

COMPOSITIONS AND METHODS FOR TREATMENT OF HUNTINGTON'S DISEASE**Sequence Listing**

5 The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on September 21, 2023, is named "51436-035WO2_Sequence_Listing_9_21_23" and is 8,809 bytes in size.

Technical Field

10 This disclosure relates to small interfering RNA (siRNA) molecules, and compositions containing the same, that target RNA transcripts (e.g., mRNA) of a Huntingtin (*HTT*) gene. The disclosure further describes methods for silencing of *HTT* and the treatment of diseases that may benefit from the silencing of *HTT* (e.g., Huntington's Disease) by delivering *HTT*-targeting siRNA molecules to a target tissue of a subject in need.

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Background

HTT (Huntingtin) encodes a protein that is implicated in the onset and progression of Huntington's Disease. Studies show that individuals with a mutated form of *HTT* characterized by an abnormal trinucleotide repeat expansion. Currently, there are no treatments that can alter the course of Huntington's Disease. Accordingly, there is a need for therapeutics capable of selectively diminishing *HTT* activity in a manner that provides effective treatment for Huntington's Disease or other *HTT*-related diseases or disorders.

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Summary of the Disclosure

25 The present disclosure provides compositions and methods for reduction of Huntingtin (*HTT*) expression by way of small interfering RNA (siRNA)-mediated silencing of *HTT* transcripts. The compositions and methods provide the benefit of exhibiting high selectivity toward *HTT* over other genes.

The siRNA molecules of the disclosure can be used to silence the *HTT* gene, thereby preventing the translation of the corresponding mRNA transcript and reducing *HTT* protein expression. This reduction of *HTT* levels thus prevents disease onset or progression. The siRNA molecules of the disclosure can be delivered directly to a subject in need of *HTT* silencing by way of, for example, injection intrathecally, intracerebroventricularly, intrastrially, intraparenchymally, intracisterna magna injection, such as by catheterization, intravenous injection, subcutaneous injection, or intramuscular injection.

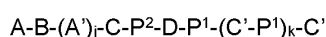
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In a first aspect, the disclosure provides an siRNA molecule that includes an antisense strand and a sense strand having complementarity to the antisense strand, wherein the antisense strand is from 10 to 30 nucleotides in length (e.g., from 10 to 29 nucleotides in length, from 10 to 28 nucleotides in length, from 10 to 27 nucleotides in length, from 10 to 26 nucleotides in length, from 10 to 25 nucleotides in length, from 10 to 24 nucleotides in length, from 10 to 23 nucleotides in length,

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from 10 to 22 nucleotides in length, from 10 to 21 nucleotides in length, or from 10 to 20 nucleotides in length, 10 nucleotides in length, 11 nucleotides in length, 12 nucleotides in length, 13 nucleotides in length, 14 nucleotides in length, 15 nucleotides in length, 16 nucleotides in length, 17 nucleotides in length, 18 nucleotides in length, 19 nucleotides in length, 20 nucleotides in length, 21 nucleotides in length, 22 nucleotides in length, 23 nucleotides in length, 24 nucleotides in length, 25 nucleotides in length, 26 nucleotides in length, 27 nucleotides in length, 28 nucleotides in length, 29 nucleotides in length, or 30 nucleotides in length) and has complementarity sufficient to hybridize to a region of equal length within a Huntingtin (*HTT*) mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. The antisense strand includes a structure represented by Formula I, wherein

Formula I is, in the 5'-to-3' direction:



Formula I;

wherein A is represented by the formula C-P¹-D-P¹;
 each A', independently, is represented by the formula C-P²-D-P²;
 B is represented by the formula C-P²-D-P²-D-P²-D-P²;
 each C, independently, is a 2'-O-methyl (2'-O-Me) ribonucleoside;
 each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside;
 each D, independently, is a 2'-F ribonucleoside;
 each P¹ is, independently, a phosphorothioate internucleoside linkage;
 each P² is, independently, a phosphodiester internucleoside linkage;
 j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and
 k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments, the antisense strand includes a structure represented by Formula A1, wherein Formula A1 is, in the 5'-to-3' direction:

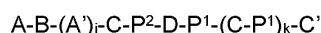


Formula A1;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In another aspect, the present disclosure provides an siRNA molecule that includes an antisense strand and a sense strand having complementarity to the antisense strand, wherein the antisense strand is from 10 to 30 nucleotides in length (e.g., from 10 to 29 nucleotides in length, from 10 to 28 nucleotides in length, from 10 to 27 nucleotides in length, from 10 to 26 nucleotides in length, from 10 to 25 nucleotides in length, from 10 to 24 nucleotides in length, from 10 to 23 nucleotides in length, from 10 to 22 nucleotides in length, from 10 to 21 nucleotides in length, or from 10 to 20 nucleotides in length, 10 nucleotides in length, 11 nucleotides in length, 12 nucleotides in length, 13 nucleotides in length, 14 nucleotides in length, 15 nucleotides in length, 16 nucleotides in length, 17

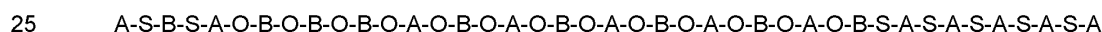
nucleotides in length, 18 nucleotides in length, 19 nucleotides in length, 20 nucleotides in length, 21 nucleotides in length, 22 nucleotides in length, 23 nucleotides in length, 24 nucleotides in length, 25 nucleotides in length, 26 nucleotides in length, 27 nucleotides in length, 28 nucleotides in length, 29 nucleotides in length, or 30 nucleotides in length) and has complementarity sufficient to hybridize to a region of equal length within a Huntingtin (*HTT*) mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. The antisense strand includes a structure represented by Formula II, wherein Formula II is, in the 5'-to-3' direction:



Formula II;

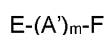
wherein A is represented by the formula C-P¹-D-P¹;
 each A', independently, is represented by the formula C-P²-D-P²;
 B is represented by the formula C-P²-D-P²-D-P²-D-P²;
 each C, independently, is a 2'-O-methyl (2'-O-Me) ribonucleoside;
 each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside;
 each D, independently, is a 2'-F ribonucleoside;
 each P¹ is, independently, a phosphorothioate internucleoside linkage;
 each P² is, independently, a phosphodiester internucleoside linkage;
 j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and
 k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments, the antisense strand has a structure represented by Formula A2, wherein Formula A2 is, in the 5'-to-3' direction:



Formula A2;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.
 In some embodiments, the sense strand includes a structure represented by Formula III, wherein Formula III is, in the 5'-to-3' direction:



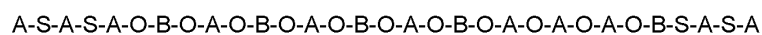
Formula III;

wherein E is represented by the formula (C-P¹)₂;
 F is represented by the formula (C-P²)₃-D-P¹-C-P¹-C, (C-P²)₃-D-P²-C-P²-C, (C-P²)₃-D-P¹-C-P¹-D, or (C-P²)₃-D-P²-C-P²-D;
 A', C, D, P¹, and P² are as defined in Formula II; and
 m is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments of any of the foregoing aspects, j is 4 and k is 4. In some embodiments, m is 4.

In some embodiments, the sense strand has a structure represented by Formula S1, wherein Formula S1 is, in the 5'-to-3' direction:

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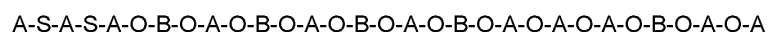
Formula S1;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

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In some embodiments, the sense strand comprises a structure represented by Formula S2, wherein Formula S2 is, in the 5'-to-3' direction:

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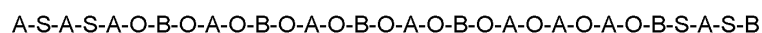


Formula S2;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

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In some embodiments, the sense strand comprises a structure represented by Formula S3, wherein Formula S3 is, in the 5'-to-3' direction:



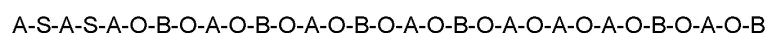
Formula S3;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

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In some embodiments, the sense strand comprises a structure represented by Formula S4, wherein Formula S4 is, in the 5'-to-3' direction:

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Formula S4;

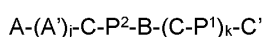
wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

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In yet another aspect, the disclosure provides an siRNA molecule that includes an antisense strand and a sense strand having complementarity to the antisense strand, wherein the antisense strand is from 10 to 30 nucleotides in length (e.g., from 10 to 29 nucleotides in length, from 10 to 28 nucleotides in length, from 10 to 27 nucleotides in length, from 10 to 26 nucleotides in length, from 10 to 25 nucleotides in length, from 10 to 24 nucleotides in length, from 10 to 23 nucleotides in length, from 10 to 22 nucleotides in length, from 10 to 21 nucleotides in length, or from 10 to 20 nucleotides

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in length, 10 nucleotides in length, 11 nucleotides in length, 12 nucleotides in length, 13 nucleotides in length, 14 nucleotides in length, 15 nucleotides in length, 16 nucleotides in length, 17 nucleotides in length, 18 nucleotides in length, 19 nucleotides in length, 20 nucleotides in length, 21 nucleotides in length, 22 nucleotides in length, 23 nucleotides in length, 24 nucleotides in length, 25 nucleotides in length, 26 nucleotides in length, 27 nucleotides in length, 28 nucleotides in length, 29 nucleotides in length, or 30 nucleotides in length) and has complementarity sufficient to hybridize to a region of equal length within a Huntingtin (*HTT*) mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. The antisense strand has a structure represented by Formula IV, wherein Formula IV is, in the 5'-to-3' direction:



Formula IV;

wherein A is represented by the formula C-P¹-D-P¹;

each A', independently, is represented by the formula C-P²-D-P²;

B is represented by the formula D-P¹-C-P¹-D-P¹;

each C, independently, is a 2'-O-Me ribonucleoside;

each C', independently, is a 2'-O-Me ribonucleoside or a 2'-F ribonucleoside;

each D, independently, is a 2'-F ribonucleoside;

each P¹ is, independently, a phosphorothioate internucleoside linkage;

each P² is, independently, a phosphodiester internucleoside linkage;

j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and

k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments, the antisense strand has a structure represented by Formula A3,

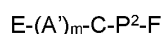
wherein Formula A3 is, in the 5'-to-3' direction:



Formula A3;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the sense strand has a structure represented by Formula V, wherein Formula V is, in the 5'-to-3' direction:



Formula V;

wherein E is represented by the formula (C-P¹)₂;

F is represented by the formula D-P¹-C-P¹-C, D-P²-C-P²-C, D-P¹-C-P¹-D, or D-P²-C-P²-D;

A', C, D, P¹ and P² are as defined in Formula IV; and

m is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

In some embodiments, j is 6 and k is 2. In some embodiments, m is 5.

In some embodiments, the sense strand has a structure represented by Formula S5, wherein Formula S5 is, in the 5'-to-3' direction:

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A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A

Formula S5;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

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In some embodiments, the sense strand has a structure represented by Formula S6, wherein Formula S6 is, in the 5'-to-3' direction:

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A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A

Formula S6;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

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In some embodiments, the sense strand has a structure represented by Formula S7, wherein Formula S7 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-B

Formula S7;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

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In some embodiments, the sense strand has a structure represented by Formula S8, wherein Formula S8 is, in the 5'-to-3' direction:

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A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B

Formula S8;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

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In another aspect, the disclosure provides an siRNA molecule that includes an antisense strand and a sense strand having complementarity to the antisense strand, wherein the antisense strand is from 10 to 30 nucleotides in length (e.g., from 10 to 29 nucleotides in length, from 10 to 28 nucleotides in length, from 10 to 27 nucleotides in length, from 10 to 26 nucleotides in length, from 10 to 25 nucleotides in length, from 10 to 24 nucleotides in length, from 10 to 23 nucleotides in length,

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from 10 to 22 nucleotides in length, from 10 to 21 nucleotides in length, or from 10 to 20 nucleotides in length, 10 nucleotides in length, 11 nucleotides in length, 12 nucleotides in length, 13 nucleotides in length, 14 nucleotides in length, 15 nucleotides in length, 16 nucleotides in length, 17 nucleotides in length, 18 nucleotides in length, 19 nucleotides in length, 20 nucleotides in length, 21 nucleotides in length, 22 nucleotides in length, 23 nucleotides in length, 24 nucleotides in length, 25 nucleotides in length, 26 nucleotides in length, 27 nucleotides in length, 28 nucleotides in length, 29 nucleotides in length, or 30 nucleotides in length) and has complementarity sufficient to hybridize to a region of equal length within a Huntingtin (*HTT*) mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. The antisense strand has a structure represented by Formula VI, wherein

Formula VI is, in the 5'-to-3' direction:



Formula VI;

wherein A is represented by the formula C-P¹-D-P¹;
 each B, independently, is represented by the formula C-P²;
 each C, independently, is a 2'-O-Me ribonucleoside;
 each C', independently, is a 2'-O-Me ribonucleoside or a 2'-F ribonucleoside;
 each D, independently, is a 2'-F ribonucleoside;
 each E, independently, is represented by the formula D-P²-C-P²;
 F is represented by the formula D-P¹-C-P¹;
 each G, independently, is represented by the formula C-P¹;
 each P¹ is, independently, a phosphorothioate internucleoside linkage;
 each P² is, independently, a phosphodiester internucleoside linkage;
 j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7);
 k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and
 l is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

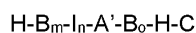
In some embodiments, the antisense strand has a structure represented by Formula A4, wherein Formula A4 is, in the 5'-to-3' direction:



Formula A4;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments, the sense strand has a structure represented by Formula VII, wherein Formula VII is, in the 5'-to-3' direction:



Formula VII;

wherein A' is represented by the formula C-P²-D-P²;

each H, independently, is represented by the formula (C-P¹)₂;

each I, independently, is represented by the formula (D-P²);

5 B, C, D, P¹ and P² are as defined in Formula VI;

m is an integer from 1 to 7;

n is an integer from 1 to 7; and

o is an integer from 1 to 7.

10 In some embodiments, j is 3, k is 6, and l is 2. In some embodiments, m is 3, n is 3, and o is 3.

In some embodiments, the sense strand has a structure represented by Formula S9, wherein Formula S9 is, in the 5'-to-3' direction:

A-S-A-S-A-O-A-O-A-O-B-O-B-O-B-O-A-O-B-O-A-O-A-O-A-O-A-S-A-S-A

15 **Formula S9;**

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

20 In some embodiments of any of the foregoing aspects, the region of equal length within the *HTT* mRNA transcript has the nucleic acid sequence of SEQ ID NO: 1. In some embodiments of any of the foregoing aspects, the region of equal length within the *HTT* mRNA transcript has the nucleic acid sequence of SEQ ID NO: 2. In some embodiments of any of the foregoing aspects, the region of equal length within the *HTT* mRNA transcript has the nucleic acid sequence of SEQ ID NO: 3.

25 In some embodiments of any of the foregoing aspects, the antisense strand has at least 70% (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) complementarity to a region of 15 contiguous nucleobases within the *HTT* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. In some embodiments, the antisense strand has at least 70% (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) complementarity to a region of 16 contiguous nucleobases within the *HTT* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. In some embodiments, the antisense strand has at least 70% (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) complementarity to a region of 17 contiguous nucleobases within the *HTT* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. In some
35 embodiments, the antisense strand has at least 70% (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) complementarity to a region of 18 contiguous nucleobases within the *HTT* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. In some
40 embodiments, the antisense strand has at least 70% (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) complementarity to a region of 19 contiguous nucleobases within the

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In some embodiments of any of the foregoing aspects, the antisense strand has at least 70% (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) complementarity to a region within the *HTT* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. In some embodiments, the antisense strand
5 has at least 75% (e.g., 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) complementarity to a region within the *HTT* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3, optionally wherein the antisense strand has at least 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementarity to the region within the *HTT*
10 mRNA transcript having the nucleic acid sequence of any one of SEQ ID Nos: 1-3.

In some embodiments, the antisense strand includes at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of
15 equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

In some embodiments, the antisense strand includes from 10 to 30 contiguous nucleotides (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length
20 within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. In some embodiments, the antisense strand includes from 12 to 30 contiguous nucleotides (e.g., 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID
25 NOs: 1-3. In some embodiments, the antisense strand includes from 15 to 30 contiguous nucleotides (e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. In some
30 embodiments, the antisense strand includes from 18 to 30 contiguous nucleotides (e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. In some
35 embodiments, the antisense strand includes from 15 to 21 contiguous nucleotides (e.g., 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. In some embodiments, the antisense strand includes 15 contiguous
40 nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. In some embodiments, the antisense strand includes 20 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the

HTT RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. In some embodiments, the antisense strand includes 21 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

5 In some embodiments, the antisense strand includes 9 or fewer nucleotide mismatches relative to a region within the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3, optionally wherein the antisense strand includes 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or only 1 mismatch relative to the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

10 In some embodiments of the foregoing aspects, the region of the *HTT* RNA transcript has the nucleic acid sequence of SEQ ID NO: 1. In some embodiments of the foregoing aspects, the region of the *HTT* RNA transcript has the nucleic acid sequence of SEQ ID NO: 2. In some embodiments of the foregoing aspects, the region of the *HTT* RNA transcript has the nucleic acid sequence of SEQ ID NO: 3.

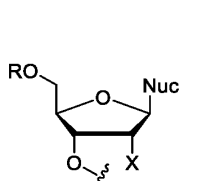
15 In some embodiments of any of the foregoing aspects, the antisense strand has a nucleic acid sequence that is at least 85% (e.g., 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) identical to the nucleic acid sequence of any one of SEQ ID NOs: 7-9. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of any one of SEQ ID NOs: 7-9. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 95% (e.g., 95, 96, 97, 98, 99, or 100%) identical to the nucleic acid sequence of SEQ ID NOs: 7-9, optionally wherein the antisense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 7-9. In some embodiments, the antisense strand has the nucleic acid sequence of any one of SEQ ID NOs: 7-9. In some embodiments, the nucleic acid sequence is SEQ ID NO: 7. In some
20
25
embodiments, the nucleic acid sequence is SEQ ID NO: 8. In some embodiments, the nucleic acid sequence is SEQ ID NO: 9.

In some embodiments of any of the foregoing aspects, the sense strand has a nucleic acid sequence that is at least 85% (e.g., 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) identical to the nucleic acid sequence of any one of SEQ ID NOs: 4-6. In some embodiments, the
30
sense strand has a nucleic acid sequence that is at least 90% (e.g., 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) identical to the nucleic acid sequence of any one of SEQ ID NOs: 4-6. In some embodiments, the sense strand has a nucleic acid sequence that is at least 90% (e.g., 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) identical to the nucleic acid sequence of any one of SEQ ID NOs: 4-6, optionally wherein the sense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or
35
99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 4-6. In some embodiments, the sense strand has the nucleic acid sequence of any one of SEQ ID NOs: 4-6. In some embodiments, the nucleic acid sequence is SEQ ID NO: 4. In some embodiments, the nucleic acid sequence is SEQ ID NO: 5. In some embodiments, the nucleic acid sequence is SEQ ID NO: 6.

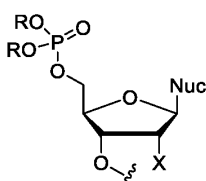
In some embodiments of any of the foregoing aspects or embodiments of the disclosure, the
40
antisense strand further includes a 5' phosphorus stabilizing moiety at the 5' end of the antisense

strand. In some embodiments of any of the foregoing aspects or embodiments of the disclosure, the sense strand further includes a 5' phosphorus stabilizing moiety at the 5' end of the sense strand.

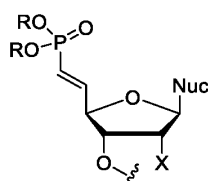
In some embodiments, each 5' phosphorus stabilizing moiety is, independently, represented by any one of Formulas IX-XVI:



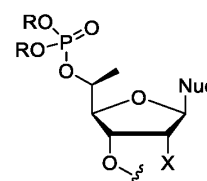
Formula IX



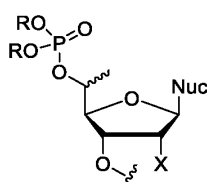
Formula X



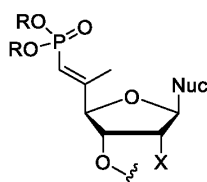
Formula XI



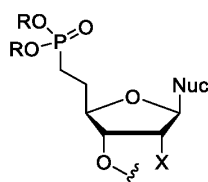
Formula XII



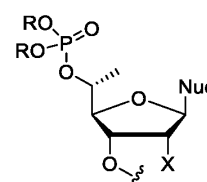
Formula XIII



Formula XIV



Formula XV



Formula XVI

wherein Nuc represents a nucleobase selected from the group consisting of adenine, uracil, guanine, thymine, and cytosine, and R represents an optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, phenyl, benzyl, a cation (e.g., a monovalent cation), or hydrogen. In

some embodiments, the nucleobase is an adenine, uracil, guanine, thymine, or cytosine.

In some embodiments, the 5' phosphorus stabilizing moiety is (E)-vinylphosphonate represented by Formula XI.

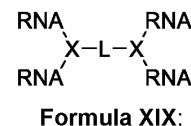
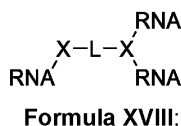
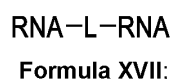
In some embodiments of any of the foregoing aspects, the siRNA molecule further includes a hydrophobic moiety at the 5' or the 3' end of the siRNA molecule. In some embodiments, the

hydrophobic moiety is selected from a group consisting of cholesterol, vitamin D, or tocopherol.

In some embodiments of any of the foregoing aspects, the length of the sense strand is between 12 and 30 nucleotides (e.g., 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides).

In some embodiments of any of the foregoing aspects, the siRNA molecule is a branched siRNA molecule. In some embodiments, the branched siRNA molecule is di-branched, tri-branched, or tetra-branched.

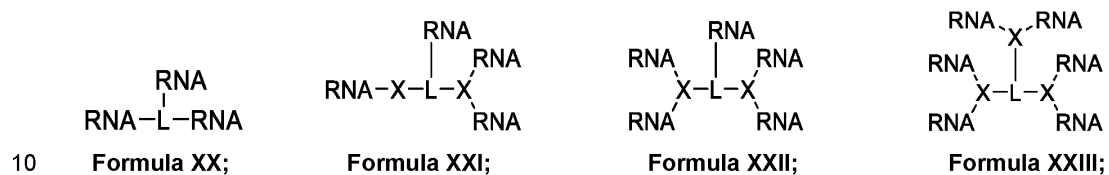
In some embodiments, the siRNA molecule is a di-branched siRNA molecule, optionally wherein the di-branched siRNA molecule is represented by any one of Formulas XVII-XIX:



wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

In some embodiments, the di-branched siRNA molecule is represented by Formula XVII. In some embodiments, the di-branched siRNA molecule is represented by Formula XVIII. In some
5 embodiments, the di-branched siRNA molecule is represented by Formula XIX.

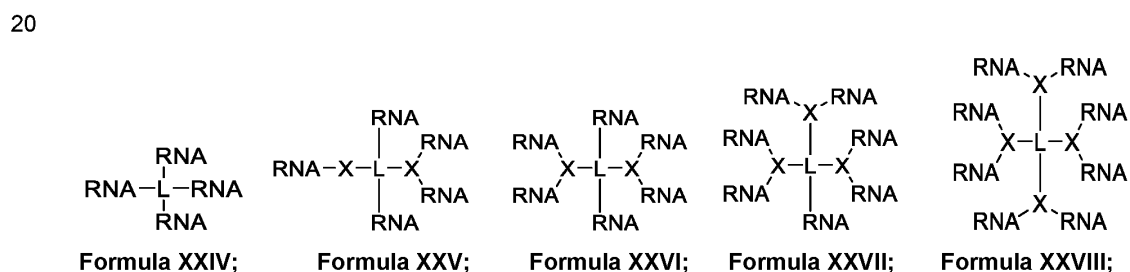
In some embodiments, the siRNA molecule is a tri-branched siRNA molecule, optionally wherein the tri-branched siRNA molecule is represented by any one of Formulas XX-XXIII:



wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

In some embodiments, the tri-branched siRNA molecule is represented by Formula XX. In
15 some embodiments, the tri-branched siRNA molecule is represented by Formula XXI. In some embodiments, the tri-branched siRNA molecule is represented by Formula XXII. In some embodiments, the tri-branched siRNA molecule is represented by Formula XXIII.

In some embodiments, the siRNA molecule is a tetra-branched siRNA molecule, optionally wherein the tetra-branched siRNA molecule is represented by any one of Formulas XXIV-XXVIII:



25 wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

In some embodiments, the tetra-branched siRNA molecule is represented by Formula XXIV. In some embodiments, the tetra-branched siRNA molecule is represented by Formula XXV. In some
30 embodiments, the tetra-branched siRNA molecule is represented by Formula XXVI. In some embodiments, the tetra-branched siRNA molecule is represented by Formula XXVII. In some embodiments, the tetra-branched siRNA molecule is represented by Formula XXVIII.

In some embodiments of the branched siRNA, the linker is selected from a group consisting of one or more contiguous subunits of an ethylene glycol (e.g., polyethylene glycol (PEG), such as, e.g., triethylene glycol (TrEG) or tetraethylene glycol (TEG)), alkyl, carbohydrate, block copolymer, peptide, RNA, and DNA.

In some embodiments, the linker is an ethylene glycol oligomer. In some embodiments, the linker is an alkyl oligomer. In some embodiments, the linker is a carbohydrate oligomer. In some embodiments, the linker is a block copolymer. In some embodiments, the linker is a peptide oligomer. In some embodiments, the linker is an RNA oligomer. In some embodiments, the linker is a DNA oligomer.

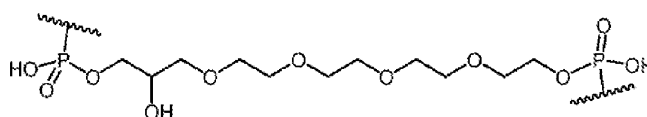
In some embodiments, the ethylene glycol oligomer is a PEG. In some embodiments, the PEG is a TrEG. In some embodiments, the PEG is a TEG.

In some embodiments, the oligomer or copolymer contains 2 to 20 contiguous subunits (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous subunits).

In some embodiments, the linker attaches one or more (e.g., 1, 2, 3, 4, or more) siRNA molecules by way of a covalent bond-forming moiety.

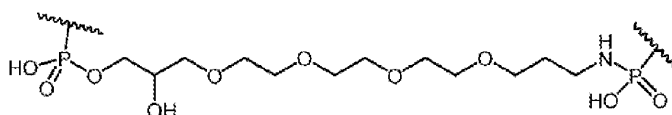
In some embodiments, the covalent bond-forming moiety is selected from the group consisting of an alkyl, ester, amide, carbamate, phosphonate, phosphate, phosphorothioate, phosphoroamidate, triazole, urea, and formacetal.

In some embodiments, the linker includes a structure of Formula L1:



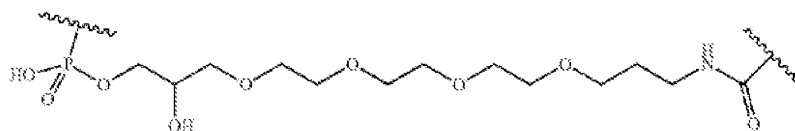
(Formula L1)

In some embodiments, the linker includes a structure of Formula L2:



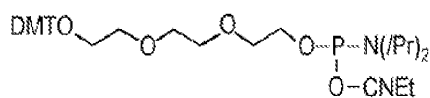
(Formula L2)

In some embodiments, the linker includes a structure of Formula L3:



(Formula L3)

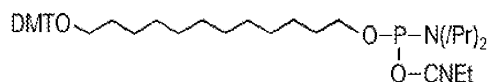
In some embodiments, the linker includes a structure of Formula L4:



(Formula L4)

In some embodiments, the linker includes a structure of Formula L5:

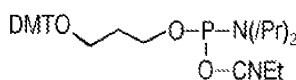
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(Formula L5)

In some embodiments, the linker includes a structure of Formula L6:

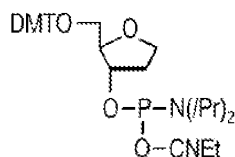
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(Formula L6)

In some embodiments, the linker includes a structure of Formula L7:

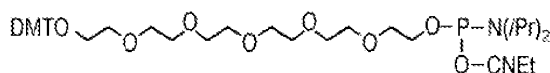
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(Formula L7)

In some embodiments, the linker includes a structure of Formula L8:

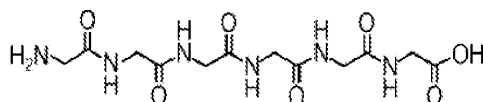
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(Formula L8)

In some embodiments, the linker includes a structure of Formula L9:

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(Formula L9)

In some embodiments of any of the siRNA molecules described herein, the siRNA is formulated as a salt containing one or more divalent cations. The siRNA molecule may contain a

plurality of cationic binding sites that are partially or fully saturated by the one or more divalent cations.

In some embodiments of the divalent cation salts, the degree of saturation of the cationic binding sites by the one or more divalent cations is from about 10% to about 100% (e.g., from about 20% to about 100%, from about 30% to about 100%, from about 40% to about 100%, from about 50% to about 100%, from about 60% to about 100%, from about 70% to about 100%, from about 80% to about 100%, or from about 90% to about 100%).

In some embodiments of the divalent cation salts, the cationic binding site is located within an internucleoside linkage, such as a phosphodiester linkage and/or a phosphorothioate linkage. For example, the cationic binding site may be an oxyanion moiety within a phosphodiester linkage or phosphorothioate linkage.

In some embodiments of the divalent cation salts, the one or more divalent cations are characterized as having an ionic radius ranging from about 30 picometers to about 150 picometers (e.g., from about 30 picometers to about 140 picometers, from about 40 picometers to about 130 picometers, from about 50 picometers to about 120 picometers, from about 60 picometers to about 110 picometers, from about 60 picometers to about 100 picometers, or from about 60 picometers to about 90 picometers).

In some embodiments of the divalent cation salts, the one or more divalent cations include a hard Lewis acid. In some embodiments, the one or more divalent cations includes Ba^{2+} , Be^{2+} , Ca^{2+} , Cu^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , or Zn^{2+} , or a combination thereof.

In some embodiments of the divalent cation salts, the one or more divalent cations includes Ba^{2+} . In some embodiments, the one or more divalent cations includes Be^{2+} . In some embodiments, the one or more divalent cations includes Ca^{2+} . In some embodiments, the one or more divalent cations includes Cu^{2+} . In some embodiments, the one or more divalent cations includes Mg^{2+} . In some embodiments, the one or more divalent cations includes Mn^{2+} . In some embodiments, the one or more divalent cations includes Ni^{2+} . In some embodiments, the one or more divalent cations includes Zn^{2+} .

In some embodiments of the divalent cation salts, the one or more divalent cations includes Ca^{2+} and Mg^{2+} , optionally wherein the ratio of Ca^{2+} to Mg^{2+} is from 1:100 to 100:1 (e.g., 1:75, 1:50, 1:25, 1:10, 1:5, 1:1, 5:1, 10:1, 25:1, 50:1, 75:1, or 100:1). In some embodiments, the Ca^{2+} and Mg^{2+} are present in a 1:1 ratio.

In some embodiments of the divalent cation salts, the one or more divalent cations displace water from a cationic binding site of the siRNA molecule.

In some embodiments of the divalent cation salts, the siRNA molecule includes one or more atoms having a negative charge and the divalent cation comprises two positive charges. In some embodiments, the ratio of negative charge to positive charge is from 0.75 to 7.5 (e.g., 0.76, 0.77, 0.78, 0.79, 0.80, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3,

7.4, or 7.5). In some embodiments, the ratio of negative charge to positive charge is from 1.0 to 2.0 (e.g., from 1.0 to 1.9, from 1.0 to 1.8, from 1.0 to 1.7, from 1.0 to 1.6, from 1.0 to 1.5, from 1.0 to 1.4, from 1.0 to 1.3, from 1.0 to 1.2, from 1.0 to 1.1, from 1.1 to 2.0, from 1.2 to 2.0, from 1.3 to 2.0, from 1.4 to 2.0, from 1.5 to 2.0, from 1.6 to 2.0, from 1.7 to 2.0, from 1.8 to 2.0, or from 1.9 to 2.0). In some
 5 embodiments, the ratio of negative charge to positive charge is from 0.75 to 6.5 (e.g., from 0.75 to 5.5, from 0.75 to 4.5, from 0.75 to 3.5, from 0.75 to 2.5, from 0.75 to 1.5, or from 0.75 to 1). In some
 embodiments, the ratio of negative charge to positive charge is from 1 to 7.5 (e.g., from 1.5 to 7.5, from 2.5 to 7.5, from 3.5 to 7.5, from 4.5 to 7.5, from 5.5 to 7.5, or from 6.5 to 7.5). In some
 embodiments, the molar ratio of siRNA molecule to divalent cation is from 1:10 to 1:100 (e.g., from
 10 1:10 to 1:50, from 1:18 to 1:38, from 1:20 to 1:25, 1:25, or 1:20). In some embodiments, the
 concentration of the one or more divalent cations is from 10 mM to 150 mM (e.g., from 20 mM to 150
 mM, from 20 mM to 100 mM, from 25 mM to 150 mM, from 25 mM to 100 mM, from 30 mM to 90 mM,
 from 35 mM to 85 mM, from 35 mM to 75 mM, from 40 mM to 70 mM, from 40 mM to 65 mM, from 40
 mM to 60 mM, or from 40 mM to 50 mM).

15 In some embodiments of any of the siRNA molecules described herein, 50% or more of the
 ribonucleotides in the antisense strand are 2'-O-Me ribonucleotides (e.g., 50%, 51%, 52%, 53%, 54%,
 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%,
 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%,
 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the ribonucleotides in the
 20 antisense strand may be 2'-O-Me ribonucleotides).

In some embodiments, 60% or more of the ribonucleotides in the antisense strand are 2'-O-
 Me ribonucleotides (e.g., 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%,
 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%,
 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the ribonucleotides in the
 25 antisense strand may be 2'-O-Me ribonucleotides).

In some embodiments, 70% or more of the ribonucleotides in the antisense strand are 2'-O-
 Me ribonucleotides (e.g., 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%,
 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or
 100% of the ribonucleotides in the antisense strand may be 2'-O-Me ribonucleotides).

30 In some embodiments, 80% or more of the ribonucleotides in the antisense strand are 2'-O-
 Me ribonucleotides (e.g., 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,
 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the ribonucleotides in the antisense strand may
 be 2'-O-Me ribonucleotides).

In some embodiments, 90% or more of the ribonucleotides in the antisense strand are 2'-O-
 35 Me ribonucleotides (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the
 ribonucleotides in the antisense strand may be 2'-O-Me ribonucleotides).

In some embodiments, 10% or less of the internucleoside linkages are phosphodiester
 linkages or phosphorothioate linkages. In some embodiments, at least 10%, 20%, 30%, 40%, 50%,
 60%, 70%, 80%, or 90% of the internucleoside linkages are phosphodiester linkages or

phosphorothioate linkages. In some embodiments, 100% of the internucleoside linkages are phosphodiester linkages or phosphorothioate linkages.

In some embodiments, 9 internucleoside linkages are phosphodiester linkages or phosphorothioate linkages.

5 In some embodiments, the length of the antisense strand is between 10 and 30 nucleotides (e.g., 10 nucleotides, 11 nucleotides, 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22
10 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides), 15 and 25 nucleotides (e.g., 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22
15 nucleotides, 23 nucleotides, 24 nucleotides, or 25 nucleotides), or 18 and 23 nucleotides (e.g., 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, or 23 nucleotides). In some embodiments, the length of the antisense strand is 20 nucleotides. In some embodiments, the length of the antisense strand is 21 nucleotides. In some embodiments, the length of the antisense
20 strand is 22 nucleotides. In some embodiments, the length of the antisense strand is 23 nucleotides. In some embodiments, the length of the antisense strand is 24 nucleotides. In some embodiments, the length of the antisense strand is 25 nucleotides. In some embodiments, the length of the antisense strand is 26 nucleotides. In some embodiments, the length of the antisense strand is 27 nucleotides. In some embodiments, the length of the antisense strand is 28 nucleotides. In some
embodiments, the length of the antisense strand is 29 nucleotides. In some embodiments, the length of the antisense strand is 30 nucleotides.

In some embodiments, the siRNA molecules of the branched compound are joined to one another by way of a linker (e.g., an ethylene glycol oligomer, such as tetraethylene glycol). In some
25 embodiments, the siRNA molecules of the branched compound are joined to one another by way of a linker between the sense strand of one siRNA molecule and the sense strand of the other siRNA molecule. In some embodiments, the siRNA molecules are joined by way of linkers between the antisense strand of one siRNA molecule and the antisense strand of the other siRNA molecule. In some
30 embodiments, the siRNA molecules of the branched compound are joined to one another by way of a linker between the sense strand of one siRNA molecule and the antisense strand of the other siRNA molecule.

In some embodiments, the length of the sense strand is between 12 and 30 nucleotides (e.g., 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18
35 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides), or 14 and 18 nucleotides (e.g., 14 nucleotides, 15 nucleotides, 16 nucleotides, 17
nucleotides, or 18 nucleotides). In some embodiments, the length of the sense strand is 15 nucleotides. In some embodiments, the length of the sense strand is 16 nucleotides. In some
40 embodiments, the length of the sense strand is 17 nucleotides. In some embodiments, the length of the sense strand is 18 nucleotides. In some embodiments, the length of the sense strand is 19 nucleotides. In some embodiments, the length of the sense strand is 20 nucleotides. In some

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[illegible]

[illegible]

[illegible]

the antisense strand is 29 nucleotides in length and the sense strand is 27 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 28 nucleotides in length. In some embodiments, the antisense strand is 29 nucleotides in length and the sense strand is 29 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 22 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 23 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 24 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 25 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 26 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 27 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 28 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 29 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 30 nucleotides in length.

In another aspect, the disclosure provides a pharmaceutical composition including the siRNA molecule of any of the preceding aspects or embodiments of the disclosure and a pharmaceutically acceptable excipient, carrier, or diluent.

In yet another aspect, the disclosure provides a method of delivering an siRNA molecule to a subject diagnosed as having Huntington's Disease, the method including administering a therapeutically effective amount of the siRNA molecule or the pharmaceutical composition of any of the preceding aspects or embodiments of the disclosure to the subject.

In still another aspect, the disclosure provides a method of treating Huntington's Disease in a subject in need thereof, the method including administering a therapeutically effective amount of the siRNA molecule or the pharmaceutical composition of any of the preceding aspects or embodiments of the disclosure to the subject.

In a further aspect, the disclosure provides a method of reducing *HTT* expression in a subject in need thereof, the method including administering a therapeutically effective amount of the siRNA molecule or the pharmaceutical composition of any of the preceding aspects or embodiments of the disclosure to the subject.

In some embodiments of the methods described herein, the siRNA molecule or the pharmaceutical composition is administered to the subject by way of intracerebroventricular, intrastratial, intraparenchymal, or intrathecal injection. In some embodiments, the siRNA molecule or the pharmaceutical composition is administered to the subject by way of intravenous, intramuscular, or subcutaneous injection.

In some embodiments, the siRNA molecule is administered to the subject in the form of an aqueous solution or in the form of a suspension. The siRNA molecule may be administered to the subject systemically or directly to the subject's central nervous system (CNS). For example, the siRNA molecule may be administered to the subject's cerebral spinal fluid (CSF), spinal cord, brain parenchyma, cortex, cerebellum, basal ganglia, caudate, putamen, thalamus, globus pallidus, substantia nigra, or another brain structure. In some embodiments, the siRNA molecule is administered intrathecally, intracerebroventricularly, intrastratially, or by intra-cisterna magna injection via catheterization. In some embodiments, the siRNA molecule is administered intrastratially. In some embodiments, the siRNA molecule is administered intrathecally. In some embodiments, the siRNA molecule is administered intracerebroventricularly.

In some embodiments of the methods described herein, the subject is a human.

In another aspect, the disclosure provides a kit including the siRNA molecule or the pharmaceutical composition of any of the preceding aspects or embodiments of the disclosure, and a package insert, wherein the package insert instructs a user of the kit to perform the method of any of the preceding aspects or embodiments of the disclosure.

Brief Description of the Drawings

FIGS. 1A and 1B show the knockdown of *HTT* mRNA (FIG. 1A) and the accumulation of an siRNA molecule (FIG. 1B) in different brain regions after 3 days in mice that were treated with siRNA molecules of the disclosure. The x axis of each graph shows the patterns of modifications of the antisense strand and sense strand and the amount of siRNA molecule that was administered. Three bars are shown for each condition tested, representing, from left to right, the results in the motor cortex, striatum, and hippocampus, respectively.

FIGS. 2A and 2B show the knockdown of *HTT* mRNA (FIG. 2A) and the accumulation of an siRNA molecule (FIG. 2B) in different brain regions after 7 days in mice that were treated with siRNA molecules of the disclosure. The x axis of each graph shows the patterns of modifications of the antisense strand and sense strand and the amount of siRNA molecule that was administered. Three bars are shown for each condition tested, representing, from left to right, the results in the motor cortex, striatum, and hippocampus, respectively.

FIGS. 3A and 3B show the knockdown of *HTT* mRNA (FIG. 3A) and the accumulation of an siRNA molecule (FIG. 3B) in different brain regions after 14 days in mice that were treated with siRNA molecules of the disclosure. The x axis of each graph shows the patterns of modifications of the antisense strand and sense strand and the amount of siRNA molecule that was administered. Four bars are shown for each condition tested, representing, from left to right, the results in the frontal cortex, motor cortex, striatum, and hippocampus, respectively.

FIGS. 4A and 4B show the knockdown of *HTT* mRNA (FIG. 4A) and the accumulation of an siRNA molecule (FIG. 4B) in different brain regions after 1 month in mice that were treated with siRNA molecules of the disclosure. The x axis of each graph shows the patterns of modifications of the antisense strand and sense strand and the amount of siRNA molecule that was administered. Four bars are shown for each condition tested, representing, from left to right, the results in the frontal cortex, motor cortex, striatum, and hippocampus, respectively.

FIGS. 5A and 5B show the knockdown of *HTT* mRNA (FIG. 5A) and the accumulation of an siRNA molecule (FIG. 5B) in different brain regions after 2 months in mice that were treated with siRNA molecules of the disclosure. The x axis of each graph shows the patterns of modifications of the antisense strand and sense strand and the amount of siRNA molecule that was administered. Four bars are shown for each condition tested, representing, from left to right, the results in the frontal cortex, motor cortex, striatum, and hippocampus, respectively.

FIGS. 6A and 6B show the knockdown of *HTT* mRNA (FIG. 6A) and the accumulation of an siRNA molecule (FIG. 6B) in different brain regions after 3 months in mice that were treated with siRNA molecules of the disclosure. The x axis of each graph shows the patterns of modifications of the antisense strand and sense strand and the amount of siRNA molecule that was administered. Four bars are shown for each condition tested, representing, from left to right, the results in the frontal cortex, motor cortex, striatum, and hippocampus, respectively.

FIGS. 7A-7D show the results over time in mice that were treated with siRNA molecules of the disclosure. The figures show *HTT* mRNA knockdown (FIG. 7A) and accumulation of the siRNA molecule (FIG. 7B) in mice that were treated with 2.5nmol of the siRNA molecule. Also shown is *HTT* mRNA knockdown (FIG. 7C) and accumulation of the siRNA molecule (FIG. 7D) in mice that were treated with 0.25nmol of the siRNA molecule. The x axis of each graph shows the patterns of modifications of the antisense strand and sense strand and the brain region tested for each condition. In FIGS. 7A, 7B, and 7C, the data for the frontal cortex has four bars which, from left to right, correspond to the results after 14 days, 1 month, 2 months, and 3 months, respectively. For the other brain regions, each bar, from left to right, correspond to the results after 3 days, 7 days, 14 days, 1 month, 2 months, and 3 months, respectively. In FIG. 7D, the data for the frontal cortex when treated with PBS and antisense A2/sense S2 has five bars, which, from left to right, correspond to the results after 7 days, 14 days, 1 month, 2 months, and 3 months, respectively. The data for the frontal cortex when treated with antisense A3/sense S6 has four bars, which, from left to right, correspond to the results after 14 days, 1 month, 2 months, and 3 months, respectively. The rest of the data in FIG. 7D each has 6 bars, which, from left to right, correspond to the results after 3 days, 7 days, 14 days, 1 month, 2 months, and 3 months, respectively.

FIGS. 8A and 8B show the knockdown of *HTT* mRNA (FIG. 8A) and the accumulation of an siRNA molecule (FIG. 8B) in different brain regions after 6 months in mice that were treated with siRNA molecules of the disclosure. The x axis of each graph shows the patterns of modifications of the antisense strand and sense strand and the amount of siRNA molecule that was administered. Four bars are shown for each condition tested, representing, from left to right, the results in the frontal cortex, motor cortex, striatum, and hippocampus, respectively.

FIGS. 9A-9F show the expression of *HTT* mRNA, HTT protein, and amount of siRNA in mice that were treated with an siRNA molecule of the disclosure. The siRNA molecule had an antisense strand of Formula A2 and a sense strand of Formula S1. The results were analyzed in mice treated with 2.5 nmol of the siRNA molecule in the motor cortex (FIG. 9A), hippocampus (FIG. 9B), and striatum (FIG. 9C). The results were also analyzed in mice treated with 0.25 nmol of the siRNA molecule in the motor cortex (FIG. 9D), hippocampus (FIG. 9E), and striatum (FIG. 9F).

FIGS. 10A and 10B show the expression of *HTT* mRNA in mice treated with an siRNA molecule of the disclosure in the kidney (FIG. 10A) and liver (FIG. 10B) over time relative to a PBS control. The siRNA molecule had an antisense strand of Formula A2 and a sense strand of Formula S1.

FIG. 11 shows the knockdown of *HTT* mRNA in different brain regions after 6 months in mice that were treated with 5nmol of siRNA molecules of the disclosure. The x axis of each graph shows the patterns of modifications of the antisense strand and sense strand and the amount of siRNA molecule that was administered. Four bars are shown for each condition tested, representing, from left to right, the results in the frontal cortex, motor cortex, striatum, and hippocampus, respectively.

FIGS. 12A-12C show the knockdown of *HTT* mRNA (FIG. 12A), HTT protein (FIG. 12B), and the accumulation of an siRNA molecule (FIG. 12C) in various brain regions of non-human primates that were treated with an siRNA molecule of the disclosure. The x axis gives the amount administered, the route of administration (IT = intrathecal; ICV = intracerebroventricular), and the brain region tested (fCtx = frontal cortex, mCtx = motor cortex, tCtx = temporal cortex, Hp = hippocampus, Put = putamen, Cd = Caudate, SN = substantia nigra, Pons, Med = medulla).

FIGS. 13A-13C show the knockdown of *HTT* mRNA (FIG. 13A), HTT protein (FIG. 13B), and the accumulation of an siRNA molecule (FIG. 13C) in the cerebellum cortex of non-human primates that were treated with an siRNA molecule of the disclosure. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular). "Ionic conditioning" means the siRNA molecule was formulated as a salt.

FIGS. 13D-13F show the knockdown of *HTT* mRNA (FIG. 13D), HTT protein (FIG. 13E), and the accumulation of an siRNA molecule (FIG. 13F) in the cerebellum deep nucleus of non-human primates that were treated with an siRNA molecule of the disclosure. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular). "Ionic conditioning" means the siRNA molecule was formulated as a salt.

FIGS. 14A-14C show the knockdown of *HTT* mRNA (FIG. 14A), HTT protein (FIG. 14B), and the accumulation of an siRNA molecule (FIG. 14C) in the liver of non-human primates that were treated with an siRNA molecule of the disclosure. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular). "IC" means the siRNA molecule was formulated as a salt.

FIGS. 15A-15C show the knockdown of *HTT* mRNA (FIG. 15A), HTT protein (FIG. 15B), and the accumulation of an siRNA molecule (FIG. 15C) in the kidney of non-human primates that were treated with an siRNA molecule of the disclosure. The x axis gives the amount administered and the

route of administration (IT = intrathecal; ICV = intracerebroventricular). "IC" means the siRNA molecule was formulated as a salt.

FIGS. 16A-16C show the knockdown of *HTT* mRNA (FIG. 16A), HTT protein (FIG. 16B), and the accumulation of an siRNA molecule (FIG. 16C) in the spinal cord of non-human primates that were treated with an siRNA molecule of the disclosure. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular). "Ionic conditioning" means the siRNA molecule was formulated as a salt.

FIGS. 17A and 17B show the concentration of siRNA molecule in the plasma (FIG. 17A) and CSF (FIG. 17B) of non-human primates treated with an siRNA molecule of the disclosure for animals in Group 2 as defined in Table 3, below.

FIGS. 17C and 17D show the concentration of siRNA molecule in the plasma (FIG. 17C) and CSF (FIG. 17C) of non-human primates treated with an siRNA molecule of the disclosure for animals in Group 3 as defined in Table 3, below.

FIGS. 17E and 17F show the concentration of siRNA molecule in the plasma (FIG. 17E) and CSF (FIG. 17F) of non-human primates treated with an siRNA molecule of the disclosure for animals in Group 4 as defined in Table 3, below.

FIGS. 17G and 17H show the concentration of siRNA molecule in the plasma (FIG. 17G) and CSF (FIG. 17H) of non-human primates treated with an siRNA molecule of the disclosure for animals in Group 6 as defined in Table 3, below.

FIGS. 17I and 17J show the concentration of siRNA molecule in the plasma (FIG. 17I) and CSF (FIG. 17J) of non-human primates treated with an siRNA molecule of the disclosure for animals in Group 7 as defined in Table 3, below.

FIGS. 17K and 17L show the concentration of siRNA molecule in the plasma (FIG. 17K) and CSF (FIG. 17L) of non-human primates treated with an siRNA molecule of the disclosure for animals in Group 8 as defined in Table 3, below.

FIGS. 17M and 17N show the concentration of siRNA molecule in the plasma (FIG. 17M) and CSF (FIG. 17N) of non-human primates treated with an siRNA molecule of the disclosure for animals in Group 5 as defined in Table 3, below.

FIGS. 18A-18C show the knockdown of *HTT* mRNA (FIG. 18A), HTT protein (FIG. 18B), and the accumulation of an siRNA molecule (FIG. 18C) in the frontal cortex of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 19A-19C show the knockdown of *HTT* mRNA (FIG. 19A), HTT protein (FIG. 19B), and the accumulation of an siRNA molecule (FIG. 19C) in the motor cortex of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 20A-20C show the knockdown of *HTT* mRNA (FIG. 20A), HTT protein (FIG. 20B), and the accumulation of an siRNA molecule (FIG. 20C) in the temporal cortex of non-human primates that

were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 21A-21C show the knockdown of *HTT* mRNA (FIG. 21A), HTT protein (FIG. 21B), and the accumulation of an siRNA molecule (FIG. 21C) in the hippocampus of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 22A-22C show the knockdown of *HTT* mRNA (FIG. 22A), HTT protein (FIG. 22B), and the accumulation of an siRNA molecule (FIG. 22C) in the putamen of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 23A-23C show the knockdown of *HTT* mRNA (FIG. 23A), HTT protein (FIG. 23B), and the accumulation of an siRNA molecule (FIG. 23C) in the caudate of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 24A-24C show the knockdown of *HTT* mRNA (FIG. 24A), HTT protein (FIG. 24B), and the accumulation of an siRNA molecule (FIG. 24C) in the substantia nigra of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 25A-25C show the knockdown of *HTT* mRNA (FIG. 25A), HTT protein (FIG. 25B), and the accumulation of an siRNA molecule (FIG. 25C) in the pons of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 26A-26C show the knockdown of *HTT* mRNA (FIG. 26A), HTT protein (FIG. 26B), and the accumulation of an siRNA molecule (FIG. 26C) in the medulla of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 27A-27C show the knockdown of *HTT* mRNA (FIG. 27A), HTT protein (FIG. 27B), and the accumulation of an siRNA molecule (FIG. 27C) in the liver of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 28A-28C show the knockdown of *HTT* mRNA (FIG. 28A), HTT protein (FIG. 28B), and the accumulation of an siRNA molecule (FIG. 28C) in the kidney of non-human primates that were

treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 29A and 29B show the knockdown of *HTT* mRNA (FIG. 29A) and HTT protein (FIG. 29B) in the spinal cord of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The values were measured in the lumbar spinal cord, thoracic spinal cord, and cervical spinal cord. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIG. 30 shows the knockdown of *HTT* mRNA in the cerebellar cortex of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIG. 31 shows the knockdown of *HTT* mRNA in the deep nucleus of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 4, below. The x axis gives the amount administered and the route of administration (IT = intrathecal; ICV = intracerebroventricular).

FIGS. 32A-32D show the accumulation of an siRNA molecule in the plasma and CSF of non-human primates that were administered siRNA molecules of the disclosure as described in Example 4, below. The results are shown for animals in group 2 in Table 4 below (FIG. 32A), group 4 in Table 4 below (FIG. 32B), group 3 in Table 4 below (FIG. 32C), and group 5 in Table 4 below (FIG. 32D).

FIGS. 33A-33C show the knockdown of *HTT* mRNA (FIG. 33A), HTT protein (FIG. 33B), and the accumulation of an siRNA molecule (FIG. 33C) in the frontal cortex of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below. These figures depict data from all animals, including animals for which poor CSF flow was observed at the time of dosing. The x axis gives the amount administered by intrathecal injection.

FIGS. 34A-34C show the knockdown of *HTT* mRNA (FIG. 34A), HTT protein (FIG. 34B), and the accumulation of an siRNA molecule (FIG. 34C) in the motor cortex of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below. These figures depict data from all animals, including animals for which poor CSF flow was observed at the time of dosing. The x axis gives the amount administered by intrathecal injection.

FIGS. 35A-35C show the knockdown of *HTT* mRNA (FIG. 35A), HTT protein (FIG. 35B), and the accumulation of an siRNA molecule (FIG. 35C) in the temporal cortex of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below. These figures depict data from all animals, including animals for which poor CSF flow was observed at the time of dosing. The x axis gives the amount administered by intrathecal injection.

FIGS. 36A-36C show the knockdown of *HTT* mRNA (FIG. 36A), HTT protein (FIG. 36B), and the accumulation of an siRNA molecule (FIG. 36C) in the caudate of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below. These figures depict data from all animals, including animals for which poor CSF flow was observed at the time of dosing. The x axis gives the amount administered by intrathecal injection.

FIGS. 37A-37C show the knockdown of *HTT* mRNA (FIG. 37A), HTT protein (FIG. 37B), and the accumulation of an siRNA molecule (FIG. 37C) in the putamen of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below. These figures depict data from all animals, including animals for which poor CSF flow was observed at the time of dosing. The x axis gives the amount administered by intrathecal injection.

FIGS. 38A-38C show the knockdown of *HTT* mRNA (FIG. 38A), HTT protein (FIG. 38B), and the accumulation of an siRNA molecule (FIG. 38C) in the hippocampus of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below. These figures depict data from all animals, including animals for which poor CSF flow was observed at the time of dosing. The x axis gives the amount administered by intrathecal injection.

FIGS. 39A-39C show the knockdown of *HTT* mRNA (FIG. 39A), HTT protein (FIG. 39B), and the accumulation of an siRNA molecule (FIG. 39C) in the cervical spinal cord of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below. These figures depict data from all animals, including animals for which poor CSF flow was observed at the time of dosing. The x axis gives the amount administered by intrathecal injection.

FIGS. 40A-40C show the knockdown of *HTT* mRNA (FIG. 40A), HTT protein (FIG. 40B), and the accumulation of an siRNA molecule (FIG. 40C) in the thoracic spinal cord of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below. These figures depict data from all animals, including animals for which poor CSF flow was observed at the time of dosing. The x axis gives the amount administered by intrathecal injection.

FIGS. 41A-41C show the knockdown of *HTT* mRNA (FIG. 41A), HTT protein (FIG. 41B), and the accumulation of an siRNA molecule (FIG. 41C) in the lumbar spinal cord of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below. These figures depict data from all animals, including animals for which poor CSF flow was observed at the time of dosing. The x axis gives the amount administered by intrathecal injection.

FIGS. 42A-42C show the knockdown of *HTT* mRNA (FIG. 42A), HTT protein (FIG. 42B), and the accumulation of an siRNA molecule (FIG. 42C) in the liver of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below. These figures depict data from all animals, including animals for which poor CSF flow was observed at the time of dosing. The x axis gives the amount administered by intrathecal injection.

FIGS. 43A-43C show the knockdown of *HTT* mRNA (FIG. 43A), HTT protein (FIG. 43B), and the accumulation of an siRNA molecule (FIG. 43C) in the kidney of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below. These figures depict data from all animals, including animals for which poor CSF flow was observed at the time of dosing. The x axis gives the amount administered by intrathecal injection.

FIGS. 44A-44C show the knockdown of *HTT* mRNA (FIG. 44A), HTT protein (FIG. 44B), and the accumulation of an siRNA molecule (FIG. 44C) in the frontal cortex of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below, wherein animals with obstructed catheters have been removed. The x axis gives the amount administered by intrathecal injection.

FIGS. 45A-45C show the knockdown of *HTT* mRNA (FIG. 45A), HTT protein (FIG. 45B), and the accumulation of an siRNA molecule (FIG. 45C) in the motor cortex of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below, wherein animals with obstructed catheters have been removed. The x axis gives the amount administered by intrathecal injection.

FIGS. 46A-46C show the knockdown of *HTT* mRNA (FIG. 46A), HTT protein (FIG. 46B), and the accumulation of an siRNA molecule (FIG. 46C) in the temporal cortex of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below, wherein animals with obstructed catheters have been removed. The x axis gives the amount administered by intrathecal injection.

FIGS. 47A-47C show the knockdown of *HTT* mRNA (FIG. 47A), HTT protein (FIG. 47B), and the accumulation of an siRNA molecule (FIG. 47C) in the caudate of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below, wherein animals with obstructed catheters have been removed. The x axis gives the amount administered by intrathecal injection.

FIGS. 48A-48C show the knockdown of *HTT* mRNA (FIG. 48A), HTT protein (FIG. 48B), and the accumulation of an siRNA molecule (FIG. 48C) in the putamen of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below, wherein animals with obstructed catheters have been removed. The x axis gives the amount administered by intrathecal injection.

FIGS. 49A-49C show the knockdown of *HTT* mRNA (FIG. 49A), HTT protein (FIG. 49B), and the accumulation of an siRNA molecule (FIG. 49C) in the hippocampus of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below, wherein animals with obstructed catheters have been removed. The x axis gives the amount administered by intrathecal injection.

FIGS. 50A-50C show the knockdown of *HTT* mRNA (FIG. 50A), HTT protein (FIG. 50B), and the accumulation of an siRNA molecule (FIG. 50C) in the cervical spinal cord of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below, wherein animals with obstructed catheters have been removed. The x axis gives the amount administered by intrathecal injection.

FIGS. 51A-51C show the knockdown of *HTT* mRNA (FIG. 51A), HTT protein (FIG. 51B), and the accumulation of an siRNA molecule (FIG. 51C) in the thoracic spinal cord of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below, wherein animals with obstructed catheters have been removed. The x axis gives the amount administered by intrathecal injection.

FIGS. 52A-52C show the knockdown of *HTT* mRNA (FIG. 52A), HTT protein (FIG. 52B), and the accumulation of an siRNA molecule (FIG. 52C) in the lumbar spinal cord of non-human primates that were treated with an siRNA molecule of the disclosure as described in Example 5, below, wherein animals with obstructed catheters have been removed. The x axis gives the amount administered by intrathecal injection.

Definitions

Unless otherwise defined herein, scientific, and technical terms used herein have the meanings that are commonly understood by those of ordinary skill in the art. In the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The use of "or" means "and/or" unless stated otherwise. The use of the term "including," as well as other forms, such as "includes" and "included," is not limiting.

As used herein, the term "nucleic acids" refers to RNA or DNA molecules consisting of a chain of ribonucleotides or deoxyribonucleotides, respectively.

As used herein, the term "therapeutic nucleic acid" refers to a nucleic acid molecule (e.g., ribonucleic acid) that has partial or complete complementarity to, and interacts with, a disease-associated target mRNA and mediates silencing of expression of the mRNA.

As used herein, the term "carrier nucleic acid" refers to a nucleic acid molecule (e.g., ribonucleic acid) that has sequence complementarity with, and hybridizes with, a therapeutic nucleic acid. As used herein, the term "3' end" refers to the end of the nucleic acid that contains an unmodified hydroxyl group at the 3' carbon of the ribose ring.

As used herein, the term "nucleoside" refers to a molecule made up of a heterocyclic base and its sugar.

As used herein, the term "nucleotide" refers to a nucleoside having a phosphate group, or a variant thereof, on its 3' or 5' sugar hydroxyl group. Examples of phosphate group variants include, but are not limited to, saturated alkyl phosphonates, unsaturated alkenyl phosphonates, phosphorothioates, and phosphoramidites.

In the context of this disclosure, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring (e.g., modified) portions that function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

As used herein, the term "siRNA" refers to small interfering RNA duplexes that induce the RNA interference (RNAi) pathway. siRNA molecules may vary in length (generally, between 10 and 30 base pairs) and may contain varying degrees of complementarity to their target mRNA. The term "siRNA" includes duplexes of two separate strands, as well as single strands that optionally form hairpin structures including a duplex region.

As used herein, the term "antisense strand" refers to the strand of the siRNA duplex that contains some degree of complementarity to the target gene.

As used herein, the term "sense strand" refers to the strand of the siRNA duplex that contains complementarity to the antisense strand.

As used herein, the term “divalent cation” refers to a positively charged ion (i.e., a cation) with a valence of 2+. Examples of divalent cations include, Ba²⁺, Be²⁺, Ca²⁺, Cu²⁺, Mg²⁺, Mn²⁺, Ni²⁺, or Zn²⁺. Because of their positive charge, divalent cations typically form ionic bonds with negatively charged atoms (e.g., oxyanion from a phosphate group or phosphorothioate group carrying a unit or partial negative charge).

As used herein, the terms “ionic radius” and “ionic radii” refer to the radius of one or more monoatomic ions (e.g., divalent cations) when measured in the form of its ionic crystal structure. The ionic radius is typically measured in units of picometers or angstroms.

As used herein, the term “salt” refers to any compound containing an ionic association between an anionic component (e.g., oxyanion from a phosphate group or phosphorothioate group carrying a unit or partial negative charge) and a cationic component (e.g., a divalent cation). Salts may have various physical forms. For example, a salt may be a solid, crystalline, ionic compound, or may be in the form of a solution in which the salt is dissolved in a solvent with which the salt’s constituent ions are miscible (e.g., water or another polar, protic solvent). Salts may also exist in suspension, such as a suspension formed by contacting (i) a homogenous solution containing the salt of interest and a first solvent with (ii) a second solvent that is not fully miscible with the first solvent. Examples of suspensions are those formed by contacting an aqueous solution containing a salt of interest with a solvent not fully miscible with water, such as an organic solvent containing one or more nonpolar functional groups. In the context of the disclosure, a “salt” includes oligonucleotides containing a plurality of cationic binding sites that are saturated by one or more divalent cations (e.g., Ba²⁺, Be²⁺, Ca²⁺, Cu²⁺, Mg²⁺, Mn²⁺, Ni²⁺, or Zn²⁺, or a combination thereof).

The term “cationic binding sites” refers to substituents in an siRNA molecule that carries either a partial negative charge or a unit negative charge (e.g., the oxyanion of a phosphate or phosphorothioate) and is capable of forming an ionic association with a cation (e.g., a divalent cation).

The term “degree of saturation” refers to the relative proportion of cationic binding sites that are ionically bound by a particular cationic species (e.g., a divalent cation).

The term “hard Lewis acid” refers to a chemical acid that is characterized by a low ionic radius, high positive charge density, strong ability to displace water, and high-energy lowest unoccupied molecular orbital (LUMO).

The term “interfering RNA molecule” refers to an RNA molecule, such as a small interfering RNA (siRNA), microRNA (miRNA), short hairpin RNA (shRNA), or an antisense oligonucleotide (ASO) that suppresses the endogenous function of a target RNA transcript.

As used herein, the terms “target,” “targeting,” and “targeted,” in the context of the design of an siRNA, refers to generating an antisense strand so as to anneal the antisense strand to a region within the mRNA transcript of interest in a manner that results in a reduction in translation of the mRNA into the protein product.

As used herein, the terms “express” and “expression” refer to one or more of the following events: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end processing); and (3) translation of an RNA into a polypeptide or protein. In the context of a gene that encodes a

protein product, the terms "gene expression" and the like are used interchangeably with the terms "protein expression" and the like. Expression of a gene or protein of interest in a patient can manifest, for example, by detecting: an increase in the quantity or concentration of mRNA encoding corresponding protein (as assessed, e.g., using RNA detection procedures described herein or known in the art, such as quantitative polymerase chain reaction (qPCR) and RNA seq techniques), an increase in the quantity or concentration of the corresponding protein (as assessed, e.g., using protein detection methods described herein or known in the art, such as enzyme-linked immunosorbent assays (ELISA), among others), and/or an increase in the activity of the corresponding protein (e.g., in the case of an enzyme, as assessed using an enzymatic activity assay described herein or known in the art) in a sample obtained from the patient. As used herein, a cell is considered to "express" a gene or protein of interest if one or more, or all, of the above events can be detected in the cell or in a medium in which the cell resides. For example, a gene or protein of interest is considered to be "expressed" by a cell or population of cells if one can detect (i) production of a corresponding RNA transcript, such as an mRNA template, by the cell or population of cells (e.g., using RNA detection procedures described herein); (ii) processing of the RNA transcript (e.g., splicing, editing, 5' cap formation, and/or 3' end processing, such as using RNA detection procedures described herein); (iii) translation of the RNA template into a protein product (e.g., using protein detection procedures described herein); and/or (iv) post-translational modification of the protein product (e.g., using protein detection procedures described herein).

As used herein, the terms "chemically modified nucleotide," "nucleotide analog," "altered nucleotide," and "modified nucleotide" refer to a non-standard nucleotide, including non-naturally occurring ribonucleotides or deoxyribonucleotides. Exemplary nucleotide analogs are modified at any position so as to alter certain chemical properties of the nucleotide yet retain the ability of the nucleotide analog to perform its intended function.

As used herein, the term "metabolically stabilized" refers to RNA molecules that contain ribonucleotides that have been chemically modified in order to decrease the rate of metabolism of an RNA molecule that is administered to a subject. Exemplary modifications include 2'-hydroxy to 2'-O-methoxy or 2'-fluoro, and phosphodiester to phosphorothioate.

As used herein, the term "phosphorothioate" refers to a phosphate group of a nucleotide that is modified by substituting one or more of the oxygens of the phosphate group with sulfur.

As used herein, the terms "internucleoside" and "internucleotide" refer to the bonds between nucleosides and nucleotides, respectively.

As used herein, the term "antagomirs" refers to nucleic acids that can function as inhibitors of miRNA activity.

As used herein, the term "gapmers" refers to chimeric antisense nucleic acids that contain a central block of deoxynucleotide monomers sufficiently long to induce RNase H cleavage. The deoxynucleotide block is flanked by ribonucleotide monomers or ribonucleotide monomers containing modifications.

As used herein, the term "mixmers" refers to nucleic acids that contain a mix of locked nucleic acids (LNAs) and DNA.

As used herein, the term "guide RNAs" refers to nucleic acids that have sequence complementarity to a specific sequence in the genome immediately or 1 base pair upstream of the protospacer adjacent motif (PAM) sequence as used in CRISPR/Cas9 gene editing systems. Alternatively, "guide RNAs" may refer to nucleic acids that have sequence complementarity (e.g., are antisense) to a specific messenger RNA (mRNA) sequence. In this context, a guide RNA may also have sequence complementarity to a "passenger RNA" sequence of equal or shorter length, which is identical or substantially identical to the sequence of mRNA to which the guide RNA hybridizes.

As used herein, the term "branched siRNA" refers to a compound containing two or more double-stranded siRNA molecules covalently bound to one another. Branched siRNA molecules may be "di-branched," also referred to herein as "di-siRNA," wherein the siRNA molecule includes 2 siRNA molecules covalently bound to one another, e.g., by way of a linker. Branched siRNA molecules may be "tri-branched," also referred to herein as "tri-siRNA," wherein the siRNA molecule includes 3 siRNA molecules covalently bound to one another, e.g., by way of a linker. Branched siRNA molecules may be "tetra-branched," also referred to herein as "tetra-siRNA," wherein the siRNA molecule includes 4 siRNA molecules covalently bound to one another, e.g., by way of a linker.

As used herein, the term "branch point moiety" refers to a chemical moiety of a branched siRNA structure of the disclosure that may be covalently linked to a 5' end or a 3' end of an antisense strand or a sense strand of an siRNA molecule and which may support the attachment of additional single- or double-stranded siRNA molecules. Non-limiting examples of branch point moieties suitable for use in conjunction with the disclosed methods and compositions include, e.g., phosphoroamidite, tosylated solketal, 1,3-diaminopropanol, pentaerythritol, and any one of the branch point moieties described in US 10,478,503.

The term "phosphate moiety" as used herein, refers to a terminal phosphate group that includes phosphates as well as modified phosphates. The phosphate moiety may be located at either terminus but is preferred at the 5'-terminal nucleoside. In one aspect, the terminal phosphate is unmodified having the formula —O—P(=O)(OH)OH . In another aspect, the terminal phosphate is modified such that one or more of the O and OH groups are replaced with H, O, S, N(R') or alkyl where R' is H, an amino protecting group or unsubstituted or substituted alkyl. In some embodiments, the 5' and or 3' terminal group may include from 1 to 3 phosphate moieties that are each, independently, unmodified (di- or tri-phosphates) or modified.

As used herein, the term "5' phosphorus stabilizing moiety" refers to a terminal phosphate group that includes phosphates as well as modified phosphates (e.g., phosphorothioates, phosphodiesteres, phosphonates). The phosphate moiety may be located at either terminus but is preferred at the 5'-terminal nucleoside. In one aspect, the terminal phosphate is unmodified having the formula —O—P(=O)(OH)OH . In another aspect, the terminal phosphate is modified such that one or more of the O and OH groups are replaced with H, O, S, N(R'), or alkyl where R' is H, an amino protecting group, or unsubstituted or substituted alkyl. In some embodiments, the 5' and or 3' terminal group may include from 1 to 3 phosphate moieties that are each, independently, unmodified (di- or tri-phosphates) or modified.

It is understood that certain internucleoside linkages provided herein, including, e.g., phosphodiester and phosphorothioate, include a formal charge of -1 at physiological pH, and that said formal charge will be balanced by a cationic moiety, e.g., an alkali metal such as sodium or potassium, an alkali earth metal such as calcium or magnesium, or an ammonium or guanidinium ion, or a plurality of divalent cations (e.g., Ba²⁺, Be²⁺, Ca²⁺, Cu²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Zn²⁺, or a combination thereof).

The phosphate group of the nucleotide may also be modified, e.g., by substituting one or more of the oxygens of the phosphate group with sulfur (e.g., phosphorothioates), or by making other substitutions which allow the nucleotide to perform its intended function such as described in, for example, Eckstein, *Antisense Nucleic Acid Drug Dev.* 10:117-21, 2000; Rusckowski et al., *Antisense Nucleic Acid Drug Dev.* 10:333-45, 2000; Stein, *Antisense Nucleic Acid Drug Dev.* 11:317-25, 2001; Vorobjev et al., *Antisense Nucleic Acid Drug Dev.* 11:77-85, 2001; and US 5,684,143. Certain of the above-referenced modifications (e.g., phosphate group modifications) preferably decrease the rate of hydrolysis of, for example, polynucleotides including said analogs in vivo or in vitro.

As used herein, the term "complementary" refers to two nucleotides that form canonical Watson-Crick base pairs. For the avoidance of doubt, Watson-Crick base pairs in the context of the present disclosure include adenine-thymine, adenine-uracil, and cytosine-guanine base pairs. A proper Watson-Crick base pair is referred to in this context as a "match," while each unpaired nucleotide, and each incorrectly paired nucleotide, is referred to as a "mismatch." Alignment for purposes of determining percent nucleic acid sequence complementarity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software.

"Percent (%) sequence complementarity" with respect to a reference polynucleotide sequence is defined as the percentage of nucleic acids in a candidate sequence that are complementary to the nucleic acids in the reference polynucleotide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence complementarity. A given nucleotide is considered to be "complementary" to a reference nucleotide as described herein if the two nucleotides form canonical Watson-Crick base pairs. For the avoidance of doubt, Watson-Crick base pairs in the context of the present disclosure include adenine-thymine, adenine-uracil, and cytosine-guanine base pairs. A proper Watson-Crick base pair is referred to in this context as a "match," while each unpaired nucleotide, and each incorrectly paired nucleotide, is referred to as a "mismatch." Alignment for purposes of determining percent nucleic acid sequence complementarity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal complementarity over the full length of the sequences being compared. As an illustration, the percent sequence complementarity of a given nucleic acid sequence, A, to a given nucleic acid sequence, B, (which can alternatively be phrased as a given nucleic acid sequence, A that has a certain percent complementarity to a given nucleic acid sequence, B) is calculated as follows:

100 multiplied by (the fraction X/Y)

where X is the number of complementary base pairs in an alignment (e.g., as executed by computer software, such as BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid sequence A is not equal to the length of nucleic acid sequence B, the percent sequence complementarity of A to B will not equal the percent sequence complementarity of B to A. As used herein, a query nucleic acid sequence is considered to be "completely complementary" to a reference nucleic acid sequence if the query nucleic acid sequence has 100% sequence complementarity to the reference nucleic acid sequence.

"Percent (%) sequence identity" with respect to a reference polynucleotide or polypeptide sequence is defined as the percentage of nucleic acids or amino acids in a candidate sequence that are identical to the nucleic acids or amino acids in the reference polynucleotide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent sequence identity values may be generated using the sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid or amino acid sequence, A, to, with, or against a given nucleic acid or amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid or amino acid sequence, A that has a certain percent sequence identity to, with, or against a given nucleic acid or amino acid sequence, B) is calculated as follows:

100 multiplied by (the fraction X/Y)

where X is the number of nucleotides or amino acids scored as identical matches by a sequence alignment program (e.g., BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid or amino acid sequence A is not equal to the length of nucleic acid or amino acid sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

The term "complementarity sufficient to hybridize," as used herein, refers to a nucleic acid sequence or a portion thereof that need not be fully complementary (e.g., 100% complementary) to a target region or a nucleic acid sequence or a portion thereof that has one or more nucleotide mismatches relative to the target region but that is still capable of hybridizing to the target region under specified conditions. For example, the nucleic acid may be, e.g., 95% complementary, 90%, complementary, 85% complementary, 80% complementary, 75% complementary, 70%

complementary, 65% complementary, 60% complementary, 55% complementary, 50% complementary, or less, but still form sufficient base pairs with the target so as to hybridize across a portion of equal length.

“Hybridization” or “annealing” of nucleic acids is achieved when one or more nucleoside residues within a polynucleotide base pairs with one or more complementary nucleosides to form a stable duplex. The base pairing is typically driven by hydrogen bonding events. Hybridization includes Watson-Crick base pairs formed from natural and/or modified nucleobases. The hybridization can also include non-Watson-Crick base pairs, such as wobble base pairs (guanosine-uracil, hypoxanthine-uracil, hypoxanthine-adenine, and hypoxanthine-cytosine) and Hoogsteen base pairs. Nucleic acids need not be 100% complementary to undergo hybridization. For example, one nucleic acid may be, e.g., 95% complementary, 90%, complementary, 85% complementary, 80% complementary, 75% complementary, 70% complementary, 65% complementary, 60% complementary, 55% complementary, 50% complementary, or less, relative to another nucleic acid, but the two nucleic acids may still form sufficient base pairs with one another so as to hybridize.

The “stable duplex” formed upon the annealing/hybridization of one nucleic acid to another is a duplex structure that is not denatured by a stringent wash. Exemplary stringent wash conditions are known in the art and include temperatures of about 5° C less than the melting temperature of an individual strand of the duplex and low concentrations of monovalent salts, such as monovalent salt concentrations (e.g., NaCl concentrations) of less than 0.2 M (e.g., 0.2 M, 0.19 M, 0.18 M, 0.17 M, 0.16 M, 0.15 M, 0.14 M, 0.13 M, 0.12 M, 0.11 M, 0.1 M, 0.09 M, 0.08 M, 0.07 M, 0.06 M, 0.05 M, 0.04 M, 0.03 M, 0.02 M, 0.01 M, or less).

The term “gene silencing” refers to the suppression of gene expression, e.g., endogenous gene expression of *HTT*, which may be mediated through processes that affect transcription and/or through processes that affect post-transcriptional mechanisms. In some embodiments, gene silencing occurs when an RNAi molecule initiates the inhibition or degradation of the mRNA transcribed from a gene of interest in a sequence-specific manner by way of RNA interference, thereby preventing translation of the gene's product.

The phrase “overactive disease driver gene,” as used herein, refers to a gene having increased activity and/or expression that contributes to or causes a disease state in a subject (e.g., a human). The disease state may be caused or exacerbated by the overactive disease driver gene directly or by way of an intermediate gene(s).

As used herein, the term “ethylene glycol chain” refers to a carbon chain with the formula $((\text{CH}_2\text{OH})_2)$.

As used herein, “alkyl” refers to a saturated hydrocarbon group. Alkyl groups may be acyclic or cyclic and contain only C and H when unsubstituted. When an alkyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be encompassed and described; thus, for example, “butyl” is meant to include *n*-butyl, *sec*-butyl, and *iso*-butyl. Examples of alkyl include ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and the like. In some embodiments, alkyl

may be substituted. Suitable substituents that may be introduced into an alkyl group include, for example, hydroxy, alkoxy, amino, alkylamino, and halo, among others.

As used herein, "alkenyl" refers to an acyclic or cyclic unsaturated hydrocarbon group having at least one site of olefinic unsaturation (i.e., having at least one moiety of the formula $C=C$). Alkenyl groups contain only C and H when unsubstituted. When an alkenyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be encompassed and described; thus, for example, "butenyl" is meant to include *n*-butenyl, *sec*-butenyl, and *iso*-butenyl. Examples of alkenyl include $-CH=CH_2$, $-CH_2-CH=CH_2$, and $-CH_2-CH=CH-CH=CH_2$. In some embodiments, alkenyl may be substituted. Suitable substituents that may be introduced into an alkenyl group include, for example, hydroxy, alkoxy, amino, alkylamino, and halo, among others.

As used herein, "alkynyl" refers to an acyclic or cyclic unsaturated hydrocarbon group having at least one site of acetylenic unsaturation (i.e., having at least one moiety of the formula $C\equiv C$). Alkynyl groups contain only C and H when unsubstituted. When an alkynyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be encompassed and described; thus, for example, "pentynyl" is meant to include *n*-pentynyl, *sec*-pentynyl, *iso*-pentynyl, and *tert*-pentynyl. Examples of alkynyl include $-C\equiv CH$ and $-C\equiv C-CH_3$. In some embodiments, alkynyl may be substituted. Suitable substituents that may be introduced into an alkynyl group include, for example, hydroxy, alkoxy, amino, alkylamino, and halo, among others.

As used herein the term "phenyl" denotes a monocyclic arene in which one hydrogen atom from a carbon atom of the ring has been removed. A phenyl group may be unsubstituted or substituted with one or more suitable substituents, wherein the substituent replaces an H of the phenyl group.

As used herein, the term "benzyl" refers to monovalent radical obtained when a hydrogen atom attached to the methyl group of toluene is removed. A benzyl group generally has the formula of phenyl- CH_2- . A benzyl group may be unsubstituted or substituted with one or more suitable substituents. For example, the substituent may replace an H of the phenyl component and/or an H of the methylene ($-CH_2-$) component.

As used herein, the term "amide" refers to an alkyl, alkenyl, alkynyl, or aromatic group that is attached to an amino-carbonyl functional group.

As used herein, the term "triazole" refers to heterocyclic compounds with the formula ($C_2H_3N_3$), having a five-membered ring of two carbons and three nitrogens, the positions of which can change resulting in multiple isomers.

As used herein, the term "terminal group" refers to the group at which a carbon chain or nucleic acid ends.

As used herein, an "amino acid" refers to a molecule containing amine and carboxyl functional groups and a side chain specific to the amino acid.

In some embodiments the amino acid is chosen from the group of proteinogenic amino acids. In some embodiments, the amino acid is an L-amino acid or a D-amino acid. In some embodiments, the amino acid is a synthetic amino acid (e.g., a beta-amino acid).

As used herein, the term "lipophilic amino acid" refers to an amino acid including a hydrophobic moiety (e.g., an alkyl chain or an aromatic ring).

As used herein, the term "target of delivery" refers to the organ or part of the body to which it is desired to deliver the branched oligonucleotide compositions.

5 As used herein, the term "between X and Y" is inclusive of the values of X and Y. For example, "between X and Y" refers to the range of values between the value of X and the value of Y, as well as the value of X and the value of Y.

As used herein, the terms "subject" and "patient" are used interchangeably and refer to an organism, such as a mammal (e.g., a human), that experiences a neurodegenerative disease or disorder (e.g., Huntington's Disease) and/or contains a gain-of-function *HTT* variant allele.

As used herein, the term "*HTT*" refers to the gene encoding Huntingtin, including any native *HTT* gene from any source. The term encompasses "full-length," unprocessed *HTT* as well as any form of *HTT* that results from processing in the cell. The term also encompasses naturally occurring variants of *HTT*, e.g., splice variants or allelic variants. The nucleic acid sequence of an exemplary *HTT* gene is shown in European Nucleotide Archive (ENA) Accession No. AB016794.1. The amino acid sequence of an exemplary protein encoded by an *HTT* gene is shown in UNIPROT™ Accession No. P42858.

As used herein, the terms "microsatellite repeat expansion disorder," "microsatellite repeat expansion disease," "nucleotide repeat expansion disorder," and "nucleotide repeat expansion disease" are used interchangeably to refer to any disease or disorder caused by the instability and expansion of specific microsatellites. "Microsatellites" are coding or non-coding DNA sequences that contain tandem repeats of base pairs. Exemplary microsatellite repeat expansion disorders include, but are not limited to, Fragile X syndrome, Fragile XE syndrome, Fragile X-associated tremor/ataxia syndrome, Fragile X-primary ovarian insufficiency, progressive myoclonic epilepsy type 1/Unverricht-Lundborg disease, spinocerebellar ataxia (SCA) 12, neuronal intranuclear inclusion disease, glutaminase deficiency, Huntington's disease, SCA1, SCA2, SCA3, SCA6, SCA7, SCA17, dentatorubral-pallidoluysian atrophy, spinal-bulbar muscular atrophy, oculopharyngeal muscular dystrophy, Huntington disease-like 2, amyotrophic lateral sclerosis, myotonic dystrophy type 2 (DM2), Friedreich ataxia, Fuchs endothelial corneal dystrophy, SCA10, SCA31, SCA36, SCA37, cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS), benign adult familial myoclonic epilepsy, SCA8, and myotonic dystrophy type 1 (DM1). Other microsatellite repeat expansion disorders are discussed in Rodriguez et al., *Neurobiology of Disease*, 130:104515, 2019, the disclosure of which is incorporated herein by reference.

As used herein, the terms "treat," "treated," and "treating" mean both therapeutic treatment and prophylactic or preventative measures wherein the object is to prevent, ameliorate, or slow down (lessen) an undesired physiological condition, disorder, or disease, or obtain beneficial or desired clinical results. Beneficial or desired clinical results include, but are not limited to, a reduction in a patient's reliance on pharmacological treatments; alleviation of symptoms; diminishment of the extent of a condition, disorder, or disease; stabilized (i.e., not worsening) state of condition, disorder, or disease; delay in onset or slowing of condition, disorder, or disease progression; amelioration of the

condition, disorder, or disease state or remission (whether partial or total), whether detectable or undetectable; an amelioration of at least one measurable physical, cognitive, or behavioral (e.g., depressive behavior or apathy) parameter, not necessarily discernible by the patient; or enhancement or improvement of condition, disorder, or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

As used herein, the terms “benefit” and “response” are used interchangeably in the context of a subject undergoing therapy for the treatment of, for example, Huntington’s Disease. For example, clinical benefits in the context of a subject having Huntington’s disease administered an siRNA molecule or siRNA composition of the disclosure include, without limitation, a reduction in involuntary movements, memory lapses, mood swings, or symptoms of anxiety and depression, and/or; a reduction in wild type *HTT* transcripts, mutant *HTT* transcripts, variant *HTT* transcripts, splice isoforms of *HTT* transcripts, and/or overexpressed *HTT* transcripts.

Detailed Description

The present disclosure provides compositions of small interfering RNA (siRNA) molecules with sequence homology to a Huntingtin (*HTT*) gene and methods for administering said siRNA molecules to a subject. Furthermore, the siRNA molecules described herein may be composed as branched siRNA structures, such as di-branched, tri-branched, and tetra-branched siRNA structures and may further include specific patterns of chemical modifications (e.g., 2’ ribose modifications or internucleoside linkage modifications) to improve resistance against nuclease enzymes, toxicity profile, and physicochemical properties (e.g., thermostability). Small interfering RNA molecules are short, double-stranded RNA molecules. They are capable of mediating RNA interference (RNAi) by degrading mRNA with a complementary nucleotide sequence, thus preventing the translation of the target gene.

The siRNA molecules of the disclosure may exhibit, for example, robust gene-specific suppression of *HTT*, relative to other genes.

The siRNA molecules of the disclosure may feature an antisense strand having a nucleic acid sequence that is complementary to a region of an *HTT* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3. The degree of complementarity of the antisense strand to the region of the *HTT* mRNA transcript may be sufficient for the antisense strand to anneal over the full length of the region of the *HTT* mRNA transcript. For example, the antisense strand may have a nucleic acid sequence that is at least 60% complementary (e.g., 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary) to the region of the *HTT* mRNA transcript. In some embodiments, the region of the *HTT* RNA transcript has the sequence of SEQ ID NO: 1. In some embodiments, the region of the *HTT* RNA transcript has the sequence of SEQ ID NO: 2. In some embodiments, the region of the *HTT* RNA transcript has the sequence of SEQ ID NO: 3.

In some embodiments, the siRNA molecules of the disclosure feature an antisense strand having the nucleic acid sequence of any one of SEQ ID NOs: 7-9, or a nucleic acid sequence that is at least 60% identical thereto. For example, the siRNA molecules of the disclosure may feature an antisense strand having a nucleic acid sequence that is at least 60% identical (e.g., 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 7-9. In some embodiments, the nucleic acid sequence is SEQ ID NO: 7. In some embodiments, the nucleic acid sequence is SEQ ID NO: 8. In some embodiments, the nucleic acid sequence is SEQ ID NO: 9.

In some embodiments, the siRNA molecules of the disclosure feature a sense strand having the nucleic acid sequence of any one of SEQ ID NOs: 4-6, or a nucleic acid sequence that is at least 60% identical thereto. For example, the siRNA molecules of the disclosure may feature a sense strand having a nucleic acid sequence that is at least 60% identical (e.g., 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 4-6. In some embodiments, the nucleic acid sequence is SEQ ID NO: 4. In some embodiments, the nucleic acid sequence is SEQ ID NO: 5. In some embodiments, the nucleic acid sequence is SEQ ID NO: 6.

Exemplary siRNA molecules of the disclosure are those shown in Table 1, below. Table 1 summarizes the antisense strands, sense strands, and corresponding regions of an *HTT* mRNA transcript that are targeted by each antisense strand.

Table 1

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
1	CUCUUUUCUUCU CA GGAUUUAAAAUU UAA U	4	UCAGUAAAGAGA UUAA	7	UUAAUCUCUUU ACUGAUUAA
2	AAAUUUAAUUA UAUC AGUAAAGAGAU UAAU	5	AUAUCAGUAAAG AGA	8	UCUCUUUACUG AUUAUUA
3	UUAAUUUAUCAG UA AAGAGAUUAAUU UA	6	CUCAGGAUUUAA AAA	9	UUUUUAAAUCC UGAGAAGAA

siRNA Structure

The siRNA molecules of the disclosure may be in the form of a single-stranded (ss) or double-stranded (ds) oligonucleotide structure. In some embodiments, the siRNA molecules may be di-branched, tri-branched, or tetra-branched molecules. Furthermore, the siRNA molecules of the disclosure may contain one or more phosphodiester internucleoside linkages and/or an analog thereof, such as a phosphorothioate internucleoside linkage. The siRNA molecules of the disclosure may further contain chemically modified nucleosides having 2' sugar modifications.

The simplest siRNAs consist of a ribonucleic acid, including a ss- or ds- structure, formed by a first strand (i.e., antisense strand), and in the case of a ds-siRNA, a second strand (i.e., sense strand). The first strand includes a stretch of contiguous nucleotides that is at least partially complementary to a target nucleic acid. The second strand also includes a stretch of contiguous
 5 nucleotides where the second stretch is at least partially identical to a target nucleic acid. The first strand and said second strand may be hybridized to each other to form a double-stranded structure. The hybridization typically occurs by Watson Crick base pairing.

Depending on the sequence of the first and second strand, the hybridization or base pairing is not necessarily complete or perfect, which means that the first and second strand are not 100% base-
 10 paired due to mismatches. One or more mismatches may also be present within the duplex without necessarily impacting the siRNA RNAi activity.

The first strand contains a stretch of contiguous nucleotides which is essentially complementary to a target nucleic acid. Typically, the target nucleic acid sequence is, in accordance with the mode of action of interfering ribonucleic acids, a ss-RNA, preferably an mRNA. Such
 15 hybridization occurs most likely through Watson Crick base pairing but is not necessarily limited thereto. The extent to which the first strand has a complementary stretch of contiguous nucleotides to a target nucleic acid sequence may be between 80% and 100%, e.g., 80%, 85%, 90%, 95%, or 100% complementary.

The siRNA molecules described herein may employ modifications to the nucleobase,
 20 phosphate backbone, ribose core, 5'- and 3'-ends, and branching, wherein multiple strands of siRNA may be covalently linked.

Lengths of Small Interfering RNA Molecules

It is within the scope of the disclosure that any length, known and previously unknown in the
 25 art, may be employed for the current invention. As described herein, potential lengths for an antisense strand of the siRNA molecules of the present disclosure is between 10 and 30 nucleotides (e.g., 10 nucleotides, 11 nucleotides, 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22
 30 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides), 15 and 25 nucleotides (e.g., 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, or 25 nucleotides), or 18 and 23 nucleotides (e.g., 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, or 23 nucleotides). In some embodiments, the antisense strand is 20 nucleotides. In some embodiments, the antisense
 35 strand is 21 nucleotides. In some embodiments, the antisense strand is 22 nucleotides. In some embodiments, the antisense strand is 23 nucleotides. In some embodiments, the antisense strand is 24 nucleotides. In some embodiments, the antisense strand is 25 nucleotides. In some embodiments, the antisense strand is 26 nucleotides. In some embodiments, the antisense strand is 27 nucleotides. In some embodiments, the antisense strand is 28 nucleotides. In some

embodiments, the antisense strand is 29 nucleotides. In some embodiments, the antisense strand is 30 nucleotides.

In some embodiments, the sense strand of the siRNA molecules of the present disclosure is between 12 and 30 nucleotides (e.g., 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, or 30 nucleotides), or 14 and 23 nucleotides (e.g., 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, or 23 nucleotides). In some embodiments, the sense strand is 15 nucleotides. In some embodiments, the sense strand is 16 nucleotides. In some embodiments, the sense strand is 17 nucleotides. In some embodiments, the sense strand is 18 nucleotides. In some embodiments, the sense strand is 19 nucleotides. In some embodiments, the sense strand is 20 nucleotides. In some embodiments, the sense strand is 21 nucleotides. In some embodiments, the sense strand is 22 nucleotides. In some embodiments, the sense strand is 23 nucleotides. In some embodiments, the sense strand is 24 nucleotides. In some embodiments, the sense strand is 25 nucleotides. In some embodiments, the sense strand is 26 nucleotides. In some embodiments, the sense strand is 27 nucleotides. In some embodiments, the sense strand is 28 nucleotides. In some embodiments, the sense strand is 29 nucleotides. In some embodiments, the sense strand is 30 nucleotides.

2' Sugar Modifications

The present disclosure may include ss- and ds- siRNA molecule compositions including at least one (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or more) nucleosides having 2' sugar modifications. Possible 2'-modifications include all possible orientations of 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 C1 to C10 alkyl or C2 to C10 alkenyl and alkynyl. In some embodiments, the modification includes a 2'-O-methyl (2'-O-Me) modification. Other potential sugar substituent groups include: C1 to C10 lower alkyl, substituted lower alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. In some embodiments, the modification includes 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE). In some embodiments, the modification includes 2'-dimethylaminoethoxyethoxy, i.e., a O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylamino-ethoxy-ethyl or 2'-DMAEOE), i.e., 2'-O-CH₂OCH₂N(CH₃)₂. Other potential sugar substituent groups include, e.g., aminopropoxy (-OCH₂CH₂CH₂NH₂), allyl (-CH₂-CH=CH₂), -O-allyl (-O-CH₂-CH=CH₂) and fluoro (F). 2'-sugar substituent groups may be in the arabino (up) position or ribo (down) position. In some embodiments, the 2'-arabino modification is 2'-F. Similar modifications may also be made at other

positions on the siRNA molecule, particularly the 3' position of the sugar on the 3' terminal nucleoside or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

5 Nucleobase Modifications

The siRNA molecules of the disclosure may also include nucleosides or other surrogate or mimetic monomeric subunits that include a nucleobase (often referred to in the art simply as "base" or "heterocyclic base moiety"). The nucleobase is another moiety that has been extensively modified or substituted and such modified and or substituted nucleobases are amenable to the present
 10 disclosure. 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 also referred herein as heterocyclic base moieties 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
 15 derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH₃) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and 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, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and
 20 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in US 3,687,808, those disclosed in Kroschwitz, J.I., ed. *The Concise Encyclopedia of*
 25 *Polymer Science and Engineering*, New York, John Wiley & Sons, 1990, pp. 858-859; those disclosed by Englisch et al., *Angewandte Chemie*, International Edition 30:613, 1991; and those disclosed by Sanghvi, Y.S., Chapter 16, *Antisense Research and Applications*, CRC Press, Gait, M.J. ed., 1993, pp. 289-302. The siRNA molecules of the present disclosure may also include polycyclic heterocyclic compounds in place of one or more heterocyclic base moieties. A number of tricyclic
 30 heterocyclic compounds have been previously reported. These compounds are routinely used in antisense applications to increase the binding properties of the modified strand to a target strand.

Representative cytosine analogs that make three hydrogen bonds with a guanosine in a second strand include 1,3-diazaphenoxazine-2-one (Kurchavov *et al.*, *Nucleosides and Nucleotides*, 16:1837-46, 1997), 1,3-diazaphenothiazine-2-one (Lin et al. *Am. Chem. Soc.*, 117:3873-4, 1995),
 35 and 6,7,8,9-tetrafluoro-1,3-diazaphenoxazine-2-one (Wang et al., *Tetrahedron Lett.*, 39:8385-8, 1998). Incorporated into oligonucleotides, these base modifications were shown to hybridize with complementary guanine and the latter was also shown to hybridize with adenine and to enhance helical thermal stability by extended stacking interactions (also see US 10/155,920 and US 10/013,295, both of which are herein incorporated by reference in their entirety). Further helix-stabilizing properties have been observed when a cytosine analog/substitute has an aminoethoxy
 40

moiety attached to the rigid 1,3-diazaphenoxazine-2-one scaffold (Lin et al., *Am. Chem. Soc.*, 120:8531-2, 1998).

Internucleoside Linkage Modifications

5 Another variable in the design of the present disclosure is the internucleoside linkage making up the phosphate backbone of the siRNA molecule. Although the natural RNA phosphate backbone may be employed here, derivatives thereof may be used which enhance desirable characteristics of the siRNA molecule. Although not limiting, of particular importance in the present disclosure is protecting parts, or the whole, of the siRNA molecule from hydrolysis. One example of a modification
10 that decreases the rate of hydrolysis is phosphorothioates. Any portion or the whole of the backbone may contain phosphate substitutions (e.g., phosphorothioates). For instance, the internucleoside linkages may be between 0 and 100% phosphorothioate, e.g., between 0 and 100%, 10 and 100%, 20 and 100%, 30 and 100%, 40 and 100%, 50 and 100%, 60 and 100%, 70 and 100%, 80 and 100%, 90 and 100%, 0 and 90%, 0 and 80%, 0 and 70%, 0 and 60%, 0 and 50%, 0 and 40%, 0 and 30%, 0
15 and 20%, 0 and 10%, 10 and 90%, 20 and 80%, 30 and 70%, 40 and 60%, 10 and 40%, 20 and 50%, 30 and 60%, 40 and 70%, 50 and 80%, or 60 and 90% phosphorothioate linkages. Similarly, the internucleoside linkages may be between 0 and 100% phosphodiester linkages, e.g., between 0 and 100%, 10 and 100%, 20 and 100%, 30 and 100%, 40 and 100%, 50 and 100%, 60 and 100% 70 and 100%, 80 and 100%, 90 and 100%, 0 and 90%, 0 and 80%, 0 and 70%, 0 and 60%, 0 and 50%, 0
20 and 40%, 0 and 30%, 0 and 20%, 0 and 10%, 10 and 90%, 20 and 80%, 30 and 70%, 40 and 60%, 10 and 40%, 20 and 50%, 30 and 60%, 40 and 70%, 50 and 80%, or 60 and 90% phosphodiester linkages.

Specific examples of some potential siRNA molecules useful in this invention include oligonucleotides containing modified e.g., non-naturally occurring internucleoside linkages. As
25 defined in this specification, oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom and internucleoside linkages that do not have a phosphorus atom. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. A preferred phosphorus containing modified
30 internucleoside linkage is the phosphorothioate internucleoside linkage. In some embodiments, the modified oligonucleotide backbones containing a phosphorus atom therein include, for example, phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and
35 aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Exemplary U.S. patents describing the preparation of phosphorus-containing linkages include but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863;
40 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 U.S.

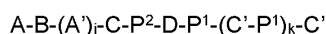
5 Pat. RE39464, the entire contents of each of which are hereby incorporated herein by reference.

In some embodiments, the modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom 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; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts. Non-limiting examples of U.S. patents that teach the preparation of non-phosphorus backbones 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, the entire contents of each of which are hereby incorporated herein by reference.

Patterns of Modifications of siRNA Molecules

The following section provides a set of exemplary scaffolds into which the siRNA molecules of the disclosure may be incorporated.

25 In some embodiments of the disclosure, the siRNA may contain an antisense strand including a region represented by Formula I, wherein Formula I is, in the 5'-to-3' direction



Formula I;

30 wherein A is represented by the formula C-P¹-D-P¹; each A' is represented by the formula C-P²-D-P²; B is represented by the formula C-P²-D-P²-D-P²-D-P²; each C is a 2'-O-methyl (2'-O-Me) ribonucleoside; each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside; each D is a 2'-F ribonucleoside; each P¹ is a phosphorothioate internucleoside linkage; each P² is a phosphodiester internucleoside linkage; j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, j is 4. In some embodiments, k is 4. In some embodiments, j is 4 and k is 4. The antisense is complementary (e.g., fully or partially complementary) to a target nucleic acid sequence.

40 In some embodiments, the antisense strand includes a structure represented by Formula A1, wherein Formula A1 is, in the 5'-to-3' direction:

A-S-B-S-A-O-B-O-B-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A-S-A-S-B-S-A

Formula A1;

- 5 wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the siRNA may contain an antisense strand including a region represented by Formula II, wherein Formula II is, in the 5'-to-3' direction:

10 A-B-(A')_j-C-P²-D-P¹-(C-P¹)_k-C'

Formula II;

- wherein A is represented by the formula C-P¹-D-P¹; each A' is represented by the formula C-P²-D-P²; B is represented by the formula C-P²-D-P²-D-P²-D-P²; each C is a 2'-O-methyl (2'-O-Me) ribonucleoside; each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside; each D is a 2'-F ribonucleoside; each P¹ is a phosphorothioate internucleoside linkage; each P² is a phosphodiester internucleoside linkage; j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, j is 4. In some embodiments, k is 4. In some embodiments, j is 4 and k is 4. The antisense is complementary (e.g., fully or partially complementary) to a target nucleic acid sequence.
- 20

In some embodiments of the disclosure, the antisense strand includes a structure represented by Formula A2, wherein Formula A2 is, in the 5'-to-3' direction:

25 A-S-B-S-A-O-B-O-B-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A-S-A-S-A-S-A

Formula A2;

- wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.
- In some embodiments of the disclosure, the antisense strand includes a structure represented by Formula A2', wherein Formula A2' is, in the 5'-to-3' direction:
- 30

A-S-B-S-A-O-B-O-B-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-B-S-A-S-A-S-A-S-B-S-A

Formula A2';

- wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.
- 35

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula III, wherein Formula III is, in the 5'-to-3' direction:

40 E-(A')_m-F

Formula III;

wherein E is represented by the formula $(C-P^1)_2$; F is represented by the formula $(C-P^2)_3-D-P^1-C-P^1-C$, $(C-P^2)_3-D-P^2-C-P^2-C$, $(C-P^2)_3-D-P^1-C-P^1-D$, or $(C-P^2)_3-D-P^2-C-P^2-D$; A¹, C, D, P¹, and P² are as defined in Formula I; and m is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, m is

- 5 4. The sense strand is complementary (e.g., fully or partially complementary) to the antisense strand.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S1, wherein Formula S1 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-S-A-S-A

10

Formula S1;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

- 15 In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S2, wherein Formula S2 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-O-A-O-A

Formula S2;

- 20 wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S3, wherein Formula S3 is, in the 5'-to-3' direction:

25

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-S-A-S-B

Formula S3;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

- 30 In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S4, wherein Formula S4 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-A-O-A-O-B-O-A-O-B

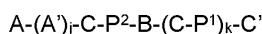
Formula S4;

35

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the siRNA may contain an antisense strand including a region represented by Formula IV, wherein Formula IV is, in the 5'-to-3' direction:

40

**Formula IV;**

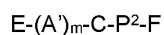
wherein A is represented by the formula C-P¹-D-P¹; each A' is represented by the formula C-P²-D-P²;
 5 B is represented by the formula D-P¹-C-P¹-D-P¹; each C is a 2'-O-Me ribonucleoside; each C',
 independently, is a 2'-O-Me ribonucleoside or a 2'-F ribonucleoside; each D is a 2'-F ribonucleoside;
 each P¹ is a phosphorothioate internucleoside linkage; each P² is a phosphodiester internucleoside
 linkage; j is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); and k is an integer from 1 to 7 (e.g., 1, 2,
 3, 4, 5, 6, or 7). In some embodiments, j is 6. In some embodiments, k is 4. In some embodiments, j
 10 is 6 and k is 4. The antisense strand is complementary (e.g., fully or partially complementary) to a
 target nucleic acid.

In some embodiments of the disclosure, the antisense strand includes a structure represented
 by Formula A3, wherein Formula A3 is, in the 5'-to-3' direction:

**Formula A3;**

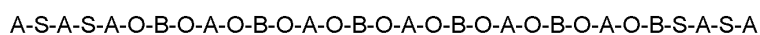
wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a
 phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

20 In some embodiments of the disclosure, the siRNA of the disclosure may have a sense strand
 represented by Formula V, wherein Formula V is, in the 5'-to-3' direction:

**Formula V;**

25 wherein E is represented by the formula (C-P¹)₂; F is represented by the formula D-P¹-C-P¹-C, D-P²-
 C-P²-C, D-P¹-C-P¹-D, or D-P²-C-P²-D; A', C, D, P¹, and P² are as defined in Formula IV; and m is an
 integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, m is 5. The sense strand is
 complementary (e.g., fully or partially complementary) to the antisense strand.

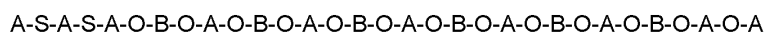
30 In some embodiments of the disclosure, the sense strand includes a structure represented by
 Formula S5, wherein Formula S5 is, in the 5'-to-3' direction:

**Formula S5;**

35 wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a
 phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

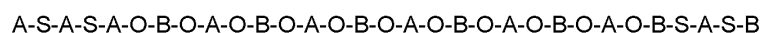
In some embodiments of the disclosure, the sense strand includes a structure represented by
 Formula S6, wherein Formula S6 is, in the 5'-to-3' direction:

40

**Formula S6;**

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a
 5 phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

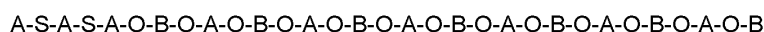
In some embodiments of the disclosure, the sense strand includes a structure represented by
 Formula S7, wherein Formula S7 is, in the 5'-to-3' direction:



10 **Formula S7;**

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a
 phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the sense strand includes a structure represented by
 15 Formula S8, wherein Formula S8 is, in the 5'-to-3' direction:

**Formula S8;**

20 wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a
 phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the siRNA may contain an antisense strand including
 a region represented by Formula VI, wherein Formula VI is, in the 5'-to-3' direction:



25 **Formula VI;**

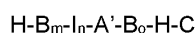
wherein A is represented by the formula C-P¹-D-P¹; each B is represented by the formula C-P²; each
 C is a 2'-O-Me ribonucleoside; each C', independently, is a 2'-O-Me ribonucleoside or a 2'-F
 30 ribonucleoside; each D is a 2'-F ribonucleoside; each E is represented by the formula D-P²-C-P²; F is
 represented by the formula D-P¹-C-P¹; each G is represented by the formula C-P¹; each P¹ is a
 phosphorothioate internucleoside linkage; each P² is a phosphodiester internucleoside linkage; j is an
 integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); k is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7);
 and l is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, j is 3. In some
 35 embodiments, k is 6. In some embodiments, l is 2. In some embodiments, j is 3, k is 6, and l is 2.
 The antisense strand is complementary (e.g., fully or partially complementary) to a target nucleic acid.

In some embodiments of the disclosure, the antisense strand includes a structure represented
 by Formula A4, wherein Formula A4 is, in the 5'-to-3' direction:

**Formula A4;**

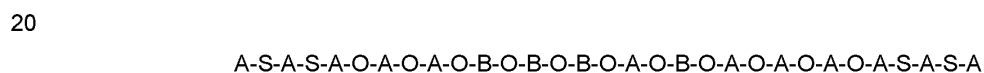
wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a
5 phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the siRNA may contain a sense strand including a region represented by Formula VII, wherein Formula VII is, in the 5'-to-3' direction:

10 **Formula VII;**

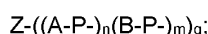
wherein A' is represented by the formula C-P²-D-P²; each H is represented by the formula (C-P¹)₂;
each I is represented by the formula (D-P²); B, C, D, P¹, and P² are as defined in Formula VI; m is an
integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7); n is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7);
15 and o is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7). In some embodiments, m is 3. In some
embodiments, n is 3. In some embodiments, o is 3. In some embodiments, m is 3, n is 3, and o is 3.
The sense strand is complementary (e.g., fully or partially complementary) to the antisense strand.

In some embodiments of the disclosure, the sense strand includes a structure represented by
Formula S9, wherein Formula S9 is, in the 5'-to-3' direction:

20 **Formula S9;**

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a
25 phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

In some embodiments of the disclosure, the siRNA may contain an antisense strand including a region that is represented by Formula VIII:

30 **Formula VIII**

wherein Z is a 5' phosphorus stabilizing moiety; each A is a 2'-O-methyl (2'-O-Me)
ribonucleoside; each B is a 2'-fluoro-ribonucleoside; each P is, independently, an internucleoside
linkage selected from a phosphodiester linkage and a phosphorothioate linkage; n is an integer from 1
35 to 5 (e.g., 1, 2, 3, 4, or 5); m is an integer from 1 to 5 (e.g., 1, 2, 3, 4, or 5); and q is an integer
between 1 and 30 (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,
26, 27, 28, 29, or 30).

Methods of siRNA Synthesis

The siRNA molecules of the disclosure 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.

5 The siRNA agent can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide including unnatural or modified nucleotides can be easily prepared. siRNA molecules of the disclosure can be prepared using solution-phase or solid-phase organic synthesis or both.

10 Further, it is contemplated that for any siRNA agent disclosed herein, further optimization could be achieved by systematically either adding or removing linked nucleosides to generate longer or shorter sequences. Further still, such optimized sequences can be adjusted by, e.g., the introduction of modified nucleosides, and/or modified internucleoside linkages as described herein or as known in the art, including alternative nucleosides, alternative sugar moieties, and/or alternative internucleoside linkages as known in the art and/or discussed herein to further optimize the molecule

15 (e.g., increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, and/or targeting to a particular location or cell type).

 siRNA molecules of the present disclosure may be prepared to include a plurality of cationic binding sites that are saturated by one or more divalent cations (e.g., Ba^{2+} , Be^{2+} , Ca^{2+} , Cu^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , or Zn^{2+} , or a combination thereof). The compositions may be prepared, for example, by

20 hybridizing the therapeutic oligonucleotide molecule in the presence of the divalent cation. Alternatively, the compositions may be prepared by hybridizing the therapeutic oligonucleotide molecule without the divalent cation, followed by addition of the divalent cation after hybridization. In the case of more than one divalent cation, the divalent cations may be added at the same time or sequentially. For example, the therapeutic oligonucleotide molecule may be hybridized in the

25 presence of two divalent cations. Alternatively, the therapeutic oligonucleotide molecule may be hybridized in the presence of one divalent cation and a second divalent cation is added after hybridization. As a further alternative, the therapeutic oligonucleotide molecule may be hybridized without a divalent cation, followed by the addition of two divalent cations.

30 Divalent Cations

 The therapeutic oligonucleotides of the disclosure may contain one or more phosphodiester internucleoside linkages and/or an analog thereof, such as a phosphorothioate internucleoside linkage, in which oxyanion moieties are electrostatically neutralized by ionic bonding to a divalent metal cation, such as Ba^{2+} , Be^{2+} , Ca^{2+} , Cu^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , or Zn^{2+} .

35 The siRNA molecules of the disclosure may include a plurality of cationic binding sites (e.g., electron-dense sites) that are saturated by one or more divalent cations (e.g., Ba^{2+} , Be^{2+} , Ca^{2+} , Cu^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , or Zn^{2+} , or a combination thereof). Because of their positive charge, divalent cations are typically reactive with negatively charged atoms (e.g., oxyanion from a phosphate group or phosphorothioate group carrying a unit or partial negative charge). The present disclosure provides

novel evidence that the saturation of cationic binding sites on a therapeutic oligonucleotide molecule with divalent cations significantly reduces toxicity when administered to the CNS of a subject.

The one or more divalent cations may have an ionic radius, when measured in the form of a crystal lattice, of about 30 picometers to about 150 picometers (e.g., from about 30 picometers to about 140 picometers, from about 40 picometers to about 130 picometers, from about 50 picometers to about 120 picometers, from about 60 picometers to about 110 picometers, from about 60 picometers to about 100 picometers, or from about 60 picometers to about 90 picometers). The calculated crystal radii of the divalent cations disclosed by R. D. Shannon, *Acta Crystallographica A*, 32:751-767, 1976, are herein incorporated by reference.

The degree of saturation of a therapeutic oligonucleotide molecule's cationic binding sites by the one or more divalent cations may range from about 10% to about 100% (e.g., from about 20% to about 100%, from about 30% to about 100%, from about 40% to about 100%, from about 50% to about 100%, from about 60% to about 100%, from about 70% to about 100%, from about 80% to about 100%, or from about 90% to about 100%).

In some embodiments, the antisense strand of the therapeutic oligonucleotide molecule may have a length of from 10 to 30 nucleotides and may be ionically bound to a total of from 10 to 30 divalent cations. For example, the molar ratio of antisense strand nucleotides to divalent cations in the therapeutic oligonucleotide molecule could range from 1:3 to 3:1 (e.g., 1:3, 1.1:3, 1.2:3, 1.3:3, 1.4:3, 1.5:3, 1.6:3, 1.7:3, 1.8:3, 1.9:3, 2:3, 2.1:3, 2.2:3, 2.3:3, 2.4:3, 2.5:3, 2.6:3, 2.7:3, 2.8:3, 2.9:3, 1:1, 3:2.9, 3:2.8, 3:2.7, 3:2.6, 3:2.5, 3:2.4, 3:2.3, 3:2.2, 3:2.1, 3:2, 3:1.9, 3:1.8, 3:1.7, 3:1.6, 3:1.5, 3:1.4, 3:1.3, 3:1.2, 3:1.1, or 3:1).

In some embodiments, the sense strand of the therapeutic oligonucleotide molecule may have a length of from 10 to 30 nucleotides and may be ionically bound to a total of from 10 to 30 divalent cations. For example, the molar ratio of sense strand nucleotides to divalent cations in the therapeutic oligonucleotide molecule could range from 1:3 to 3:1 (e.g., 1:3, 1.1:3, 1.2:3, 1.3:3, 1.4:3, 1.5:3, 1.6:3, 1.7:3, 1.8:3, 1.9:3, 2:3, 2.1:3, 2.2:3, 2.3:3, 2.4:3, 2.5:3, 2.6:3, 2.7:3, 2.8:3, 2.9:3, 1:1, 3:2.9, 3:2.8, 3:2.7, 3:2.6, 3:2.5, 3:2.4, 3:2.3, 3:2.2, 3:2.1, 3:2, 3:1.9, 3:1.8, 3:1.7, 3:1.6, 3:1.5, 3:1.4, 3:1.3, 3:1.2, 3:1.1, or 3:1).

The therapeutic oligonucleotide molecules of the disclosure may be combined with one or more divalent cations in a specific molar ratio. The specific molar ratio of therapeutic oligonucleotide molecule to divalent cation may be relevant to the toxicity benefit achieved by the divalent cation. For example, the molar ratio of therapeutic oligonucleotide molecule to divalent cation may range from 1:10 to 1:50 (e.g., 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:21, 1:22, 1:23, 1:24, 1:25, 1:26, 1:27, 1:28, 1:29, 1:30, 1:31, 1:32, 1:33, 1:34, 1:35, 1:36, 1:37, 1:38, 1:39, 1:40, 1:41, 1:42, 1:43, 1:44, 1:45, 1:46, 1:47, 1:48, 1:49, or 1:50). In some embodiments, the molar ratio of therapeutic oligonucleotide molecule to divalent cation may range from 1:18 to 1:38 (e.g., 1:18, 1:19, 1:20, 1:21, 1:22, 1:23, 1:24, 1:25, 1:26, 1:27, 1:28, 1:29, 1:30, 1:31, 1:32, 1:33, 1:34, 1:35, 1:36, 1:37, or 1:38). In some embodiments, the molar ratio of therapeutic oligonucleotide molecule to divalent cation may range from 1:20 to 1:25 (e.g., 1:20, 1:21, 1:22, 1:23, 1:24, or 1:25). In some

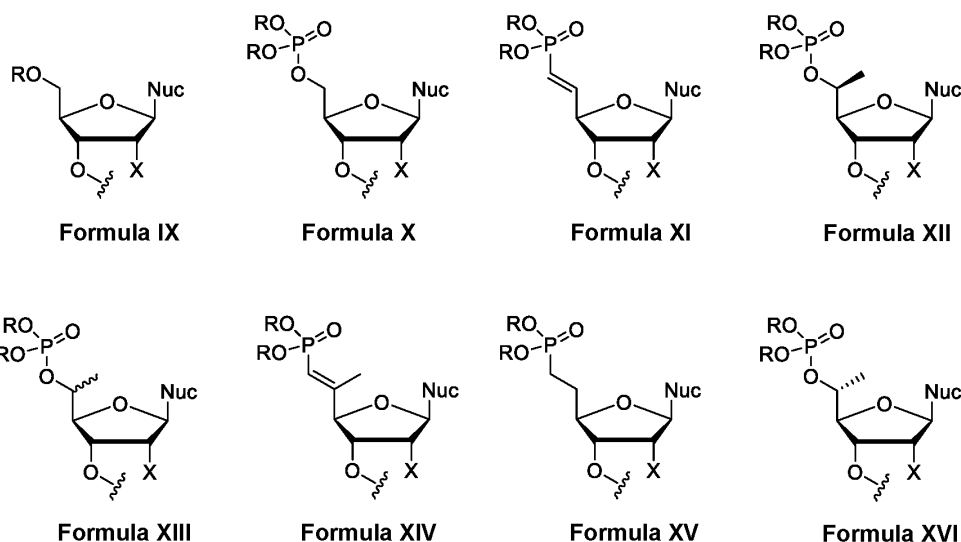
embodiments, the molar ratio of therapeutic oligonucleotide to divalent cation may be 1:20. In some embodiments, the molar ratio of therapeutic oligonucleotide to divalent cation may be 1:25.

The therapeutic oligonucleotides of the disclosure may be combined with one or more divalent cations in which the divalent cation is present in a specific concentration or range of concentrations. The concentration of the divalent cation may be relevant to the toxicity benefit achieved by the divalent cation. For example, the concentration of the divalent cation may be from 20 mM to 150 mM (e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, or 150 mM). In some embodiments, the concentration of the divalent cation is from 20 mM to 100 mM (e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 mM). In some embodiments, the concentration of the divalent cation is from 35 mM to 75 mM (e.g., 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, or 75 mM). In some embodiments, the concentration of the divalent cation may be from 40 mM to 70 mM (e.g., 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 mM).

The therapeutic oligonucleotide may include one or more atoms having a negative charge and the divalent cation may include a positive charge. In some embodiments, the therapeutic oligonucleotide and divalent cation are present in an amount so that there is a specific ratio of negative to positive charge present within the composition. Methods of determining the negative to positive charge ratio are known in the art, for example, in Furst et al., *Electrophoresis*, 37:2685-2691, 2016, the disclosure of which is hereby incorporated by reference. In some embodiments, the ratio of negative charge to positive charge is from 0.75 to 7.5 (e.g., 0.76, 0.77, 0.78, 0.79, 0.80, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, or 7.5). In some embodiments, the ratio of negative charge to positive charge is from 1.0 to 2.0 (e.g., from 1.0 to 1.9, from 1.0 to 1.8, from 1.0 to 1.7, from 1.0 to 1.6, from 1.0 to 1.5, from 1.0 to 1.4, from 1.0 to 1.3, from 1.0 to 1.2, from 1.0 to 1.1, from 1.1 to 2.0, from 1.2 to 2.0, from 1.3 to 2.0, from 1.4 to 2.0, from 1.5 to 2.0, from 1.6 to 2.0, from 1.7 to 2.0, from 1.8 to 2.0, or from 1.9 to 2.0). In some embodiments, the ratio of negative charge to positive charge is from 0.75 to 6.5 (e.g., from 0.75 to 5.5, from 0.75 to 4.5, from 0.75 to 3.5, from 0.75 to 2.5, from 0.75 to 1.5, or from 0.75 to 1). In some embodiments, the ratio of negative charge to positive charge is from 1 to 7.5 (e.g., from 1.5 to 7.5, from 2.5 to 7.5, from 3.5 to 7.5, from 4.5 to 7.5, from 5.5 to 7.5, or from 6.5 to 7.5).

5' Phosphorus Stabilizing Moieties

To further protect the siRNA molecules of this disclosure from degradation, a 5'-phosphorus stabilizing moiety may be employed. A 5'-phosphorus stabilizing moiety replaces the 5'-phosphate to prevent hydrolysis of the phosphate. Hydrolysis of the 5'-phosphate prevents binding to RISC, a necessary step in gene silencing. Any replacement for phosphate that does not impede binding to RISC is contemplated in this disclosure. In some embodiments, the replacement for the 5'-phosphate is also stable to *in vivo* hydrolysis. Each strand of a siRNA molecule may independently and optionally employ any suitable 5'-phosphorus stabilizing moiety.



Some exemplary endcaps are demonstrated in Formulas IX-XVI. Nuc in Formulas IX-XVI represents a nucleobase or nucleobase derivative or replacement as described herein. X in formula IX-XVI represents a 2'-modification as described herein. Some embodiments employ hydroxy as in Formula IX, phosphate as in Formula X, vinylphosphonates as in Formula XI and XIV, 5'-methyl-substituted phosphates as in Formula XII, XIII, and XVI, methylenephosphonates as in Formula XV, or vinyl 5'-vinylphosphonate as a 5'-phosphorus stabilizing moiety as demonstrated in Formula XI.

Hydrophobic Moieties

The present disclosure further provides siRNA molecules having one or more hydrophobic moieties attached thereto. The hydrophobic moiety may be covalently attached to the 5' end or the 3' end of the siRNA molecules of the disclosure. Non-limiting examples of hydrophobic moieties suitable for use with the siRNA molecules of the disclosure may include cholesterol, vitamin D, tocopherol, phosphatidylcholine (PC), docosahexaenoic acid, docosanoic acid, PC-docosanoic acid, eicosapentaenoic acid, lithocholic acid or any combination of the aforementioned hydrophobic moieties with PC.

siRNA Branching

The siRNA molecules of the disclosure may be branched. For example, the siRNA molecules of the disclosure may have one of several branching patterns, as described herein.

According to the present disclosure, the siRNA molecules disclosed herein may be branched siRNA molecules. The siRNA molecule may not be branched, or may be di-branched, tri-branched, or tetra-branched, connected through a linker. Each main branch may be further branched to allow for 2, 3, 4, 5, 6, 7, or 8 separate RNA single- or double-strands. The branch points on the linker may stem from the same atom, or separate atoms along the linker. Some exemplary embodiments are listed in Table 2.

10

Table 2. Branched siRNA structures

Di-branched	Tri-branched	Tetra-branched
RNA-L-RNA Formula XVII	RNA RNA-L-RNA Formula XX	RNA RNA-L-RNA RNA Formula XXIV
RNA X-L-X RNA Formula XVIII	RNA RNA RNA-X-L-X RNA Formula XXI	RNA RNA RNA-X-L-X RNA Formula XXV
RNA RNA X-L-X RNA Formula XIX	RNA RNA RNA RNA X-L-X RNA Formula XXII	RNA RNA RNA RNA X-L-X RNA Formula XXVI
	RNA RNA RNA RNA X X-L-X RNA Formula XXIII	RNA RNA RNA RNA X X-L-X RNA Formula XXVII
		RNA RNA RNA RNA X X-L-X RNA X RNA Formula XXVIII

In some embodiments, the siRNA molecule is a branched siRNA molecule. In some embodiments, the branched siRNA molecule is di-branched, tri-branched, or tetra-branched. In some

embodiments, the di-branched siRNA molecule is represented by any one of Formulas XVII-XIX, wherein each RNA, independently, is an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety (e.g., phosphoroamidite, tosylated solketal, 1,3-diaminopropanol, pentaerythritol, or any one of the branch point moieties described in US 10,478,503).

5 In some embodiments, the tri-branched siRNA molecule represented by any one of Formulas XX-XXIII, wherein each RNA, independently, is an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

In some embodiments, the tetra-branched siRNA molecule represented by any one of Formulas XXIV-XXVIII, wherein each RNA, independently, is an siRNA molecule, L is a linker, and
10 each X, independently, represents a branch point moiety.

Linkers

Multiple strands of siRNA described herein may be covalently attached by way of a linker. The effect of this branching improves, *inter alia*, cell permeability allowing better access into cells
15 (e.g., neurons or glial cells) in the CNS. Any linking moiety may be employed which is not incompatible with the siRNAs of the present invention. Linkers include ethylene glycol chains of 2 to 10 subunits (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 subunits), alkyl chains, carbohydrate chains, block copolymers, peptides, RNA, DNA, and others. In some embodiments, any carbon or oxygen atom of the linker is optionally replaced with a nitrogen atom, bears a hydroxyl substituent, or bears an oxo
20 substituent. In some embodiments, the linker is a poly-ethylene glycol (PEG) linker. The PEG linkers suitable for use with the disclosed compositions and methods include linear or non-linear PEG linkers. Examples of non-linear PEG linkers include branched PEGs, linear forked PEGs, or branched forked PEGs.

PEG linkers of various weights may be used with the disclosed compositions and methods.
25 For example, the PEG linker may have a weight that is between 5 and 500 Daltons. In some embodiments, a PEG linker having a weight that is between 500 and 1,000 Dalton may be used. In some embodiments, a PEG linker having a weight that is between 1,000 and 10,000 Dalton may be used. In some embodiments, a PEG linker having a weight that is between 200 and 20,000 Dalton may be used. In some embodiments, the linker is covalently attached to a sense strand of the siRNA.
30 In some embodiments, the linker is covalently attached to an antisense strand of the siRNA. In some embodiments, the PEG linker is a triethylene glycol (TrEG) linker. In some embodiments, the PEG linker is a tetraethylene glycol (TEG) linker.

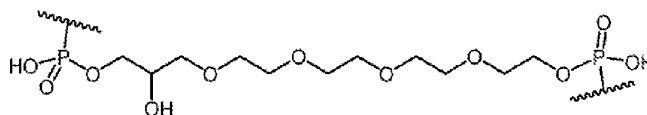
In some embodiments, the linker is an alkyl chain linker. In some embodiments, the linker is a peptide linker. In some embodiments, the linker is an RNA linker. In some embodiments, the linker
35 is a DNA linker.

Linkers may covalently link 2, 3, 4, or 5 unique siRNA strands. The linker may covalently bind to any part of the siRNA oligomer. In some embodiments, the linker attaches to the 3' end of nucleosides of each siRNA strand. In some embodiments, the linker attaches to the 5' end of nucleosides of each siRNA strand. In some embodiments, the linker attaches to a nucleoside of an
40 siRNA strand (e.g., sense or antisense strand) by way of a covalent bond-forming moiety. In some

embodiments, the covalent-bond-forming moiety is selected from the group consisting of an alkyl, ester, amide, carbonate, carbamate, triazole, urea, formacetal, phosphonate, phosphate, and phosphate derivative (e.g., phosphorothioate, phosphoramidate, etc.).

In some embodiments, the linker has a structure of Formula L1:

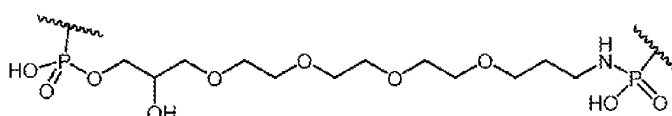
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(Formula L1)

In some embodiments, the linker has a structure of Formula L2:

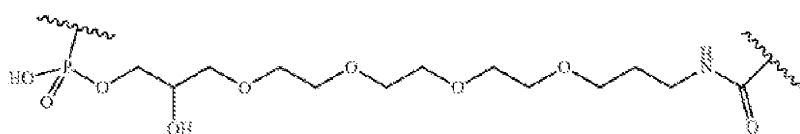
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(Formula L2)

In some embodiments, the linker has a structure of Formula L3:

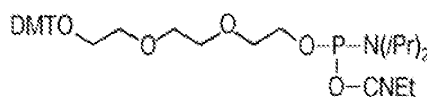
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(Formula L3)

In some embodiments, the linker has a structure of Formula L4:

20



(Formula L4)

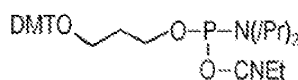
In some embodiments, the linker has a structure of Formula L5:

25



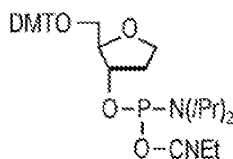
(Formula L5)

In some embodiments, the linker has a structure of Formula L6:



(Formula L6)

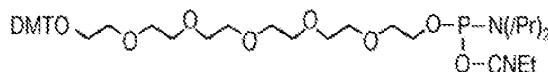
In some embodiments, the linker has a structure of Formula L7, as is shown below:



(Formula L7)

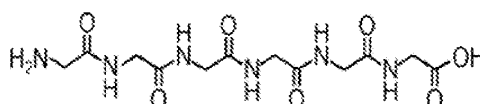
In some embodiments, the linker has a structure of Formula L8:

5



(Formula L8)

In some embodiments, the linker has a structure of Formula L9:



(Formula L9)

In some embodiments, the selection of a linker for use with one or more of the branched siRNA molecules disclosed herein may be based on the hydrophobicity of the linker, such that, e.g., desirable hydrophobicity is achieved for the one or more branched siRNA molecules of the disclosure.

15 For example, a linker containing an alkyl chain may be used to increase the hydrophobicity of the branched siRNA molecule as compared to a branched siRNA molecule having a less hydrophobic linker or a hydrophilic linker.

The siRNA agents disclosed herein may be synthesized and/or modified by methods well established in the art, such as those described in Beaucage, S. L. et al. (eds.), *Current Protocols in Nucleic Acid Chemistry*, John Wiley & Sons, Inc., New York, N.Y., 2000, which is hereby incorporated herein by reference.

Methods of Treatment

The *HTT*-targeting siRNA molecules of the disclosure may be delivered to a subject, thereby treating Huntington's Disease and/or mitigating Huntington's disease associated phenotypes (e.g., movement disorders such as chorea and dystonia, cognitive impairments, and psychiatric disorders such as depression and anxiety). Furthermore, the siRNA molecules of the disclosure may also be delivered to a subject having a variant of the *HTT* gene for which siRNA-mediated gene silencing of the *HTT* variant gene reduces the expression level of *HTT* transcript, thereby treating Huntington's Disease.

The disclosure provides methods of treating a subject by way of *HTT* gene silencing with one or more of the siRNA molecules described herein. The gene silencing may be performed in a subject to silence wild type *HTT* transcripts, mutant *HTT* transcripts, splice isoforms of *HTT* transcripts, and/or overexpressed *HTT* transcripts thereof, relative to a healthy subject. The method may include

delivering to the CNS or affected tissues of the subject (e.g., a human) the siRNA molecules of the disclosure or a pharmaceutical composition containing the same by any appropriate route of administration (e.g., intracerebroventricular, intrathecal injection, intrastriatal injection, intra-cisterna magna injection by catheterization, intraparenchymal injection, intravenous injection, subcutaneous injection, or intramuscular injection). The active compound can be administered in any suitable dose. The actual dosage amount of a composition of the present disclosure administered to a patient can be determined by physical and physiological factors such as body weight, severity of condition, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. Depending upon the dosage and the route of administration, the number of administrations of a preferred dosage and/or an effective amount may vary according to the response of the subject. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject. Administration may occur any suitable number of times per day, and for as long as necessary. Subjects may be adult or pediatric humans, with or without comorbid diseases.

Selection of Subjects

Subjects that may be treated with the siRNA molecules disclosed herein are subjects in need of treatment of, for example, Huntington's Disease, and/or any other medical risk(s) associated with a pathological mutation in the *HTT* gene. Subjects that may be treated with the siRNA molecules disclosed herein may include, for example, humans, monkeys, rats, mice, pigs, and other mammals containing at least one orthologous copy of the *HTT* gene. Subjects may be adult or pediatric humans, with or without comorbid diseases.

Osmolality

Administration of an siRNA molecule of the disclosure may influence the osmolality of a subject (e.g., of cerebrospinal fluid (CSF)). CSF osmolality of subjects being treated with an siRNA molecule of the disclosure may be, for example, from 250 to 450 mOsmol/kg. In some embodiments, the CSF osmolality is from 250 to 350 mOsmol/kg. In an siRNA molecule formulated as a salt with one or more divalent cations, the CSF osmolality of the subject may be affected by the concentration of the divalent cation. A person overseeing treatment of a subject may be able to monitor the CSF osmolality of the subject and adjust the dosage accordingly. For example, the dose can be decreased in a subject exhibiting a higher-than-normal osmolality.

Alternatively, the concentration of sodium ions in the composition containing the siRNA molecule can be altered. For example, in a liquid formulation of an siRNA molecule, the concentration of sodium may be modulated to increase or decrease the resulting osmolality, without having a negative effect on the toxicity benefit of the divalent cation. Reducing the level of sodium in a formulation may allow for the maintenance of normal physiological osmolality levels in subjects undergoing treatment with a therapeutic oligonucleotide of the disclosure.

Pharmaceutical Compositions

The siRNA molecules in the present disclosure may be formulated into a pharmaceutical composition for administration to a subject in a biologically compatible form suitable for administration *in vivo*. Accordingly, the present disclosure provides a pharmaceutical composition containing a siRNA molecule of the disclosure in admixture with a suitable diluent, carrier, or excipient. The siRNA molecules may be administered, for example, directly into the CNS or affected tissues of the subject (e.g., by way of intracerebroventricular, intrastriatally, intrathecal injection, intra-cisterna magna injection by catheterization, intraparenchymal injection, intravenous injection, subcutaneous injection, or intramuscular injection).

Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington, J.P. *The Science and Practice of Pharmacy*, Easton, PA. Mack Publishers, 2012, 22nd ed. and in The United States Pharmacopeial Convention, *The National Formulary*, United States Pharmacopeial, 2015, USP 38 NF 33).

Under ordinary conditions of storage and use, a pharmaceutical composition may contain a preservative, e.g., to prevent the growth of microorganisms. Pharmaceutical compositions may include sterile aqueous solutions, dispersions, or powders, e.g., for the extemporaneous preparation of sterile solutions or dispersions. In all cases the form may be sterilized using techniques known in the art and may be fluidized to the extent that may be easily administered to a subject in need of treatment.

A pharmaceutical composition may be administered to a subject, e.g., a human subject, alone or in combination with pharmaceutically acceptable carriers, as noted herein, the proportion of which may be determined by the solubility and/or chemical nature of the compound, chosen route of administration, and standard pharmaceutical practice.

Dosing Regimens

A physician having ordinary skill in the art can readily determine an effective amount of the siRNA molecule for administration to a mammalian subject (e.g., a human) in need thereof. For example, a physician could start prescribing doses of one the siRNA molecules of the disclosure at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. Alternatively, a physician may begin a treatment regimen by administering one of the siRNA molecules of the disclosure at a high dose and subsequently administer progressively lower doses until reaching a minimal dosage at which a therapeutic effect is achieved (e.g., a reduction in expression of a target gene sequence). In general, a suitable daily dose of one of the siRNA molecules of the disclosure will be an amount of the siRNA molecule which is the lowest dose effective to produce a therapeutic effect. The ss- or ds-siRNA molecules of the disclosure may be administered by injection, e.g., intrathecally, intracerebroventricularly, by intra-cisterna magna injection by catheterization, intraparenchymally, intravenously, subcutaneously, or intramuscularly. A daily dose of a therapeutic composition of the siRNA molecules of the disclosure may be administered as a single dose or as two, three, four, five, six or more doses administered separately at appropriate intervals throughout the day, week, month, or year, optionally, in unit

dosage forms. While it is possible for the siRNA molecules of the disclosure to be administered alone, it may also be administered as a pharmaceutical formulation in combination with excipients, carriers, and optionally, additional therapeutic agents.

5 *Routes of Administration*

The method of the disclosure contemplates any route of administration tolerated by the therapeutic composition. Some embodiments of the method include injection intrathecally, intracerebroventricularly, intrastratially, intraparenchymally, or by intra-cisterna magna injection by catheterization.

10 Intrathecal injection is the direct injection into the spinal column or subarachnoid space. By injecting directly into the CSF of the spinal column the siRNA molecules of the disclosure have direct access to cells (e.g., neurons and glial cells) in the spinal column and a route to access the cells in the brain by bypassing the blood brain barrier.

15 Intracerebroventricular (ICV) injection is a method to directly inject into the CSF of the cerebral ventricles. Similar to intrathecal injection, ICV is a method of injection which bypasses the blood brain barrier. Using ICV allows the advantage of access to the cells of the brain and spinal column without the danger of the therapeutic being degraded in the blood.

20 Intrastratial injection is the direct injection into the striatum, or corpus striatum. The striatum is an area in the subcortical basal ganglia in the brain. Injecting into the striatum bypasses the blood brain barrier and the pharmacokinetic challenges of injection into the blood stream and allows for direct access to the cells of the brain.

25 Intraparenchymal administration is the direct injection into the parenchyma (e.g., the brain parenchyma). Injection into the brain parenchyma allows for injection directly into brain regions affected by a disease or disorder while bypassing the blood brain barrier.

30 Intra-cisterna magna injection by catheterization is the direct injection into the cisterna magna. The cisterna magna is the area of the brain located between the cerebellum and the dorsal surface of the medulla oblongata. Injecting into the cisterna magna results in more direct delivery to the cells of the cerebellum, brainstem, and spinal cord.

35 In some embodiments of the methods described herein, the therapeutic composition may be delivered to the subject by way of systemic administration, e.g., intravenously, intramuscularly, or subcutaneously.

40 Intravenous (IV) injection is a method to directly inject into the bloodstream of a subject. The IV administration may be in the form of a bolus dose or by way of continuous infusion, or any other method tolerated by the therapeutic composition.

 Intramuscular (IM) injection is injection into a muscle of a subject, such as the deltoid muscle or gluteal muscle. IM may allow for rapid absorption of the therapeutic composition.

 Subcutaneous injection is injection into subcutaneous tissue. Absorption of compositions delivered subcutaneously may be slower than IV or IM injection, which may be beneficial for compositions requiring continuous absorption.

Examples

The following examples are put forth so as to provide those of ordinary skill in the art with a description of how the compositions and methods described herein may be used, made, and evaluated, and are intended to be purely exemplary of the disclosure and are not intended to limit the scope of what the inventors regard as their disclosure.

Example 1. *HTT* Knockdown with siRNA Molecules of the Disclosure in Mice

Introduction

The purpose of these experiments was to test the ability of siRNA molecules of the disclosure to silence *HTT*. The siRNA molecules targeted a portion of the *HTT* mRNA transcript having a nucleobase sequence of any one of SEQ ID NOs: 1-3. The siRNA molecules had a sense strand having a nucleobase sequence of any one of SEQ ID NOs: 4-6 and an antisense strand having a nucleobase sequence of any one of SEQ ID NOs: 7-9. The siRNA molecules were branched (e.g., a di-branched siRNA molecule having the structure of Formula XVII, Formula XVIII, or Formula XIX, a tri-branched siRNA molecule having the structure of Formula XX, Formula XXI, Formula XXII, or Formula XXIII, or a tetra-branched siRNA molecule having the structure of Formula XXIV, Formula XXV, Formula XXVI, Formula XXVII, or Formula XXVIII). Each siRNA molecule was a ds-siRNA molecule having an antisense strand and a sense strand. The antisense strands had patterns of modifications (e.g., the general structure of Formula I, Formula II, Formula IV, or Formula VI, or the specific structure of Formula A1, A2, A3, or A4). The sense strands also had patterns of modifications (e.g., the general structure of Formula III, Formula V, or Formula VII, or the specific structure of Formula S1, S2, S3, S4, S5, S6, S7, S8, or S9).

Materials and Methods

Mice were treated with either 0.25 or 2.5 nmol of a di-branched siRNA molecule having the structure of Formula XVII. The antisense strand had the nucleobase sequence of SEQ ID NO: 7 and the sense strand had the nucleobase sequence of SEQ ID NO: 4. The siRNA molecules were administered to FBV/NJ mice by way of a bilateral intracerebroventricular injection. 5 μ L was administered per side of the bilateral injection at a flow rate of 0.5 μ L/minute. *HTT* mRNA knockdown was quantified by qRT-PCR. *HTT* protein knockdown was quantified by Western Blot. siRNA accumulation was quantified by stem loop PCR.

Results – HTT mRNA knockdown

Mice treated with an siRNA molecule having the nucleobase sequence as described above and (i) an antisense strand of Formula II (particularly Formula A2) and a sense strand of Formula III (particularly Formula S2), or (ii) an antisense strand of Formula IV (particularly Formula A3) and a sense strand of Formula V (particularly Formula S6). The mice were analyzed for the levels of *HTT* mRNA in various brain regions (frontal cortex, motor cortex, striatum, and hippocampus). The accumulation of the siRNA molecule in the same brain regions was also analyzed. Data was collected after 3 days (FIGS. 1A and 1B), 7 days (FIGS. 2A and 2B), 14 days (FIGS. 3A and 3B).

These patterns each showed dose dependent knockdown of *HTT* and accumulation of the siRNA molecules.

After 1 month, mice that were treated with patterns (i) and (ii) above were analyzed in addition to (iii) an antisense strand of Formula II/Formula A2' and a sense strand of Formula III/Formula S2.

5 Again, dose dependent knockdown (FIG. 4A) and accumulation (FIG. 4B) were observed.

After 2 months and 3 months, mice that were treated with patterns (i), (ii), and (iii) above were analyzed in addition to (iv) an antisense strand of Formula II (particularly Formula A2) and a sense strand of Formula III (particularly Formula S1), (v) an antisense strand of Formula IV (particularly Formula A3) and a sense strand of Formula V (particularly Formula S8). Yet again, dose dependent
10 knockdown (FIGS. 5A and 6A) and accumulation (FIGS. 5B and 6B) was observed. These data show that there is a correlation between knockdown and accumulation across the different patterns, and that pattern (iv) shows the best knockdown of all tested compounds.

The above data for patterns (i) and (ii) was plotted together showing the time course of the knockdown at the 2.5nmol dose level (FIG. 7A) and the siRNA accumulation at the 2.5nmol dose level
15 (FIG. 7B) across the different brain regions. The same was plotted for the 0.25 nmol dose (FIGS. 7C and 7D). These data show a time-dependent decrease in the *HTT* transcript across brain regions with peak knockdown being achieved at one month. There was also a time-dependent decrease in accumulation.

Patterns (i), (ii), (iii), (iv), and (v) were tested over 6M, with the results shown in FIG. 8A (mRNA knockdown) and FIG. 8B (siRNA accumulation). These data indicate that pattern (iv), having
20 an antisense strand of Formula A2 and a sense strand of Formula S1, exhibited superior properties. This pattern was plotted in isolation at the 2.5 and 0.25 nmol dose across several brain regions (FIGS. 9A-9F). *HTT* mRNA levels, *HTT* protein levels, and the amount of siRNA were calculated. At 2.5nmol, strong knockdown was observed, with the effect more pronounced when measuring the total
25 protein. The amount of protein and transcript returned toward baseline approximately in parallel.

Finally, *HTT* knockdown in the kidney (FIG. 10A) and liver (FIG. 10B) was measured. At all time points, <20% knockdown was observed in the liver with <10% observed in the kidney.

Example 2. *HTT* Knockdown with siRNA Molecules of the Disclosure in Mice

30 Introduction

The purpose of these experiments was to test the ability of different siRNA molecules of the disclosure at different doses from those tested in Example 1 to silence *HTT*. The siRNA molecules targeted a portion of the *HTT* mRNA transcript having a nucleobase sequence of any one of SEQ ID
35 NOs: 1-3. The siRNA molecules had a sense strand having a nucleobase sequence of any one of SEQ ID NOs: 4-6 and an antisense strand having a nucleobase sequence of any one of SEQ ID NOs: 7-9. The siRNA molecules were branched (e.g., a di-branched siRNA molecule having the structure of Formula XVII, Formula XVIII, or Formula XIX, a tri-branched siRNA molecule having the structure of Formula XX, Formula XXI, Formula XXII, or Formula XXIII, or a tetra-branched siRNA molecule having the structure of Formula XXIV, Formula XXV, Formula XXVI, Formula XXVII, or Formula
40 XXVIII). Each siRNA molecule was a ds-siRNA molecule having an antisense strand and a sense

strand. The antisense strands had patterns of modifications (e.g., the general structure of Formula I, Formula II, Formula IV, or Formula VI, or the specific structure of Formula A1, A2, A3, or A4). The sense strands also had patterns of modifications (e.g., the general structure of Formula III, Formula V, or Formula VII, or the specific structure of Formula S1, S2, S3, S4, S5, S6, S7, S8, or S9).

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Materials and Methods

Mice were treated with either 0.2, 1.0, or 5.0 nmol of a di-branched siRNA molecule having the structure of Formula XVII. The antisense strand had the nucleobase sequence of SEQ ID NO: 7 and the sense strand had the nucleobase sequence of SEQ ID NO: 4. The siRNA molecules were administered to FBV/NJ mice by way of a unilateral intracerebroventricular injection. 5µL was administered at a flow rate of 0.5 µL/minute. *HTT* mRNA knockdown was quantified by qRT-PCR. *HTT* protein knockdown was quantified by Western Blot. siRNA accumulation was quantified by stem loop PCR.

15 *Results*

Mice were treated with 5 nmol of an siRNA molecule having the patterns of (i) an antisense strand of Formula II (particularly Formula A2) and a sense strand of Formula III (particularly Formula S2), (ii) an antisense strand of Formula IV (particularly Formula A3) and a sense strand of Formula V (particularly Formula S6), (iv) an antisense strand of Formula II (particularly Formula A2) and a sense strand of Formula III (particularly Formula S1), (v) an antisense strand of Formula III (particularly Formula A3) and a sense strand of Formula V (particularly Formula S8), (vi) an antisense strand of Formula IV (particularly Formula A3) and a sense strand of Formula V (particularly Formula S5), (vii) an antisense strand of Formula IV (particularly Formula A3) and a sense strand of Formula V (particularly Formula S7), (viii) an antisense strand of Formula II (particularly Formula A2) and a sense strand of Formula III (particularly Formula S4), and (ix) an antisense strand of Formula VI (particularly Formula A4) and a sense strand of Formula VII (particularly Formula S9). The results are shown in FIG. 11. Each of these patterns exhibited knockdown of *HTT* after six months across various brain regions, with the effect most pronounced in the hippocampus.

30 **Example 3. *HTT* Knockdown, Pharmacokinetics, and Pharmacodynamics of siRNA Molecules of the Disclosure in Non-human Primates**

Introduction

The purpose of these experiments was to test the ability of different siRNA molecules of the disclosure to silence *HTT*. The pharmacokinetic (PK) and pharmacodynamic (PD) properties were also investigated. The siRNA molecules targeted a portion of the *HTT* mRNA transcript having a nucleobase sequence of any one of SEQ ID NOs: 1-3. The siRNA molecules had a sense strand having a nucleobase sequence of any one of SEQ ID NOs: 4-6 and an antisense strand having a nucleobase sequence of any one of SEQ ID NOs: 7-9. The siRNA molecules were branched (e.g., a di-branched siRNA molecule having the structure of Formula XVII, Formula XVIII, or Formula XIX, a tri-branched siRNA molecule having the structure of Formula XX, Formula XXI, Formula XXII, or

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Formula XXIII, or a tetra-branched siRNA molecule having the structure of Formula XXIV, Formula XXV, Formula XXVI, Formula XXVII, or Formula XXVIII). Each siRNA molecule was a ds-siRNA molecule having an antisense strand and a sense strand. The antisense strands had patterns of modifications (e.g., the general structure of Formula I, Formula II, Formula IV, or Formula VI, or the specific structure of Formula A1, A2, A3, or A4). The sense strands also had patterns of modifications (e.g., the general structure of Formula III, Formula V, or Formula VII, or the specific structure of Formula S1, S2, S3, S4, S5, S6, S7, S8, or S9).

Materials and Methods

Animals were treated with varying doses of a di-branched siRNA molecule having the structure of Formula XVII. The antisense strand had the nucleobase sequence of SEQ ID NO: 7 and the sense strand had the nucleobase sequence of SEQ ID NO: 4. The siRNA molecules were administered by way of either an intrathecal or intracerebroventricular injection. The antisense strand had the structure of Formula II (particularly Formula A2), and the sense strand had the structure of Formula III (particularly Formula S1). In some experiments, the siRNA molecule was formulated as a salt. In these experiments, the cation was Mg²⁺, the ratio of di-siRNA to Mg²⁺ was 1:25, and the concentration of Mg²⁺ was 33 mM. The conditions tested are given in Table 3, below.

Table 3

Group No.	No. Animals	Treatment	Dose Level (mg)*	Route of Administration	Time point
1	3	Vehicle	N/A	IT-L	1 mo.
2	1	di-siRNA	50	IT-L	Sac.
	1		18	IT-L	1 mo.
	1		12.5	IT-L	1 mo.
3	3		12.5	IT-L	2 mo.
4	3		12.5	IT-L	3 mo.
5	3	di-siRNA formulated as a salt	100	ICV	3 mo.
6	3		100	IT-L	1 mo.
7	3		100	IT-L	2 mo.
8	3		100	IT-L	3 mo.

Results

Various brain regions were analyzed for percent *HTT* mRNA expression relative to PBS control, percent *HTT* protein expression relative to PBS control, and amount of siRNA. Regions examined were the frontal cortex, motor cortex, temporal cortex, hippocampus, caudate, putamen, substantia nigra, pons, and medulla (FIGS. 12A-12C), as well as the cerebellum cortex (FIGS. 13A-

13C) and cerebellum deep nucleus (FIGS. 13D-13F). Other tissues analyzed were the liver (FIGS. 14A-14C), kidney (FIGS. 15A-15C), and spinal cord (FIGS. 16A-16C). The amount of siRNA in the cerebrospinal fluid (CSF) and plasma was also determined (FIGS. 17A-17N) for all groups treated.

Taken together, these data show *HTT* was silenced at all doses tested. Furthermore, formulating the siRNA molecule as a salt enabled dosing at 100mg with no acute neurological symptoms. The siRNA molecules exhibited durable gene silencing throughout the brain and spinal cord when administered as a salt, even in deep brain regions, while also exhibiting limited pharmacodynamic effect in the liver and kidney. Intrathecal administration behaved equivalently to intracerebroventricular administration. Furthermore, animals with strong target silencing had more siRNA molecule found in the CSF and less in plasma. A high concentration of siRNA molecule in the CSF was observed and sustained for 1 week.

Example 4. *HTT* Knockdown with siRNA Molecules of the Disclosure in a 1-Month Study in Non-human Primates

Introduction

The purpose of these experiments was to test the ability of different siRNA molecules of the disclosure to silence *HTT*. The pharmacokinetic (PK) and pharmacodynamic (PD) properties were also investigated. The siRNA molecules targeted a portion of the *HTT* mRNA transcript having a nucleobase sequence of any one of SEQ ID NOs: 1-3. The siRNA molecules had a sense strand having a nucleobase sequence of any one of SEQ ID NOs: 4-6 and an antisense strand having a nucleobase sequence of any one of SEQ ID NOs: 7-9. The siRNA molecules were branched (e.g., a di-branched siRNA molecule having the structure of Formula XVII, Formula XVIII, or Formula XIX, a tri-branched siRNA molecule having the structure of Formula XX, Formula XXI, Formula XXII, or Formula XXIII, or a tetra-branched siRNA molecule having the structure of Formula XXIV, Formula XXV, Formula XXVI, Formula XXVII, or Formula XXVIII). Each siRNA molecule was a ds-siRNA molecule having an antisense strand and a sense strand. The antisense strands had patterns of modifications (e.g., the general structure of Formula I, Formula II, Formula IV, or Formula VI, or the specific structure of Formula A1, A2, A3, or A4). The sense strands also had patterns of modifications (e.g., the general structure of Formula III, Formula V, or Formula VII, or the specific structure of Formula S1, S2, S3, S4, S5, S6, S7, S8, or S9).

Materials and Methods

Naïve animals were treated with varying doses of a di-branched siRNA molecule having the structure of Formula XVII. The antisense strand had the nucleobase sequence of SEQ ID NO: 7 and the sense strand had the nucleobase sequence of SEQ ID NO: 4. The siRNA molecules were administered by way of either an intrathecal (IT) or intracerebroventricular (ICV) injection. The antisense strand had the structure of Formula II (particularly Formula A2), and the sense strand had the structure of Formula III (particularly Formula S1). In some experiments, the siRNA molecule was formulated as a salt. In these experiments, the cation was Mg^{2+} and the ratio of di-siRNA to Mg^{2+} was 1:25. The concentration of Mg^{2+} was 16.7 mM for animals dosed at 50mg, 8.3 mM for animals dosed

at 25mg, and 4.2 mM for animals dosed at 12.5 mg. The conditions tested are given in Table 4, below.

Table 4

Group No.	No. Animals	Treatment	Dose Level (mg)*	Route of Administration	Tissue Collection
1	3	vehicle	N/A	IT-L: Slow	1 month
2	4	di-siRNA formulated as a salt	50	bolus injection through a pre-implanted intrathecal catheter	1 month
3	4		25		1 month
4	4		12.5		1 month
5	4	di-siRNA formulated as a salt	25	ICV	1 month

5

Results

Various brain regions were analyzed for percent *HTT* mRNA expression relative to PBS control, percent *HTT* protein expression relative to PBS control, and amount of siRNA. Regions examined were the frontal cortex (FIGS. 18A-18C), motor cortex (FIGS. 19A-19C), temporal cortex (FIGS. 20A-20C), hippocampus (FIGS. 21A-21C), putamen (FIGS. 22A-22C), caudate (FIGS. 23A-23C), substantia nigra (FIGS. 24A-24C), pons (FIGS. 25A-25C), and medulla (FIGS. 26A-26C). Other tissues analyzed were the liver (FIGS. 27A-27C), and kidney (FIGS. 28A-28C), which did not show substantial silencing. *HTT* mRNA and *HTT* protein expression were also measured in the spinal cord (FIGS. 29A and 29B). Finally, *HTT* mRNA expression was measured in the cerebellar cortex (FIG. 30) and deep nucleus (FIG. 31). Furthermore, accumulation and clearance of the di-siRNA molecule in the plasma and CSF was tested in each of the four treatment groups (group numbers 2-5 in table 4, above; FIGS. 32A-32B).

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Taken together, these data show *HTT* was silenced at all doses tested. Consistent with the data presented in Example 3, 50-75% protein knockdown was observed in all cortical areas and in the hippocampus. 25-50% protein knockdown was observed in the caudate putamen, substantia nigra, pons, and medulla when the siRNA was administered via IT-L delivery, whereas approximately 50% knockdown was observed when the siRNA was administered via intracerebroventricular delivery. Furthermore, low amounts of knockdown were observed in liver and kidney, and the siRNA molecule was cleared from the plasma within hours, whereas it persisted in the CSF for approximately 2 weeks.

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Example 5. Evaluation of siRNA molecules at various time points in non-human primates

Introduction

The purpose of these experiments was to evaluate the tissue distribution and pharmacokinetics (PK) of siRNA molecules of the disclosure to silence *HTT*. The siRNA molecules

targeted a portion of the *HTT* mRNA transcript having a nucleobase sequence of any one of SEQ ID NOs: 1-3. The siRNA molecules had a sense strand having a nucleobase sequence of any one of SEQ ID NOs: 4-6 and an antisense strand having a nucleobase sequence of any one of SEQ ID NOs: 7-9. The siRNA molecules were branched (e.g., a di-branched siRNA molecule having the structure of Formula XVII, Formula XVIII, or Formula XIX, a tri-branched siRNA molecule having the structure of Formula XX, Formula XXI, Formula XXII, or Formula XXIII, or a tetra-branched siRNA molecule having the structure of Formula XXIV, Formula XXV, Formula XXVI, Formula XXVII, or Formula XXVIII). Each siRNA molecule was a ds-siRNA molecule having an antisense strand and a sense strand. The antisense strands had patterns of modifications (e.g., the general structure of Formula I, Formula II, Formula IV, or Formula VI, or the specific structure of Formula A1, A2, A3, or A4). The sense strands also had patterns of modifications (e.g., the general structure of Formula III, Formula V, or Formula VII, or the specific structure of Formula S1, S2, S3, S4, S5, S6, S7, S8, or S9).

Materials and Methods

Naïve cynomolgus macaques were treated with varying doses of a di-branched siRNA molecule having the structure of Formula XVII. The antisense strand had the nucleobase sequence of SEQ ID NO: 7 and the sense strand had the nucleobase sequence of SEQ ID NO: 4.

The siRNA molecules were administered as either a single, 50mg dose (groups 2-5, below) of the siRNA molecule or three separate 50mg doses of the siRNA molecule administered, with the second and third doses being given 3 months and 6 months after the initial dose, respectively. The doses were administered as an intrathecal bolus injection. The antisense strand had the structure of Formula II (particularly Formula A2), and the sense strand had the structure of Formula III (particularly Formula S1). The conditions tested are given in Table 5, below.

Table 5

Group	Dose (mg)	Time Point (months)	N
1	N/A (vehicle)	3	3
2	50	1	5
3		3	5
4		4	5
5		6	5
6	3x 50*	7	4

Animals dosed in this experiment were 3-7 year old males. Animals were dosed as indicated in Table 5, above, through a surgically pre-implanted CSF (cerebrospinal fluid) catheter and port system with the catheter tip positioned in the mid thoracic region. One animal from group 3 and two

animals from group 4 displayed obstructed catheters at the time of dosing. These animals were preidentified to be at risk of diminished drug administered into the intrathecal space. Upon study completion, it was determined that there was little to no di-siRNA exposure in CNS, including all brain regions evaluated and lumbar spinal cord. An alternate analysis of the CNS tissues is also provided, where these animals (referred to as “outlier animals”) have been removed from the data set.

HTT mRNA expression was determined by quantitative real-time PCR (qRT-PCR). Data was normalized to housekeeping gene (PPIA), and % expression was calculated based on PBS-treated animals. HTT protein expression was determined by enzyme-linked immunosorbent assay (ELISA). Data was normalized to total protein content using BCA assay, and % expression was calculated based on PBS-treated animals. siRNA was quantified using a locked nucleic acid-based hybridization assay using Meso-scale Discovery for detection.

Results

Various brain regions were analyzed for percent *HTT* mRNA expression relative to PBS control, percent HTT protein expression relative to PBS control, and amount of siRNA. Regions examined were the frontal cortex (FIGS. 33A-33C), motor cortex (FIGS. 34A-34C), temporal cortex (FIGS. 35A-35C), caudate (FIGS. 36A-36C), putamen (FIGS. 37A-37C), hippocampus (FIGS. 38A-38C), cervical spinal cord (FIGS. 39A-39C), thoracic spinal cord (FIGS. 40A-40C), and lumbar spinal cord (FIGS. 41A-41C). The liver (FIGS. 42A-42C), and kidney (FIGS. 43A-43C), were also measured. A further analysis was conducted in which the outlier animals were removed, with those results shown for the frontal cortex (FIGS. 44A-44C), motor cortex (FIGS. 45A-45C), temporal cortex (FIGS. 46A-46C), caudate (FIGS. 47A-47C), putamen (FIGS. 48A-48C), hippocampus (FIGS. 49A-49C), cervical spinal cord (FIGS. 50A-50C), thoracic spinal cord (FIGS. 51A-51C), and lumbar spinal cord (FIGS. 52A-52C).

Taken together, these data show *HTT* was silenced at all doses tested. Additionally, the silencing persisted even at 6-months following administration.

Example 6. Generating *HTT*-targeting siRNA Molecules

The siRNA molecules of the disclosure 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.

The siRNA agent can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide including unnatural or modified nucleotides can be easily prepared. Specific examples of siRNA molecules, with the nucleotide sequence of the sense and antisense strand, as well as the Huntingtin (*HTT*) mRNA target sequence, are shown in Table 1, above. It is appreciated that one of skill in the art could anneal the antisense (AS) strand to the corresponding sense (S) strand to yield a ds-siRNA molecule. Alternatively, one of skill in the art could derive a ss-siRNA molecule using antisense strand only.

Example 7. Optimizing *HTT*-targeting siRNA Molecules

It is contemplated that for any small interfering RNA (siRNA) agent disclosed herein, modifications to the siRNA may further optimize the molecule's efficacy or biophysical properties (e.g., increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, and/or targeting to a particular location or cell type). Such optimization could be achieved by systematically either adding or removing linked nucleosides to generate longer or shorter sequences. Further siRNA optimization could include the incorporation of, for example, one or more alternative nucleosides, alternative 2' sugar moieties, and/or alternative internucleoside linkages. Further still, such optimized siRNA molecules may include the introduction of hydrophobic and/or stabilizing moieties at the 5' and/or 3' ends.

siRNA Optimization with Alternative Nucleosides

Optimization of the siRNA molecules of the disclosure may include one or more of the following nucleoside modifications: 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 (-C≡C-CH₃) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and 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, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine, and/or 3-deazaguanine and 3-deazaadenine. The siRNA molecules may also include nucleobases in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine, and/or 2-pyridone. Further optimization of the siRNA molecules of the disclosure may include nucleobases disclosed in US 3,687,808; Kroschwitz, J.I., ed. *The Concise Encyclopedia of Polymer Science and Engineering*, New York, John Wiley & Sons, 1990, pp. 858-859; Englisch et al., *Angewandte Chemie*, International Edition 30:613, 1991; and Sanghvi, Y.S., Chapter 16, *Antisense Research and Applications*, CRC Press, Gait, M.J. ed., 1993, pp. 289-302.

siRNA Optimization with Alternative Sugar Modifications

Optimization of the siRNA molecules of the disclosure may include one or more of the following 2' sugar modifications: 2'-O-methyl (2'-O-Me), 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE), 2'-dimethylaminoethoxy, i.e., a O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, and/or 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylamino-ethoxy-ethyl or 2'-DMAEOE), i.e., 2'-O-CH₂OCH₂N(CH₃)₂. Other possible 2'-modifications that can optimize the siRNA molecules of the disclosure include all possible orientations of 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 C1 to C10 alkyl or C2 to C10 alkenyl and alkynyl. Other potential sugar substituent groups include, e.g., aminopropoxy (-OCH₂CH₂CH₂NH₂), allyl (-CH₂-CH=CH₂), -O-allyl (-O-CH₂-CH=CH₂) and fluoro (F). 2'-sugar

substituent groups may be in the arabino (up) position or ribo (down) position. In some embodiments, the 2'-arabino modification is 2'-F. Similar modifications may also be made at other positions on the siRNA molecule, particularly the 3' position of the sugar on the 3' terminal nucleoside or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

siRNA Optimization with Alternative Internucleoside Linkages

Optimization of the siRNA molecules of the disclosure may include one or more of the following internucleoside modifications: phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage.

siRNA Optimization with Hydrophobic Moieties

Optimization of the siRNA molecules of the disclosure may include hydrophobic moieties covalently attached to the 5' end or the 3' end. Non-limiting examples of hydrophobic moieties suitable for use with the siRNA molecules of the disclosure may include cholesterol, vitamin D, tocopherol, phosphatidylcholine (PC), docosahexaenoic acid, docosanoic acid, PC-docosanoic acid, eicosapentaenoic acid, lithocholic acid or any combination of the aforementioned hydrophobic moieties with PC.

siRNA Optimization with Stabilizing Moieties

Optimization of the siRNA molecules of the disclosure may include a 5'-phosphorous stabilizing moiety that protects the siRNA molecules from degradation. A 5'-phosphorus stabilizing moiety replaces the 5'-phosphate to prevent hydrolysis of the phosphate. Hydrolysis of the 5'-phosphate prevents binding to RISC, a necessary step in gene silencing. Any replacement for phosphate that does not impede binding to RISC is contemplated in this disclosure. In some embodiments, the replacement for the 5'-phosphate is also stable to *in vivo* hydrolysis. Each siRNA strand may independently and optionally employ any suitable 5'-phosphorus stabilizing moiety. Non-limiting examples of 5' stabilizing moieties suitable for use with the siRNA molecules of the disclosure may include those demonstrated by Formulas IX-XVI above.

siRNA Optimization with Branched siRNA

Optimization of the siRNA molecules of the disclosure may include the incorporation of branching patterns, such as, for example, di-branched, tri-branched, or tetra-branched siRNAs connected by way of a linker. Each main branch may be further branched to allow for 2, 3, 4, 5, 6, 7, or 8 separate RNA single- or double-strands. The branch points on the linker may stem from the

same atom, or separate atoms along the linker. Some exemplary embodiments are listed in Table 2, above.

The siRNA composition of the disclosure may be optimized to be in the form of: di-branched siRNA molecules, as represented by any one of Formulas XVII-XIX; tri-branched siRNA molecules, as represented by any one of Formulas XX-XXIII; and/or tetra-branched siRNA molecules, as represented by any one of Formulas XXIV-XXVIII, wherein each RNA, independently, is an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety (e.g., phosphoroamidite, tosylated solketal, 1,3-diaminopropanol, pentaerythritol, or any one of the branch point moieties described in US 10,478,503).

Example 8. Preparing and Administering *HTT*-targeting siRNA Molecules

The siRNA molecules in the present disclosure may be formulated into a pharmaceutical composition for administration to a subject in a biologically compatible form suitable for administration *in vivo*. For example, the siRNA molecules of the disclosure may be administered in a suitable diluent, carrier, or excipient, and may further contain a preservative, e.g., to prevent the growth of microorganisms. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington, J.P. *The Science and Practice of Pharmacy*, Easton, PA. Mack Publishers, 2012, 22nd ed. and in The United States Pharmacopeial Convention, *The National Formulary*, United States Pharmacopeial, 2015, USP 38 NF 33).

The method of the disclosure contemplates any route of administration to the subject that is tolerated by the siRNA compositions of the disclosure. Non-limiting examples of siRNA injections into the CNS include intrathecally, intracerebroventricularly, intrastrially, intraparenchymally, or intracisterna magna injection by catheterization. Examples of systemic administration include intravenous, intramuscular, and subcutaneous injection. A physician having ordinary skill in the art can readily determine an effective route of administration.

Example 9. Methods for the Treatment of Huntington's Disease Using *HTT*-targeting siRNA Molecules

A subject in need of treatment of a Huntington's Disease is treated with a dosage of the siRNA molecule or siRNA composition of the disclosure, formulated as a salt, at frequency determined by a practitioner. A physician having ordinary skill in the art can readily determine an effective amount of the siRNA molecule for administration to a mammalian subject (e.g., a human) in need thereof. For example, a physician could start prescribing doses of one of the siRNA molecules of the disclosure at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. Alternatively, a physician may begin a treatment regimen by administering one of the siRNA molecules of the disclosure at a high dose and subsequently administer progressively lower doses until a minimum dose that produces a therapeutic effect (e.g., a reduction in expression of *HTT* mRNA or suitable biomarker) is achieved. In general, a suitable daily dose of one of one of the siRNA molecules of the disclosure will be an amount which is the lowest dose effective to produce a therapeutic effect. The ss- or ds-siRNA

molecules of the disclosure may be administered by injection, e.g., intrathecally, intracerebroventricularly, intrastrially, intraparenchymally, intravenously, intramuscularly, subcutaneously, or by intra-cisterna magna injection via catheterization. A daily dose of a therapeutic composition of one of the siRNA molecules of the disclosure may be administered as a single dose or
5 as two, three, four, five, six or more doses administered separately at appropriate intervals throughout the day, week, month, or year, optionally, in unit dosage forms. While it is possible for any of the siRNA molecules of the disclosure to be administered alone, it may also be administered as a pharmaceutical formulation in combination with excipients, carriers, and optionally, additional therapeutic agents. Dosage and frequency are determined based on the subject's height, weight,
10 age, sex, and other disorders.

The siRNA molecule(s) of the disclosure is selected by the practitioner for compatibility with the subject. Single- or double-stranded siRNA molecules (e.g., non-branched siRNA, di-branched siRNA, tri-branched siRNA, tetra-branched siRNA) are available for selection. The siRNA molecule chosen has an antisense strand and may have a sense strand with a sequence and RNA
15 modifications (e.g., natural and non-natural internucleoside linkages, modified sugars, 5'-phosphorus stabilizing moieties, hydrophobic moieties, and/or branching structures) best suited to the patient.

The siRNA molecule is delivered by the route best suited the patient (e.g., intrathecally, intracerebroventricularly, intrastrially, intraparenchymally, intravenously, intramuscularly, subcutaneously, or by intra-cisterna magna injection via catheterization) and condition at a rate
20 tolerable to the patient until the subject has reached a maximum tolerated dose, or until symptoms are ameliorated satisfactorily.

Other Embodiments

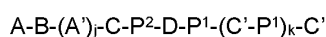
All publications, patents, and patent applications mentioned in this specification are
25 incorporated herein by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the
30 invention and including such departures from the invention that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are within the claims.

Claims

1. A small interfering RNA (siRNA) molecule comprising an antisense strand and a sense strand having complementarity to the antisense strand, wherein the antisense strand has complementarity sufficient to hybridize to a region of equal length within a Huntingtin (*HTT*) mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3, wherein the antisense strand comprises a structure represented by Formula I, wherein Formula I is, in the 5'-to-3' direction:

**Formula I;**

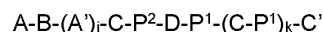
wherein A is represented by the formula C-P¹-D-P¹;
 each A', independently, is represented by the formula C-P²-D-P²;
 B is represented by the formula C-P²-D-P²-D-P²-D-P²;
 each C, independently, is a 2'-O-methyl (2'-O-Me) ribonucleoside;
 each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside;
 each D, independently, is a 2'-F ribonucleoside;
 each P¹ is, independently, a phosphorothioate internucleoside linkage;
 each P² is, independently, a phosphodiester internucleoside linkage;
 j is an integer from 1 to 7; and
 k is an integer from 1 to 7.

2. The siRNA molecule of claim 1, wherein the antisense strand comprises a structure represented by Formula A1, wherein Formula A1 is, in the 5'-to-3' direction:

**Formula A1;**

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

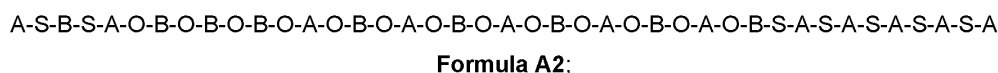
3. An siRNA molecule comprising an antisense strand and a sense strand having complementarity to the antisense strand, wherein the antisense strand has complementarity sufficient to hybridize to a region of equal length within a Huntingtin (*HTT*) mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3, wherein the antisense strand comprises a structure represented by Formula II, wherein Formula II is, in the 5'-to-3' direction:

**Formula II;**

wherein A is represented by the formula C-P¹-D-P¹;

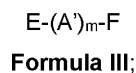
each A', independently, is represented by the formula C-P²-D-P²;
 B is represented by the formula C-P²-D-P²-D-P²-D-P²;
 each C, independently, is a 2'-O-methyl (2'-O-Me) ribonucleoside;
 each C', independently, is a 2'-O-Me ribonucleoside or a 2'-fluoro (2'-F) ribonucleoside;
 each D, independently, is a 2'-F ribonucleoside;
 each P¹ is, independently, a phosphorothioate internucleoside linkage;
 each P² is, independently, a phosphodiester internucleoside linkage;
 j is an integer from 1 to 7; and
 k is an integer from 1 to 7.

4. The siRNA molecule of claim 3, wherein the antisense strand comprises a structure represented by Formula A2, wherein Formula A2 is, in the 5'-to-3' direction:



wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

5. The siRNA molecule of any one of claims 1-4, wherein the sense strand comprises a structure represented by Formula III, wherein Formula III is, in the 5'-to-3' direction:

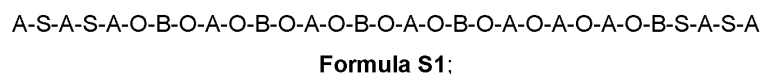


wherein E is represented by the formula (C-P¹)₂;
 F is represented by the formula (C-P²)₃-D-P¹-C-P¹-C, (C-P²)₃-D-P²-C-P²-C, (C-P²)₃-D-P¹-C-P¹-D, or (C-P²)₃-D-P²-C-P²-D;
 A', C, D, P¹, and P² are as defined in Formula II; and
 m is an integer from 1 to 7.

6. The siRNA molecule of any one of claims 1-5, wherein j is 4 and k is 4.

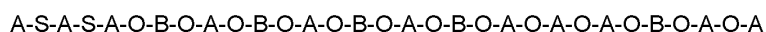
7. The siRNA molecule of claim 5 or claim 6, wherein m is 4.

8. The siRNA molecule of claim 5, wherein the sense strand comprises a structure represented by Formula S1, wherein Formula S1 is, in the 5'-to-3' direction:



wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

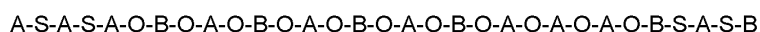
9. The siRNA molecule of claim 5, wherein the sense strand comprises a structure represented by Formula S2, wherein Formula S2 is, in the 5'-to-3' direction:



Formula S2;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

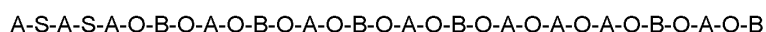
10. The siRNA molecule of claim 5, wherein the sense strand comprises a structure represented by Formula S3, wherein Formula S3 is, in the 5'-to-3' direction:



Formula S3;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

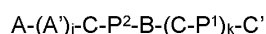
11. The siRNA molecule of claim 5, wherein the sense strand comprises a structure represented by Formula S4, wherein Formula S4 is, in the 5'-to-3' direction:



Formula S4;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

12. An siRNA molecule comprising an antisense strand and a sense strand having complementarity to the antisense strand, wherein the antisense strand has complementarity sufficient to hybridize to a region of equal length within a Huntingtin (*HTT*) mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3, wherein the antisense strand comprises a structure represented by Formula IV, wherein Formula IV is, in the 5'-to-3' direction:



Formula IV;

wherein A is represented by the formula C-P¹-D-P¹;
 each A', independently, is represented by the formula C-P²-D-P²;
 B is represented by the formula D-P¹-C-P¹-D-P¹;
 each C, independently, is a 2'-O-Me ribonucleoside;
 each C', independently, is a 2'-O-Me ribonucleoside or a 2'-F ribonucleoside;
 each D, independently, is a 2'-F ribonucleoside;
 each P¹ is, independently, a phosphorothioate internucleoside linkage;
 each P² is, independently, a phosphodiester internucleoside linkage;
 j is an integer from 1 to 7; and
 k is an integer from 1 to 7.

13. The siRNA molecule of claim 12, wherein the antisense strand comprises a structure represented by Formula A3, wherein Formula A3 is, in the 5'-to-3' direction:

A-S-B-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-B-S-A-S-A-S-A

Formula A3;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

14. The siRNA molecule of claim 12 or claim 13, wherein the sense strand comprises a structure represented by Formula V, wherein Formula V is, in the 5'-to-3' direction:

E-(A')_m-C-P²-F

Formula V;

wherein E is represented by the formula (C-P¹)₂;
 F is represented by the formula D-P¹-C-P¹-C, D-P²-C-P²-C, D-P¹-C-P¹-D, or D-P²-C-P²-D;
 A', C, D, P¹ and P² are as defined in Formula IV; and
 m is an integer from 1 to 7.

15. The siRNA molecule of claim 14, wherein j is 6 and k is 2.

16. The siRNA molecule of claim 14 or claim 15, wherein m is 5.

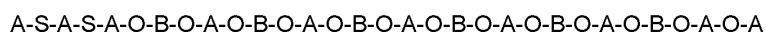
17. The siRNA molecule of claim 14, wherein the sense strand comprises a structure represented by Formula S5, wherein Formula S5 is, in the 5'-to-3' direction:

A-S-A-S-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-O-A-O-B-S-A-S-A

Formula S5;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

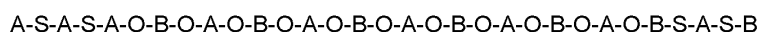
18. The siRNA molecule of claim 14, wherein the sense strand comprises a structure represented by Formula S6, wherein Formula S6 is, in the 5'-to-3' direction:



Formula S6;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

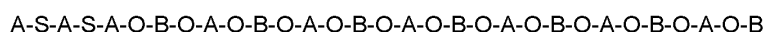
19. The siRNA molecule of claim 14, wherein the sense strand comprises a structure represented by Formula S7, wherein Formula S7 is, in the 5'-to-3' direction:



Formula S7;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

20. The siRNA molecule of claim 14, wherein the sense strand comprises a structure represented by Formula S8, wherein Formula S8 is, in the 5'-to-3' direction:



Formula S8;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

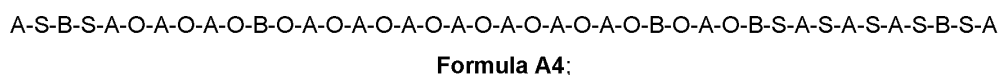
21. An siRNA molecule comprising an antisense strand and a sense strand having complementarity to the antisense strand, wherein the antisense strand has complementarity sufficient to hybridize to a region of equal length within a Huntingtin (*HTT*) mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3, wherein the antisense strand comprises a structure represented by Formula VI, wherein Formula VI is, in the 5'-to-3' direction:



Formula VI;

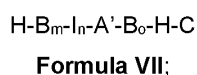
wherein A is represented by the formula C-P¹-D-P¹;
 each B, independently, is represented by the formula C-P²;
 each C, independently, is a 2'-O-Me ribonucleoside;
 each C', independently, is a 2'-O-Me ribonucleoside or a 2'-F ribonucleoside;
 each D, independently, is a 2'-F ribonucleoside;
 each E, independently, is represented by the formula D-P²-C-P²;
 F is represented by the formula D-P¹-C-P¹;
 each G, independently, is represented by the formula C-P¹;
 each P¹ is, independently, a phosphorothioate internucleoside linkage;
 each P² is, independently, a phosphodiester internucleoside linkage;
 j is an integer from 1 to 7;
 k is an integer from 1 to 7; and
 l is an integer from 1 to 7.

22. The siRNA molecule of claim 21, wherein the antisense strand comprises a structure represented by Formula A4, wherein Formula A4 is, in the 5'-to-3' direction:



wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

23. The siRNA molecule of claim 21 or claim 22, wherein the sense strand comprises is represented by Formula VII, wherein Formula VII is, in the 5'-to-3' direction:

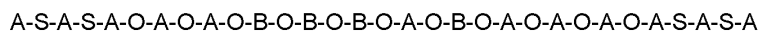


wherein A' is represented by the formula C-P²-D-P²;
 each H, independently, is represented by the formula (C-P¹)₂;
 each I, independently, is represented by the formula (D-P²);
 B, C, D, P¹ and P² are as defined in Formula VI;
 m is an integer from 1 to 7;
 n is an integer from 1 to 7; and
 o is an integer from 1 to 7.

24. The siRNA molecule of claim 23, wherein j is 3, k is 6, and l is 2.

25. The siRNA molecule of claim 23 or claim 24, wherein m is 3, n is 3, and o is 3.

26. The siRNA molecule of claim 23, wherein the sense strand comprises a structure represented by Formula S9, wherein Formula S9 is, in the 5'-to-3' direction:



Formula S9;

wherein A represents a 2'-O-Me ribonucleoside, B represents a 2'-F ribonucleoside, O represents a phosphodiester internucleoside linkage, and S represents a phosphorothioate internucleoside linkage.

27. The siRNA molecule of any one of claims 1-26, wherein the region of equal length within the *HTT* mRNA transcript has the nucleic acid sequence of SEQ ID NO: 1.

28. The siRNA molecule of any one of claims 1-26, wherein the region of equal length within the *HTT* mRNA transcript has the nucleic acid sequence of SEQ ID NO: 2.

29. The siRNA molecule of any one of claims 1-26, wherein the region of equal length within the *HTT* mRNA transcript has the nucleic acid sequence of SEQ ID NO: 3.

30. The siRNA molecule of any one of claims 1-29, wherein the antisense strand has at least 70% complementarity to a region of 19, 20, 21, or more contiguous nucleobases within the *HTT* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3, optionally wherein the antisense strand has at least 70% complementarity to the *HTT* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

31. The siRNA molecule of claim 30, wherein the antisense strand has at least 75% complementarity to a region within the *HTT* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3, optionally wherein the antisense strand has at least 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementarity to the region within the *HTT* mRNA transcript having the nucleic acid sequence of any one of SEQ ID Nos: 1-3.

32. The siRNA molecule of any one of claims 1-31, wherein the antisense strand comprises at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

33. The siRNA molecule of claim 32, wherein the antisense strand comprises from 10 to 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

34. The siRNA molecule of claim 33, wherein the antisense strand comprises from 12 to 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

35. The siRNA molecule of claim 34, wherein the antisense strand comprises from 15 to 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

36. The siRNA molecule of claim 35, wherein the antisense strand comprises from 18 to 30 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

37. The siRNA molecule of claim 36, wherein the antisense strand comprises from 18 to 21 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

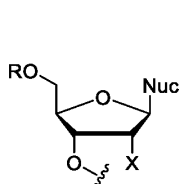
38. The siRNA molecule of claim 35, wherein the antisense strand comprises 15 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

39. The siRNA molecule of claim 37, wherein the antisense strand comprises 20 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

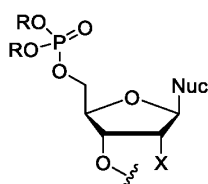
40. The siRNA molecule of claim 37, wherein the antisense strand comprises 21 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.

41. The siRNA molecule of any one of claims 1-40, wherein the antisense strand comprises 9 or fewer nucleotide mismatches relative to a region within the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3, optionally wherein the antisense strand comprises 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or only 1 mismatch relative to the region of the *HTT* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-3.
42. The siRNA molecule of any one of claims 30-41, wherein the region of the *HTT* RNA transcript has the nucleic acid sequence of SEQ ID NO: 1.
43. The siRNA molecule of any one of claims 30-41, wherein the region of the *HTT* RNA transcript has the nucleic acid sequence of SEQ ID NO: 2.
44. The siRNA molecule of any one of claims 30-41, wherein the region of the *HTT* RNA transcript has the nucleic acid sequence of SEQ ID NO: 3.
45. The siRNA molecule of any one of claims 1-44, wherein the antisense strand has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of any one of SEQ ID NOs: 7-9.
46. The siRNA molecule of claim 45, wherein the antisense strand has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of any one of SEQ ID NOs: 7-9.
47. The siRNA molecule of claim 46, wherein the antisense strand has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NOs: 7-9, optionally wherein the antisense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 7-9.
48. The siRNA molecule of claim 47, wherein the antisense strand has the nucleic acid sequence of any one of SEQ ID NOs: 7-9.
49. The siRNA molecule of any one of claims 45-48, wherein the nucleic acid sequence is SEQ ID NO: 7.
50. The siRNA molecule of any one of claims 45-48, wherein the nucleic acid sequence is SEQ ID NO: 8.
51. The siRNA molecule of any one of claims 45-48, wherein the nucleic acid sequence is SEQ ID NO: 9.

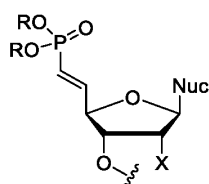
52. The siRNA molecule of any one of claims 1-51, wherein the sense strand has a nucleic acid sequence that is at least 85% identical to the nucleic acid sequence of any one of SEQ ID NOs: 4-6.
53. The siRNA molecule of claim 52, wherein the sense strand has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of any one of SEQ ID NOs: 4-6.
54. The siRNA molecule of claim 53, wherein the sense strand has a nucleic acid sequence that is at least 90% identical to the nucleic acid sequence of any one of SEQ ID NOs: 4-6, optionally wherein the sense strand has a nucleic acid sequence that is at least 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of any one of SEQ ID NOs: 4-6.
55. The siRNA molecule of claim 54, wherein the sense strand has the nucleic acid sequence of any one of SEQ ID NOs: 4-6.
56. The siRNA molecule of any one of claims 52-55, wherein the nucleic acid sequence is SEQ ID NO: 4.
57. The siRNA molecule of any one of claims 52-55, wherein the nucleic acid sequence is SEQ ID NO: 5.
58. The siRNA molecule of any one of claims 52-55, wherein the nucleic acid sequence is SEQ ID NO: 6.
59. The siRNA molecule of any one of claims 1-58, wherein the antisense strand further comprises a 5' phosphorus stabilizing moiety at the 5' end of the antisense strand.
60. The siRNA molecule of any one of claims 1-59, wherein the sense strand further comprises a 5' phosphorus stabilizing moiety at the 5' end of the sense strand.
61. The siRNA molecule of claim 59 or 60, wherein each 5' phosphorus stabilizing moiety is, independently, represented by any one of Formulas IX-XVI:



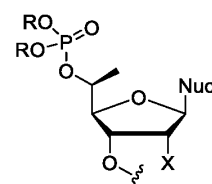
Formula IX



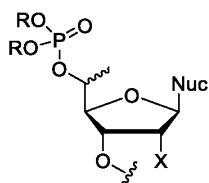
Formula X



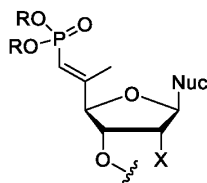
Formula XI



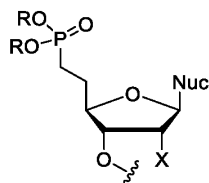
Formula XII



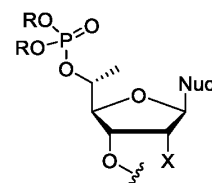
Formula XIII



Formula XIV



Formula XV



Formula XVI

wherein Nuc represents a nucleobase, optionally wherein the nucleobase is selected from the group consisting of adenine, uracil, guanine, thymine, and cytosine, and R represents an optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, phenyl, benzyl, a cation, or hydrogen.

62. The siRNA molecule of claim 61, wherein the nucleobase is an adenine, uracil, guanine, thymine, or cytosine.

63. The siRNA molecule of any one of claims 59-62, wherein the 5' phosphorus stabilizing moiety is (E)-vinylphosphonate represented by Formula XI.

64. The siRNA molecule of any one of claims 1-63, wherein the siRNA molecule further comprises a hydrophobic moiety at the 5' or the 3' end of the siRNA molecule.

65. The siRNA molecule of claim 64, wherein the hydrophobic moiety is selected from a group consisting of cholesterol, vitamin D, or tocopherol.

66. The siRNA molecule of any one of claims 1-65, wherein the length of the sense strand is between 10 and 30 nucleotides.

67. The siRNA molecule of claim 66, wherein the length of the sense strand is between 10 and 25 nucleotides.

68. The siRNA molecule of claim 67, wherein the length of the sense strand is between 12 and 25 nucleotides.

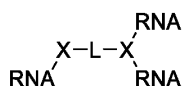
69. The siRNA molecule of claim 68, wherein the length of the sense strand is between 12 and 20 nucleotides.
70. The siRNA molecule of claim 69, wherein the length of the sense strand is between 12 and 19 nucleotides.
71. The siRNA molecule of claim 70, wherein the length of the sense strand is 15 nucleotides.
72. The siRNA molecule of claim 70, wherein the length of the sense strand is 16 nucleotides.
73. The siRNA molecule of claim 70, wherein the length of the sense strand is 18 nucleotides.
74. The siRNA molecule of any one of claims 1-73, wherein the length of the antisense strand is between 10 and 30 nucleotides.
75. The siRNA molecule of claim 74, wherein the length of the antisense strand is between 12 and 30 nucleotides.
76. The siRNA molecule of claim 75, wherein the length of the antisense strand is between 15 and 30 nucleotides.
77. The siRNA molecule of claim 76, wherein the length of the antisense strand is between 18 and 30 nucleotides.
78. The siRNA molecule of claim 77, wherein the length of the antisense strand is between 18 and 25 nucleotides.
79. The siRNA molecule of claim 78, wherein the length of the antisense strand is between 18 and 21 nucleotides.
80. The siRNA molecule of claim 79, wherein the length of the antisense strand is 18 nucleotides.
81. The siRNA molecule of claim 79, wherein the length of the antisense strand is 20 nucleotides.
82. The siRNA molecule of claim 79, wherein the length of the antisense strand is 21 nucleotides.
83. The siRNA molecule of any one of claims 1-82, wherein the siRNA molecule is a branched siRNA molecule.

84. The siRNA molecule of claim 83, wherein the branched siRNA molecule is di-branched, tri-branched, or tetra-branched.

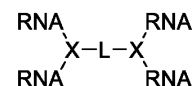
85. The siRNA molecule of claim 84, wherein the siRNA molecule is a di-branched siRNA molecule, optionally wherein the di-branched siRNA molecule is represented by any one of Formulas XVII-XIX:



Formula XVII;



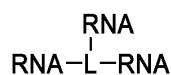
Formula XVIII;



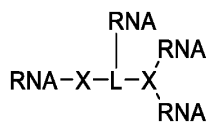
Formula XIX;

wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

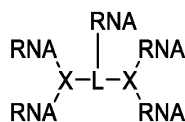
86. The siRNA molecule of claim 84, wherein the siRNA molecule is a tri-branched siRNA molecule, optionally wherein the tri-branched siRNA molecule is represented by any one of Formulas XX-XXIII:



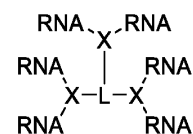
Formula XX;



Formula XXI;



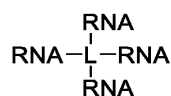
Formula XXII;



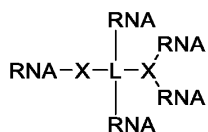
Formula XXIII;

wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

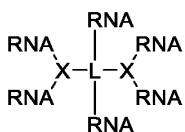
87. The siRNA molecule of claim 84, wherein the siRNA molecule is a tetra-branched siRNA molecule, optionally wherein the tetra-branched siRNA molecule is represented by any one of Formulas XXIV-XXVIII:



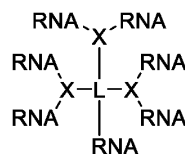
Formula XXIV;



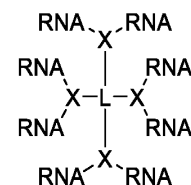
Formula XXV;



Formula XXVI;



Formula XXVII;



Formula XXVIII;

wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

88. The siRNA molecule of any one of claims 85-87, wherein the linker is selected from a group consisting of one or more contiguous subunits of an ethylene glycol, alkyl, carbohydrate, block copolymer, peptide, RNA, and DNA.
89. The siRNA molecule of claim 88, wherein the one or more contiguous subunits is 2 to 20 contiguous subunits.
90. The siRNA molecule of any one of claims 1-89, wherein the siRNA molecule is formulated as a salt comprising one or more divalent cations.
91. The siRNA molecule of claim 90, wherein the siRNA molecule comprises a plurality of cationic binding sites that are partially or fully saturated by the one or more divalent cations.
92. The siRNA molecule of claim 90 or 91, wherein the one or more divalent cations comprise Ba^{2+} , Be^{2+} , Ca^{2+} , Cu^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , or Zn^{2+} , or a combination thereof.
93. The siRNA molecule of claim 92, wherein the one or more divalent cations comprise Mg^{2+} .
94. The siRNA molecule of any one of claims 90-93, wherein the siRNA molecule comprises one or more atoms having a negative charge and the divalent cation comprises two positive charges, and wherein the ratio of negative charge to positive charge is from 0.75 to 7.5, optionally wherein the ratio of negative charge to positive charge is from 1.0 to 2.0.
95. The siRNA molecule of claim 94, wherein:
- the ratio of negative charge to positive charge is from 0.75 to 6.5, optionally wherein the ratio of negative charge to positive charge is from 0.75 to 5.5, from 0.75 to 4.5, from 0.75 to 3.5, from 0.75 to 2.5, from 0.75 to 1.5, or from 0.75 to 1; or
 - the ratio of negative charge to positive charge is from 1 to 7.5, from 1.5 to 7.5, from 2.5 to 7.5, from 3.5 to 7.5, from 4.5 to 7.5, from 5.5 to 7.5, or from 6.5 to 7.5.
96. The siRNA molecule of any one of claims 90-95, wherein the molar ratio of the siRNA molecule to the one or more divalent cations is from 1:10 to 1:100.
97. The siRNA molecule of claim 96, wherein the molar ratio of the siRNA molecule to the one or more divalent cations is from 1:10 to 1:50, optionally wherein the molar ratio of the siRNA molecule to the one or more divalent cations is from 1:18 to 1:38, optionally wherein the molar ratio of the siRNA molecule to the one or more divalent cations is from 1:20 to 1:25, optionally wherein the molar ratio of the siRNA molecule to the one or more divalent cations is about 1:20, optionally wherein the molar ratio of the siRNA molecule to the one or more divalent cations is about 1:25.

98. A pharmaceutical composition comprising the siRNA molecule of any one of claims 1-97 and a pharmaceutically acceptable excipient, carrier, or diluent.
99. A method of delivering an siRNA molecule to a subject diagnosed as having Huntington's Disease, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-97 or the pharmaceutical composition of claim 98 to the subject.
100. A method of treating Huntington's Disease in a subject in need thereof, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-97 or the pharmaceutical composition of claim 98 to the subject.
101. A method of reducing *HTT* expression in a subject in need thereof, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-97 or the pharmaceutical composition of claim 98 to the subject.
102. The method of any one of claims 99-101, wherein the siRNA molecule or the pharmaceutical composition is administered to the subject by way of intracerebroventricular, intrastriatal, intraparenchymal, or intrathecal injection.
103. The method of any one of claims 99-101, wherein the siRNA molecule or the pharmaceutical composition is administered to the subject by way of intravenous, intramuscular, or subcutaneous injection.
104. The method of any one of claims 99-103, wherein the subject is a human.
105. A kit comprising the siRNA molecule of any one of claims 1-97, or the pharmaceutical composition of claim 98, and a package insert, wherein the package insert instructs a user of the kit to perform the method of any one of claims 99-104.