisense strand.

In other examples, the sense strand is 21 nucleotides in length, the antisense strand is 23 nucleotides in length, and the lipophilic moiety is conjugated to position 21, position 20, position 15, position 1, position 7, position 6, or position 2 of the sense strand or position 16 of the antisense strand.

In some examples, the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound.

In some examples, the lipophilic moiety is selected from the group consisting of 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.

In some examples, the lipophilic moiety contains a saturated or unsaturated C4-C30 hydrocarbon chain, and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

In some examples, the lipophilic moiety contains a saturated or unsaturated C6-C18 hydrocarbon chain.

In some examples, the lipophilic moiety contains a saturated or unsaturated C16 hydrocarbon chain. In some examples, the saturated or unsaturated C16 hydrocarbon chain is conjugated to position 6, counting from the 5'-end of the strand.

In some examples, the lipophilic moiety is conjugated *via* a carrier that replaces one or more nucleotide(s) in the internal position(s) or the double stranded region. In some examples, 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.

In some examples, the lipophilic moiety is conjugated to the dsRNA 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.

In some examples, the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

In some examples, the dsRNA agent comprises at least one modified nucleotide. In some examples, no more than five of the sense strand nucleotides and no more than five of the nucleotides of the antisense strand are unmodified nucleotides. In other examples, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

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

In other examples, the modified nucleotide is selected from the group consisting of a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, 3'-terminal deoxy-thymine nucleotides (dT), a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

In some examples, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), and, a vinyl-phosphonate nucleotide; and combinations thereof.

In some examples, at least one of the modifications on the nucleotides is a thermally destabilizing nucleotide modification. In some examples, the thermally destabilizing nucleotide modification is selected from the group consisting of an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), and a glycerol nucleic acid (GNA)

In some examples, the modified nucleotide comprises a short sequence of 3'-terminal deoxy-thymine nucleotides (dT).

In some examples, the modifications on the nucleotides are 2'-O-methyl, GNA and 2'fluoro modifications.

In some examples, the dsRNA agent further comprises at least one phosphorothioate internucleotide linkage. In some examples, the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages. In one example, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another example, the strand is the sense strand. In a related example, the

phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. Optionally, the strand is the antisense strand. In another example, the strand is the sense strand. In another example, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another example, the strand is the sense strand.

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

In some examples, at least one strand comprises a 3' overhang of at least 1 nucleotide or a 3' overhang of at least 2 nucleotides.

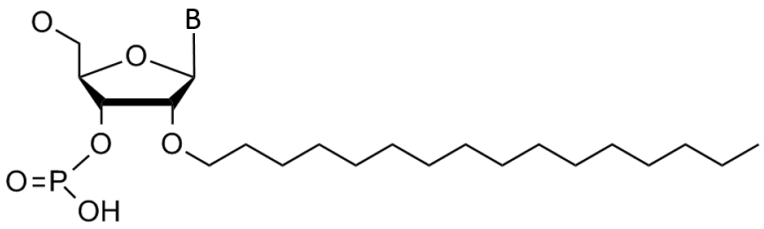
The double stranded region may be 15-30 nucleotide pairs in length; 17-23 nucleotide pairs in length; 17-25 nucleotide pairs in length; 23-27 nucleotide pairs in length; 19-21 nucleotide pairs in length; or 21-23 nucleotide pairs in length.

Each strand may be 19-30 nucleotides; 19-23 nucleotides; or 21-23 nucleotides.

In some examples, the dsRNA agent further comprises a targeting ligand that targets a liver tissue. In some examples, the targeting ligand is a GalNAc conjugate.

In certain examples, the double-stranded RNAi agent further includes a targeting ligand that targets a receptor which mediates delivery to a CNS tissue, *e.g*., a hydrophilic ligand.

In certain examples, the targeting ligand is a C16 ligand. In one example, the ligand is



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where B is a nucleotide base or a nucleotide base analog, optionally where B is adenine, guanine, cytosine, thymine or uracil.

In some examples, 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.

In some examples, 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.

In some examples, the dsRNA agent further comprises a terminal, chiral modification occurring 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 occurring 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 occurring 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.

In some examples, the dsRNA agent further comprises a terminal, chiral modification occurring 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 occurring 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 occurring at the first internucleotide linkage at the 5’ end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In some examples, the dsRNA agent further comprises a terminal, chiral modification occurring 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 occurring 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 occurring at the first internucleotide linkage at the 5’ end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In some examples, the dsRNA agent further comprises a terminal, chiral modification occurring 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 occurring 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 occurring 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 occurring at the first internucleotide linkage at the 5’ end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In some examples, the dsRNA agent further comprises a terminal, chiral modification occurring 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 occurring 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 occurring at the first internucleotide linkage at the 5’ end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In some examples, the dsRNA agent further comprises a phosphate or phosphate mimic at the 5’-end of the antisense strand. In some examples, the phosphate mimic is a 5’-vinyl phosphonate (VP).

In some examples, the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

In some examples, the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

An additional example of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a huntingtin (HTT) gene, where the double stranded RNAi agent targeted to HTT includes a sense strand and an antisense strand forming a double stranded region, where the sense strand includes at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 1-5 and the antisense strand includes at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 6-10; where a substitution of a uracil for any thymine in the sequences provided in the SEQ ID NOs: 1-10 (when comparing aligned sequences) does not count as a difference that contributes to the differing by no more than 3 nucleotides from any one of the nucleotide sequences provided in SEQ ID NOs: 1-10, where substantially all of the nucleotides of the sense strand include a modification that is a 2'-O-methyl modification, a GNA or a 2'-fluoro modification, where the sense strand includes two phosphorothioate internucleotide linkages at the 5'-terminus, where substantially all of the nucleotides of the antisense strand include a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, where the antisense strand includes two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and where the sense strand is conjugated to one or more lipophilic, *e.g.*, C16, ligands.

Another example of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a huntingtin (HTT) gene, where the double stranded RNAi agent targeted to HTT includes a sense strand and an antisense strand forming a double stranded region, where the sense strand includes at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 1-5 and the antisense strand includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 6-10, where a substitution of a uracil for any thymine in the sequences provided in the SEQ ID NOs: 1-10 (when comparing aligned sequences) does not count as a difference that contributes to the differing by no more than 3 nucleotides from any one of the nucleotide sequences provided in SEQ ID NOs:1-10; where the sense strand includes at least one 3'-terminal deoxy-thymine nucleotide (dT), and where the antisense strand includes at least one 3'-terminal deoxy-thymine nucleotide (dT).

An additional example of the instant disclosure provides a double stranded ribonucleic acid (RNAi) agent for inhibiting expression of a huntingtin (HTT) gene, where the RNAi agent possesses a sense strand and an antisense strand, and where the antisense strand includes a region of complementarity which includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides), *e.g.*, at least 15 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides), at least 19 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides), from any one of the antisense strand nucleobase sequences of Tables 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21, 24, 25, 27-30, 32 and 33. In one example, the RNAi agent includes one or more of the following

modifications: a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-C-alkyl-modified nucleotide, a nucleotide comprising a glycol nucleic acid (GNA), a phosphorothioate (PS) and a vinyl phosphonate (VP). Optionally, the RNAi agent includes at least one of each of the following modifications: a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-C-alkyl-modified nucleotide, a nucleotide comprising a glycol nucleic acid (GNA), a phosphorothioate and a vinyl phosphonate (VP).

In another example, the RNAi agent includes four or more PS modifications, optionally six to ten PS modifications, optionally eight PS modifications.

In an additional example, each of the sense strand and the antisense strand of the RNAi agent possesses a 5'-terminus and a 3'-terminus, and the RNAi agent includes eight PS modifications positioned at each of the penultimate and ultimate internucleotide linkages from the respective 3'- and 5'-termini of each of the sense and antisense strands of the RNAi agent.

In another example, each of the sense strand and the antisense strand of the RNAi agent includes a 5'-terminus and a 3'-terminus, and the RNAi agent includes only one nucleotide including a GNA. Optionally, the nucleotide including a GNA is positioned on the antisense strand at the seventh nucleobase residue from the 5'-terminus of the antisense strand.

In an additional example, each of the sense strand and the antisense strand of the RNAi agent includes a 5'-terminus and a 3'-terminus, and the RNAi agent includes one to four 2'-C-alkyl-modified nucleotides. Optionally, the 2'-C-alkyl-modified nucleotide is a 2'-C16-modified nucleotide. Optionally, the RNAi agent includes a single 2'- C-alkyl, *e.g.*, C16-modified nucleotide. Optionally, the single 2'- C-alkyl, *e.g.*, C16-modified nucleotide is located on the sense strand at the sixth nucleobase position from the 5'-terminus of the sense strand.

In another example, each of the sense strand and the antisense strand of the RNAi agent includes a 5'-terminus and a 3'-terminus, and the RNAi agent includes two or more 2'-fluoro modified nucleotides. Optionally, each of the sense strand and the antisense strand of the RNAi agent includes two or more 2'-fluoro modified nucleotides. Optionally, the 2'-fluoro modified nucleotides are located on the sense strand at nucleobase positions 7, 9, 10 and 11 from the 5'-terminus of the sense strand and on the antisense strand at nucleobase positions 2, 14 and 16 from the 5'-terminus of the antisense strand.

In an additional example, each of the sense strand and the antisense strand of the RNAi agent includes a 5'-terminus and a 3'-terminus, and the RNAi agent includes one or more VP modifications. Optionally, the RNAi agent includes a single VP modification at the 5'-terminus of the antisense strand.

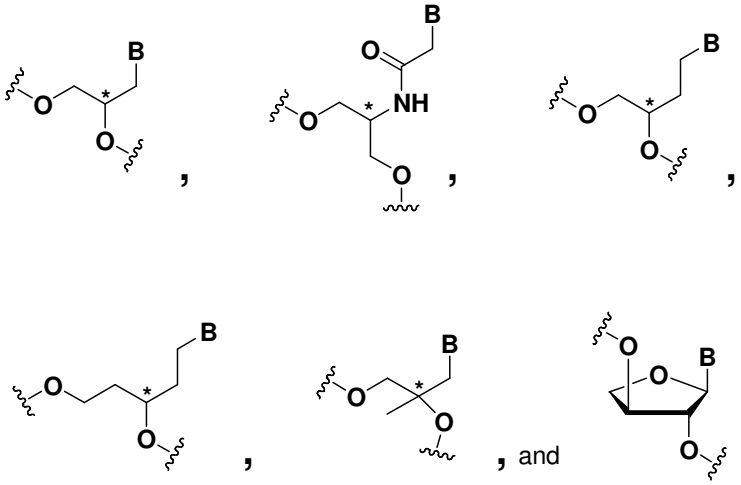
In another example, each of the sense strand and the antisense strand of the RNAi agent includes a 5'-terminus and a 3'-terminus, and the RNAi agent includes two or more 2'-O-methyl modified nucleotides. Optionally, the RNAi agent includes 2'-O-methyl modified nucleotides at all nucleobase locations not modified by a 2'-fluoro, a 2'-C-alkyl or a glycol nucleic acid (GNA). Optionally, the two or more 2'-O-methyl modified nucleotides are located on the sense strand at positions 1, 2, 3, 4, 5, 8, 12, 13, 14, 15, 16, 17, 18, 19, 20 and 21 from the 5'-terminus of the sense

strand and on the antisense strand at positions 1, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 15, 17, 18, 19, 20, 21, 22 and 23 from the 5'-terminus of the antisense strand.

In one example, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.

In another example, each strand has 19-30 nucleotides.

In certain examples, the antisense strand of the RNAi agent includes at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions of the 5′ region or a precursor thereof. Optionally, the thermally destabilizing modification of the duplex is one or more of



where B is nucleobase.

The present disclosure further provides cells containing any of the dsRNA agents of the disclosure and pharmaceutical compositions for inhibiting expression of a gene encoding HTT, comprising any of the dsRNA agents ot he disclosure.

In one example, the double stranded RNAi agent is in an unbuffered solution. Optionally, the unbuffered solution is saline or water. In another example, the double stranded RNAi agent is in a buffer solution. Optionally, the buffer solution includes acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof. In another example, the buffer solution is phosphate buffered saline (PBS). Another example of the disclosure provides a pharmaceutical composition that includes a double stranded RNAi agent of the instant disclosure and a lipid formulation. In one example, the lipid formulation includes a lipid nanoparticle (LNP).

An additional example of the disclosure provides a method of inhibiting expression of an HTT gene in a cell, the method including (a) contacting the cell with a double stranded RNAi agent of the instant disclosure or a pharmaceutical composition of of the instant disclosure; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of an HTT gene, thereby inhibiting expression of the HTT gene in the cell.

In one example, the cell is within a subject. Optionally, the subject is a human.

In certain examples, the subject is a rhesus monkey, a cynomolgous monkey, a mouse, or a rat. In certain examples HTT expression is inhibited by at least about 50% by the RNAi agent.

In certain examples, the human subject has been diagnosed with an HTT-associated disease, *e.g.,* Huntington's disease.

Another example of the disclosure provides a method of treating a subject diagnosed with an HTT-associated disease, *e.g.*, Huntington's disease, the method including administering to the subject a therapeutically effective amount of a double stranded RNAi agent of the disclosure or a pharmaceutical composition of the disclosure, thereby treating the subject.

In one example, treating comprises amelioration of at least on sign or symptom of the disease. In another example, treating comprises prevention of progression of the disease.

In some examples, the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

In some examples, the dsRNA agent is administered to the subject intrathecally. In one example, the method reduces the expression of an HTT gene in a brain (*e.g.,* striatum) or spine tissue. Optionally, the brain or spine tissue is striatum, cortex, cerebellum, cervical spine, lumbar spine, or thoracic spine.

In some examples, the method further comprises measuring a level of HTT in a sample obtained from the subject.

Another example of the instant disclosure provides a method of inhibiting the expression of huntingtin (HTT) in a subject, the method involving: administering to the subject a therapeutically effective amount of a double stranded RNAi agent of the disclosure or a pharmaceutical composition of the disclosure, thereby inhibiting the expression of HTT in the subject.

In some example, the method further comprises administering to the subject an additional agent suitable for treatment or prevention of an HTT-associated disorder.

BRIEF DESCRIPTIONS OF THE FIGURES

FIG.1 is a graph showing wild-type human HTT mRNA levels in the livers of mice expressing a portion of wild-type human HTT (via AAV). These mice were subcutaneously administered a single 3 mg/kg dose of the indicated dsRNA duplexes targeting exon 1 of the human HTT transcript at Day 14 post AAV dose. Human HTT levels shown are normalized to AAV treated controls 14 days post siRNA dose.

FIG.2 is a graph showing wild-type human HTT mRNA levels in the livers of mice expressing a portion of wild-type human HTT (via AAV). These mice were subcutaneously administered a single 3 mg/kg dose of the indicated dsRNA duplexes targeting exon 1 of the human HTT transcript at Day 14 post AAV dose. Human HTT levels shown are normalized to AAV treated controls 14 days post siRNA dose.

FIG. 3A is a graph showing full length mutant human HTT mRNA levels in the livers of YAC128 mice and subcutaneously administered a single 10 mg/kg dose of the indicated dsRNA duplexes targeting exon 1 of the human HTT transcript at Day 7 post-dose. Human HTT levels shown are normalized to PBS treatment levels.

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Huntingtin (HTT), wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the complement of nucleotides 142-195 of SEQ ID NO:1, and

wherein one or more lipophilic moieties are conjugated to one or more internal positions on at least one of the sense strand or the antisense strand.

2. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Huntingtin (HTT), wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO: 1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO: 6, and

wherein one or more lipophilic moieties are conjugated to one or more internal positions on at least one of the sense strand or the antisense strand.

3. The dsRNA agent of claim 2, wherein the nucleotide sequence of the sense strand comprises any one of the sense strand nucleotide sequences in any one of Tables 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21, 24, 25, 27-30, 32 or 33.

4. The dsRNA agent of any one of claims 1-3, wherein the lipophilic moiety is conjugated *via* a linker or a carrier.

5. A double stranded ribonucleic acid (dsRNA) for inhibiting expression of Huntingtin (HTT) in a cell, wherein the dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding HTT, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21, 24, 25, 27-30, 32 or 33.

6. The dsRNA agent of claim 5, wherein the sense strand, the antisense strand, or both the sense strand and the antisense strand is conjugated to one or more lipophilic moieties.

7. The dsRNA agent of claim 6, wherein the lipophilic moiety is conjugated to one or more internal positions in the double stranded region of the dsRNA agent.

8. The dsRNA agent of claim 6 or 7, wherein the one or more lipophilic moieties are conjugated to one or more internal positions on the antisense strand.

9. The dsRNA agent of claim 6 or 7, wherein the one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand *via* a linker or carrier.

10. The dsRNA agent of any one of claims 1-4, 6 and 9, wherein the one or more lipophilic moieties are conjugated to one or more of the internal positions selected from the group consisting of positions 4-8 and 13-18 on the sense strand.

11. The dsRNA agent of claim 10, wherein the one or more lipophilic moieties are conjugated to one or more of the internal positions selected from the group consisting of positions 5, 6, 7, 15, and 17 on the sense strand.

12. The dsRNA agent of any one of claims 1-4 and 6-11, wherein the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound.

13. The dsRNA agent of claim 12, wherein the lipophilic moiety is selected from the group consisting of 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.

14. The dsRNA agent of claim 13, wherein the lipophilic moiety contains a saturated or unsaturated C4-C30 hydrocarbon chain, and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

15. The dsRNA agent of claim 14, wherein the lipophilic moiety contains a saturated or unsaturated C6-C18 hydrocarbon chain.

16. The dsRNA agent of claim 15, wherein the lipophilic moiety contains a saturated or unsaturated C16 hydrocarbon chain.

17. The dsRNA agent of claim 16, wherein the saturated or unsaturated C16 hydrocarbon chain is conjugated to position 6, counting from the 5’-end of the strand.

18. The dsRNA agent of any one of claims 1-4 and 6-17, wherein the lipophilic moiety is conjugated *via* a carrier that replaces one or more nucleotide(s) in the internal position(s) or the double stranded region.

19. The dsRNA agent of claim 18, 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.

20. The dsRNA agent of any one of claims 1-4 and 6-19, wherein the lipophilic moiety is conjugated to the dsRNA 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.

21. The dsRNA agent of any one of claims 1-4 and 6-20, wherein the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

22. The dsRNA agent of any one of claims 1-21, wherein the dsRNA agent comprises at least one modified nucleotide.

23. The dsRNA agent of claim 22, wherein no more than five of the sense strand nucleotides and no more than five of the nucleotides of the antisense strand are unmodified nucleotides

24. The dsRNA agent of claim 22, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.

25. The dsRNA agent of any one of claims 22-24, wherein at least one of the modified nucleotides is selected from the group a deoxy-nucleotide, a 3’-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2’-amino-modified nucleotide, a 2’-O-allyl-modified nucleotide, 2’-C-alkyl-modified nucleotide, 2’-hydroxly-modified nucleotide, a 2’-methoxyethyl modified nucleotide, a 2’-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5'-methylphosphonate group, a nucleotide

comprising a 5’ phosphate or 5’ phosphate mimic, a nucleotide comprising vinyl phosphonate, a nucleotide comprising adenosine-glycol nucleic acid (GNA), a nucleotide comprising thymidine-glycol nucleic acid (GNA) S-Isomer, a nucleotide comprising 2-hydroxymethyl-tetrahydrofurane-5-phosphate, a nucleotide comprising 2’-deoxythymidine-3’phosphate, a nucleotide comprising 2’-deoxyguanosine-3’-phosphate, and a terminal nucleotide linked to a cholesteryl derivative, a dodecanoic acid bisdecylamide group; acytidine-2`-phosphate, a guanosine-2`-phosphate, a uridine-2`-phosphate, a adenosine-2`-phosphate, a 2'-O-hexadecyl-adenosine-3'-phosphate, a 2'-O-hexadecyl-cytidine-3'-phosphate, a 2'-O-hexadecyl-guanosine-3'-phosphate, and a 2'-O-hexadecyl-uridine-3'-phosphate, and combinations thereof.

26. The dsRNA agent of claim 25, wherein the modified nucleotide is selected from the group consisting of a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, 3’-terminal deoxy-thymine nucleotides (dT), a locked nucleotide, an abasic nucleotide, a 2’-amino-modified nucleotide, a 2’-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

27. The dsRNA of claim 25, wherein at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), and, a vinyl-phosphonate nucleotide; and combinations thereof.

28. The dsRNA of claim 25, wherein at least one of the modifications on the nucleotides is a thermally destabilizing nucleotide modification.

29. The dsRNA agent of any one of claims 1-28, further comprising at least one phosphorothioate internucleotide linkage.

30. The dsRNA agent of claim 29, wherein the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages.

31. The dsRNA agent of any one of claims 1-30, wherein each strand is no more than 30 nucleotides in length.

32. The dsRNA agent of any one of claims 1-31, wherein at least one strand comprises a 3’ overhang of at least 1 nucleotide.

33. The dsRNA agent of any one of claims 1-32, wherein the double stranded region is 15-30 nucleotide pairs in length.

34. The dsRNA agent of any one of claims 1-33, wherein each strand is independently 19-30 nucleotides in length.

35. The dsRNA agent of any one of claims 1-4 and 6-34, 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.

36. The dsRNA agent of any one of claims 1-35, further comprising a phosphate or phosphate mimic at the 5'-end of the antisense strand.

37. The dsRNA agent of claim 36, wherein the phosphate mimic is a 5'-vinyl phosphonate (VP).

38. The dsRNA agent of any one of claims 1-37, wherein the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

39. A cell containing the dsRNA agent of any one of claims 1-38.

40. A pharmaceutical composition for inhibiting expression of a gene encoding HTT, comprising the dsRNA agent of any one of claims 1-38.

41. A pharmaceutical composition comprising the dsRNA agent of any one of claims 1-38 and a lipid formulation.

42. An *in vitro* method of inhibiting expression of a huntingtin (HTT) gene in a cell, the method comprising:
(a) contacting the cell with the dsRNA agent of any one of claims 1-38, or the pharmaceutical composition of claim 40 or 41; and
(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the HTT gene, thereby inhibiting expression of the HTT gene in the cell.

43. The method of claim 42, wherein the expression of HTT is inhibited by at least 50%.

44. Use of the dsRNA agent of any one of claims 1-38, or the pharmaceutical composition of claim 40 or 41 in the manufacture of a medicament for treating a subject diagnosed with an HTT-associated disease.

45. The use of claim 44, wherein the HTT-associated disease is Huntington's disease.

46. The use of claim 44 or 45, wherein the dsRNA agent is to be administered to the subject at a dose of 0.01 mg/kg to 50 mg/kg.

47. The use of any one of claims 44-46, wherein the dsRNA agent is to be administered to the subject intrathecally.

48. The use of any one of claims 44-47, further comprising that an additional agent is to be administered to the subject for treatment or prevention of an HTT-associated disorder.