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(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF NEUROLOGICAL DISORDERS

(57) Abstract: The present disclosure provides single- or double-stranded interfering RNA molecules (e.g., siRNA) that target a chromosome 9 open reading frame 72 (*C9orf72*) gene. The interfering RNA molecules may contain specific patterns of nucleoside modifications and internucleoside linkage modifications, as pharmaceutical compositions including the same. The siRNA molecules may be branched siRNA molecules, such as di-branched, tri-branched, ortetra-branched siRNA molecules. The disclosed siRNA molecules may further feature a 5' phosphorus stabilizing moiety and/or a hydrophobic moiety. Additionally, the disclosure provides methods for delivering the siRNA molecule of the disclosure to the central nervous system of a subject, such as a subject identified as having amyotrophic lateral sclerosis or frontotemporal dementia.

COMPOSITIONS AND METHODS FOR TREATMENT OF NEUROLOGICAL DISORDERS

Sequence Listing

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 20, 2023, is named "51436-037WO2_Sequence_Listing_9_20_23" and is 1,011,545 bytes in size.

Technical Field

This disclosure relates to small interfering RNA (siRNA) molecules, and compositions containing the same, that target RNA transcripts (e.g., mRNA) of a chromosome 9 open reading frame 72 (*C9orf72*) gene. The disclosure further describes methods for silencing of *C9orf72* and the treatment of diseases that may benefit from the silencing of *C9orf72* (e.g., frontotemporal dementia or amyotrophic lateral sclerosis) by delivering *C9orf72*-targeting siRNA molecules to a target tissue of a subject in need.

Background

C9orf72 (chromosome 9 open reading frame 72) encodes a protein that is implicated in the pathology of diseases including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Studies have shown that repeat expansions within the *C9orf72* can cause ALS and FTD. There is a need for therapeutics capable of selectively diminishing *C9orf72* activity in a manner that provides effective treatment for ALS, FTD, or other *C9orf72*-related diseases or disorders.

Summary of the Invention

The present disclosure provides compositions and methods for reduction of chromosome 9 open reading frame 72 (*C9orf72*) expression by way of small interfering RNA (siRNA)-mediated silencing of *C9orf72* transcripts. The compositions and methods provide the benefit of exhibiting high selectivity toward *C9orf72* over other genes.

The siRNA molecules of the disclosure can be used to silence the *C9orf72* gene, thereby preventing the translation of the corresponding mRNA transcript and reducing C9orf72 protein expression. This reduction of C9orf72 levels thus prevents disease onset or progression, as the hexanucleotide repeat expansion GGGGCC in the *C9orf72* gene is a common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The siRNA molecules of the disclosure can be delivered directly to a subject in need of *C9orf72* silencing by way of, for example, injection intrathecally, intracerebroventricularly, intrastriatally, intraparenchymally, intra-cisterna magna injection, such as by catheterization, intravenous injection, subcutaneous injection, or intramuscular injection.

In an aspect, the disclosure provides an siRNA molecule containing an antisense strand and sense strand having complementarity to the antisense strand. The antisense strand has complementarity sufficient to hybridize to a region within an *C9orf72* mRNA transcript having the

nucleic acid sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the antisense strand has complementarity sufficient to hybridize to a region within an *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the antisense strand has complementarity sufficient to hybridize to a region within an *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 193-384. The antisense strand may be, for example, from 10 to 50 nucleotides in length (e.g., from 10 to 45 nucleotides in length, from 10 to 40 nucleotides in length, from 10 to 35 nucleotides in length, from 10 to 30 nucleotides in length, 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). In some embodiments, the antisense strand is 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, 30 nucleotides in length, or more.

In some embodiments of any of the foregoing aspects, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) complementarity to a region of 15 contiguous nucleobases within the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384, SEQ ID NOs: 1-192, or SEQ ID NOs: 193-384. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) complementarity to a region of 16 contiguous nucleobases within the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) complementarity to a region of 17 contiguous nucleobases within the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384, SEQ ID NOs: 1-192, or SEQ ID NOs: 193-384. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least

80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) complementarity to a region of 18 contiguous nucleobases within the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384, SEQ ID NOs: 1-192, or SEQ ID NOs: 193-384. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) complementarity to a region of 19 contiguous nucleobases within the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384, SEQ ID NOs: 1-192, or SEQ ID NOs: 193-384. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) complementarity to a region of 20 contiguous nucleobases within the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384, SEQ ID NOs: 1-192, or SEQ ID NOs: 193-384. In some embodiments, the antisense strand has at least 70% (e.g., at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) complementarity to a region of 21 contiguous nucleobases within the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384, SEQ ID NOs: 1-192, or SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has at least 70% (e.g., at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) complementarity to the region within the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the region within the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region within *the C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has at least 75% complementarity to the region within the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. For example, the antisense strand may have 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 *C9orf72* mRNA transcript having the nucleic acid

sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the region within the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region within the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has 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 *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has 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 within the region of the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has from 12 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 within the region of the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has 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 *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has from 18 to 30 contiguous nucleotides (e.g., 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 *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has from 18 to 25 contiguous nucleotides (e.g., 18, 19, 20, 21, 22, 23, 24, or 25 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID Nos: 1-384. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has from 18 to 21 contiguous nucleotides (e.g., 18, 19, 20, or 21 contiguous nucleotides) that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has 21 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has 9 or fewer nucleotide mismatches relative to a region of 21 contiguous nucleobases of the *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384, optionally wherein the antisense strand contains 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 *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region of the *C9orf72* mRNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

In some embodiments, the antisense strand has a nucleic acid sequence that is at least 85% identical (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: 769-1152. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 85% identical (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: 769-960. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 85% identical (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: 961-1152.

In some embodiments, the antisense strand has a nucleic acid sequence that is at least 90% identical (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: 769-1152. In some embodiments, the antisense

strand has a nucleic acid sequence that is at least 90% identical (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: 769-960. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 90% identical (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: 961-1152.

In some embodiments, the antisense strand has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 769-1152, 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: 769-1152. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 769-960. In some embodiments, the antisense strand has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 961-1152.

In some embodiments, the antisense strand has the nucleic acid sequence of any one of SEQ ID NOs: 769-1152. In some embodiments, the antisense strand has the nucleic acid sequence of any one of SEQ ID NOs: 769-960. In some embodiments the antisense strand has the nucleic acid sequence of any one of SEQ ID NOs: 961-1152.

In some embodiments, the sense strand has a nucleic acid sequence that is at least 85% identical (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: 385-768. In some embodiments, the sense strand has a nucleic acid sequence that is at least 85% identical (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: 385-576. In some embodiments, the sense strand has a nucleic acid sequence that is at least 85% identical (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: 577-768.

In some embodiments, the sense strand has a nucleic acid sequence that is at least 90% identical (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: 385-768. In some embodiments, the sense strand has a nucleic acid sequence that is at least 90% identical (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: 385-576. In some embodiments, the sense strand has a nucleic acid sequence that is at least 90% identical (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: 577-768.

In some embodiments, the sense strand has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 385-768, 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: 385-768. In some embodiments, the sense strand has a nucleic acid sequence that is at least 95%

identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 385-576. In some embodiments, the sense strand has a nucleic acid sequence that is at least 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, or 100% identical) to the nucleic acid sequence of any one of SEQ ID NOs: 577-768.

In some embodiments, the siRNA molecule has a sense strand having the nucleic acid sequence of any one of SEQ ID NOs: 385-768. In some embodiments, the sense strand has a nucleic acid sequence of any one of SEQ ID NOs: 385-576. In some embodiments, the sense strand has a nucleic acid sequence of any one of SEQ ID NOs: 577-768.

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

$$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C'\text{-}P^1)_k\text{-}C'$$

Formula I;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
B is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$;
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 P^1 is a phosphorothioate internucleoside linkage;
each P^2 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, the antisense strand has 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-O-A-O-B-S-A-S-A-S-A-S-B-S-A

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

$$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C\text{-}P^1)_k\text{-}C'$$

Formula II;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;

B is represented by the formula C-P^2-D-P^2-D-P^2-D-P^2;
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 P^1 is a phosphorothioate internucleoside linkage;
each P^2 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, antisense strand has a structure represented by Formula A2, wherein Formula A2 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-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, the sense strand has a structure represented by Formula III, wherein Formula III is, in the 5'-to-3' direction:

$$E\text{-}(A')_m\text{-}F$$

Formula III;

wherein E is represented by the formula $(C\text{-}P^1)_2$;
F is represented by the formula $(C\text{-}P^2)_3$-D-P^1-C-P^1-C, $(C\text{-}P^2)_3$-D-P^2-C-P^2-C, $(C\text{-}P^2)_3$-D-P^1-C-P^1-D, or $(C\text{-}P^2)_3$-D-P^2-C-P^2-D;
A', C, D, P^1, and P^2 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, the sense strand has 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

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.

In some embodiments, the sense strand has 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;

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 S3, wherein Formula S3 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-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.

In some embodiments, the sense strand has 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;

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

$A\text{-}(A')_j\text{-}C\text{-}P^2\text{-}B\text{-}(C\text{-}P^1)_k\text{-}C'$

Formula IV;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
B is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1\text{-}D\text{-}P^1$;
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 P^1 is a phosphorothioate internucleoside linkage;
each P^2 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, the antisense strand has 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-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.

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

$$E\text{-}(A')_m\text{-}C\text{-}P^2\text{-}F$$

Formula V;

wherein E is represented by the formula $(C\text{-}P^1)_2$;
F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, or $D\text{-}P^2\text{-}C\text{-}P^2\text{-}D$;
A', C, D, P^1 and P^2 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, the sense strand has 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.

In some embodiments, the sense strand has a structure represented by Formula S6, wherein Formula S6 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-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.

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.

In some embodiments, the sense strand has a structure represented by Formula S8, wherein Formula S8 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-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.

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

$A\text{-}B_j\text{-}E\text{-}B_k\text{-}E\text{-}F\text{-}G_l\text{-}D\text{-}P^1\text{-}C'$

Formula VI;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each B is represented by the formula $C\text{-}P^2$;
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 E is represented by the formula $D\text{-}P^2\text{-}C\text{-}P^2$;
F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1$;
each G is represented by the formula $C\text{-}P^1$;
each P^1 is a phosphorothioate internucleoside linkage;
each P^2 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, the antisense strand has a structure represented by Formula A4, wherein Formula A4 is, in the 5'-to-3' direction:

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

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:

$$H\text{-}B_m\text{-}I_n\text{-}A'\text{-}B_o\text{-}H\text{-}C$$

Formula VII;

wherein A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
each H is represented by the formula $(C\text{-}P^1)_2$;
each I is represented by the formula $(D\text{-}P^2)$;
B, C, D, P^1 and P^2 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); and
o is an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, or 7).

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

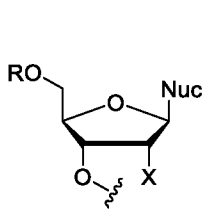
Formula S9;

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.

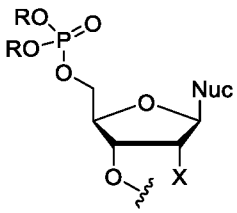
In some embodiments, the antisense strand also has a 5' phosphorus stabilizing moiety at the 5' end of the antisense strand.

In some embodiments, the sense strand also has a 5' phosphorus stabilizing moiety at the 5' end of the sense strand.

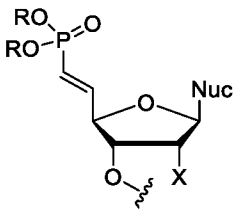
In some embodiments, each 5' phosphorus stabilizing moiety is, independently, represented by any one of Formulas IX, XX, XI, XII, XIII, XIV, XV, or XVI:



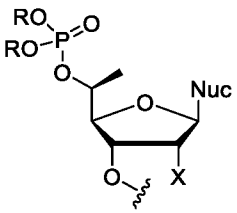
Formula IX



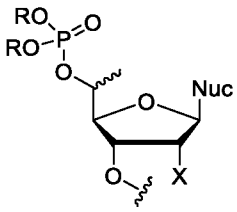
Formula X



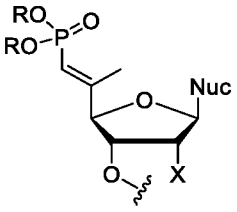
Formula XI



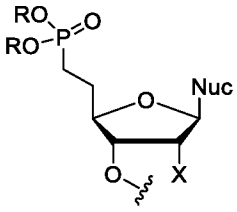
Formula XII



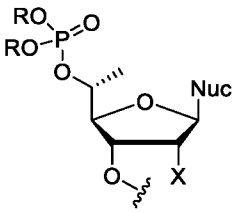
Formula XIII



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 (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, the siRNA molecule also has 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.

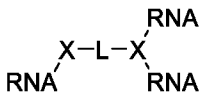
In some embodiments, the siRNA molecule is a branched siRNA molecule.

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

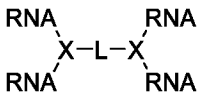
In some embodiments, the siRNA molecule is di-branched, optionally wherein the di-branched siRNA molecule is represented by any one of Formulas XVII, XVIII, or XIX:

RNA–L–RNA

Formula XVII;



Formula XVIII;

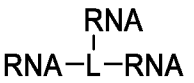


Formula XIX;

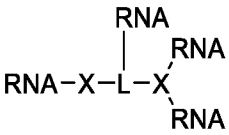
wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

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 embodiments, the di-branched siRNA molecule is represented by Formula XIX.

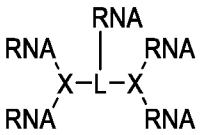
In some embodiments, the siRNA molecule is tri-branched, optionally wherein the tri-branched siRNA molecule is represented by any one of Formulas XX, XXI, XXII, or XXIII:



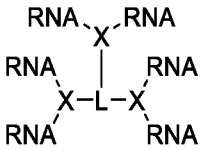
Formula XX;



Formula XXI;



Formula XXII;

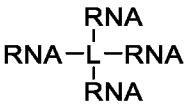


Formula XXIII;

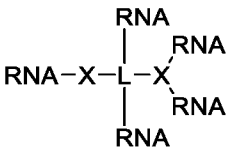
wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

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

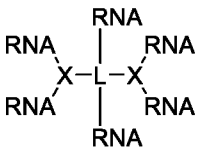
In some embodiments, the siRNA molecule is tetra-branched, optionally wherein the tetra-branched siRNA molecule is represented by any one of Formulas XXIV, XXV, XXVI, XXVII, or XXVIII:



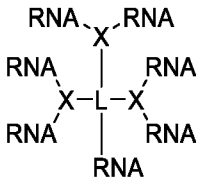
Formula XXIV;



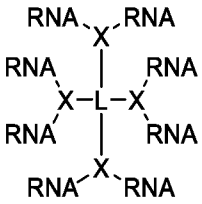
Formula XXV;



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.

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 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.

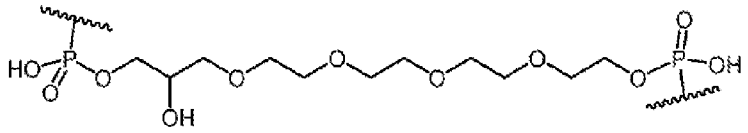
In some embodiments, the ethylene glycol oligomer is a PEG. In some embodiments, the PEG is a TrEG. In some embodiments, the PEG is a TEG.

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.

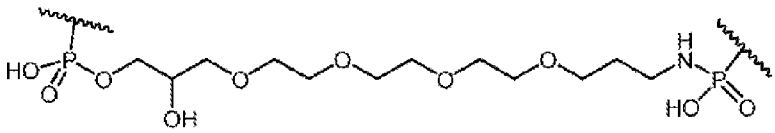
In some embodiments, the covalent bond-forming moiety is selected from the group consisting of an alkyl, ester, amide, carbamate, phosphonate, phosphate, phosphorothioate, phosphoroamidate, triazole, urea, and formacetal.

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



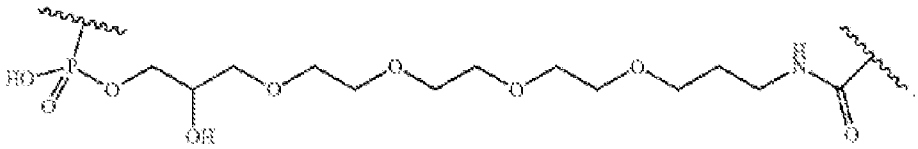
(**Formula L1**)

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



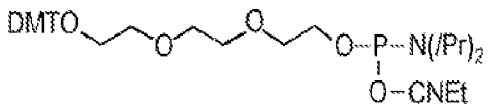
(**Formula L2**)

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



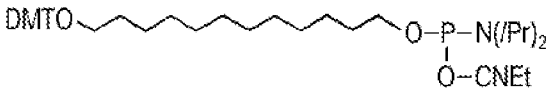
(**Formula L3**)

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



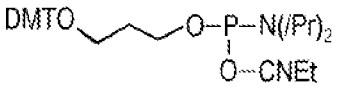
(**Formula L4**)

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



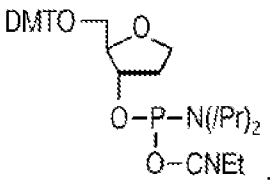
(**Formula L5**)

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



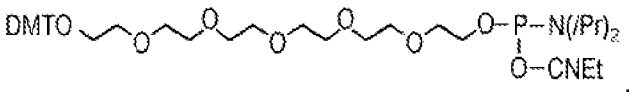
(**Formula L6**)

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



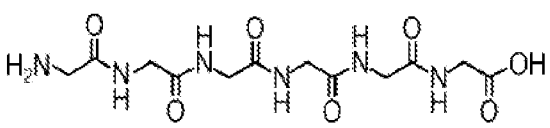
(**Formula L7**)

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



(**Formula L8**)

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



(**Formula L9**)

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 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 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).

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-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.

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 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 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 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 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 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 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 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 embodiments, the length of the sense strand is 21 nucleotides. In some embodiments, the length of the sense strand is 22 nucleotides. In some embodiments, the length of the sense strand is 23 nucleotides. In some embodiments, the length of the sense strand is 24 nucleotides. In some embodiments, the length of the sense strand is 25 nucleotides. In some embodiments, the length of the sense strand is 26 nucleotides. In some embodiments, the length of the sense strand is 27 nucleotides. In some embodiments, the length of the sense strand is 28 nucleotides. In some embodiments, the length of the sense strand is 29 nucleotides. In some embodiments, the length of the sense strand is 30 nucleotides.

In some embodiments, four internucleoside linkages are phosphorothioate linkages.

In some embodiments of the siRNA molecules described herein, the antisense strand is 18 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 18 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 18 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 18 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 18 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments,

the antisense strand is 19 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 19 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 20 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 21 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 17 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 18 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 19 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 20 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 21 nucleotides in length. In some embodiments, the antisense strand is 22 nucleotides in length and the sense strand is 22 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 14 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 15 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 16 nucleotides in length. In some embodiments, the antisense strand is 23 nucleotides in length and the sense strand is 17 nucleotides

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

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

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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 a further aspect, the disclosure provides a pharmaceutical composition containing an siRNA molecule of any of the preceding aspects or embodiments of the disclosure, and a pharmaceutically acceptable excipient, carrier, or diluent.

In a further aspect, the disclosure provides a method of delivering an siRNA molecule to a subject diagnosed as having ALS by administering a therapeutically effective amount of the siRNA molecule or a 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 delivering an siRNA molecule to a subject diagnosed as having FTD by administering a therapeutically effective amount of the siRNA molecule or a 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 treating ALS in a subject in need thereof by administering a therapeutically effective amount of an siRNA molecule or a 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 treating FTD in a subject in need thereof by administering a therapeutically effective amount of an siRNA molecule or a pharmaceutical composition of any of the preceding aspects or embodiments of the disclosure to the subject.

In another aspect, the disclosure provides a method of reducing C9orf72 expression in a subject in need thereof by administering a therapeutically effective amount of an siRNA or pharmaceutical composition of any of the preceding aspects or embodiments of the disclosure.

In some embodiments, the siRNA molecule or the pharmaceutical composition is administered to the subject by way of intracerebroventricular, intrastriatal, 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 subject is a human.

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

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.

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 "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 “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 "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, phosphodiesters, 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.

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 its 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 *C9orf72*, 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 $((CH_2OH)_2)$.

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≡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≡CH and $–C≡C-CH_3$. 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.

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., amyotrophic lateral sclerosis of frontotemporal dementia) and/or contains a gain-of-function *C9orf72* variant allele or a repeat expansion of the *C9orf72* gene.

As used herein, the terms “neuroinflammatory disease” and “neuroinflammatory disorder” are used interchangeably to refer to any condition that is in some way caused by neuroinflammation. “Neuroinflammation” refers to a range of immune responses in the central nervous system (e.g., in microglia). Neuroinflammation may be brain-derived or result from a systemic inflammatory response.

As used herein, the terms “neurodegenerative disease” and “neurodegenerative disorder” are used interchangeably to refer to any condition that is in some way caused by a loss of function or death of cells of the central nervous system or peripheral nervous system. Exemplary neurodegenerative diseases are Alzheimer’s disease, Huntington’s disease, amyotrophic lateral sclerosis, frontotemporal dementia, and spinocerebellar ataxias.

As used herein, the term “*C9orf72*” refers to the gene encoding chromosome, including any native *C9orf72* gene from any source. The term encompasses “full-length,” unprocessed *C9orf72* as well as any form of *C9orf72* that results from processing in the cell. The term also encompasses naturally occurring variants of *C9orf72*, e.g., splice variants or allelic variants. The nucleic acid sequence of an exemplary *C9orf72* gene is shown in European Nucleotide Archive (ENA) Accession No. JN681271.1. The amino acid sequence of an exemplary protein encoded by an *C9orf72* gene is shown in UNIPROT™ Accession No. Q96LT7.

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, amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD). For example, clinical benefits in the context of a subject having ALS administered an siRNA molecule or siRNA composition of the disclosure include, without limitation, a reduction in involuntary movements, muscle cramps, weakness, loss of motor control. As a further example, clinical benefits in the context of a subject having FTD administered an siRNA molecule or siRNA composition of the disclosure include, without limitation, a reduction in memory problems, behavioral problems, and language problems. “Benefit” and “response” are also used interchangeably to refer to, for example, a reduction in wild type *C9orf72* transcripts, mutant *C9orf72* transcripts, variant *C9orf72* transcripts, splice isoforms of *C9orf72* transcripts, and/or overexpressed *C9orf72* transcripts.

Detailed Description

The present disclosure provides compositions of small interfering RNA (siRNA) molecules with sequence homology to a chromosome 9 open reading frame 72 (*C9orf72*) 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 *C9orf72*, relative to other human genes.

The siRNA molecules of the disclosure may feature an antisense strand having a nucleic acid sequence that is complementary to a region of a *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384. The degree of complementarity of the antisense strand to the region of the *C9orf72* mRNA transcript may be sufficient for the antisense strand to anneal over the full length of the region of the *C9orf72* 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 *C9orf72* mRNA transcript. In some embodiments,

the region of the *C9orf72* RNA transcript has the sequence of any one of SEQ ID NOs: 1-192. In some embodiments, the region of the *C9orf72* RNA transcript has the sequence of any one of SEQ ID NOs: 193-384.

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: 769-1152, 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: 769-1152. In some embodiments, the nucleic acid sequence is any one of SEQ ID NOs: 769-960. In some embodiments, the nucleic acid sequence is any one of SEQ ID NOs: 961-1152.

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: 385-768, 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: 385-768. In some embodiments, the nucleic acid sequence is any one of SEQ ID NOs: 385-576. In some embodiments, the nucleic acid sequence is any one of SEQ ID NOs: 577-768.

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 a *C9orf72*mRNA transcript that are targeted by each antisense strand.

Table 1. Nucleotide sequences for gene-specific *C9orf72*-targeting siRNA

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
1	UCCAGCUGUUG CCAAGACAGA	385	CUGUUGCCAAGA CAGA	769	UCUGUCUUGGCAACA GCUGGA
2	CAGCUGUUGCC AAGACAGAGA	386	GUUGCCAAGACA GAGA	770	UCUCUGUCUUGGCAA CAGCUG
3	GCUGUUGCCAA GACAGAGAUU	387	UGCCAAGACAGA GAUA	771	UAUCUCUGUCUUGGC AACAGC
4	CUGUUGCCAAG ACAGAGAUUG	388	GCCAAGACAGAG AUUA	772	UAAUCUCUGUCUUGG CAACAG
5	UGUUGCCAAGA CAGAGAUUGC	389	CCAAGACAGAGAU UGA	773	UCAAUCUCUGUCUUG GCAACA
6	GUUGCCAAGAC AGAGAUUGCU	390	CAAGACAGAGAUU GCA	774	UGCAAUCUCUGUCUU GGCAAC
7	UUGCCAAGACA GAGAUUGCUU	391	AAGACAGAGAUU GCUA	775	UAGCAAUCUCUGUCU UGGCAA

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
8	CCAAGACAGAGA UUGCUUUAA	392	ACAGAGAUUGCU UUAA	776	UUAAAGCAAUCUCUG UCUUGG
9	ACAGAGAUUGC UUUAAGUGGC	393	GAUUGCUUUAAG UGGA	777	UCCACUUAAAGCAAU CUCUGU
10	AAUCACCUUUAU UAGCAGCUA	394	CCUUUAUUAGCA GCUA	778	UAGCUGCUAAUAAAG GUGAUU
11	GGACAAUAUUC UUGGUCCUAG	395	AUAUUCUUGGUC CUAA	779	UUAGGACCAAGAAUA UUGUCC
12	GACAAUAUUCUU GGUCCUAGA	396	UAUUCUUGGUCC UAGA	780	UCUAGGACCAAGAAU AUUGUC
13	CAAUAUUCUUG GUCCUAGAGU	397	UUCUUGGUCCUA GAGA	781	UCUCUAGGACCAAGA AUAUUG
14	AUAUUCUUGGU CCUAGAGUAA	398	CUUGGUCCUAGA GUAA	782	UUACUCUAGGACCAA GAAUAU
15	UUCUUGGUCCU AGAGUAAGGC	399	GGUCCUAGAGUA AGGA	783	UCCUUACUCUAGGAC CAAGAA
16	UCUUGGUCCUA GAGUAAGGCA	400	GUCCUAGAGUAA GGCA	784	UGCCUUACUCUAGGA CCAAGA
17	CUUGGUCCUAG AGUAAGGCAC	401	UCCUAGAGUAAG GCAA	785	UUGCCUUACUCUAGG ACCAAG
18	GCACAUUUGGG CUCCAAAGAC	402	UUUGGGCUCCAA AGAA	786	UUCUUUGGAGCCCAA AUGUGC
19	CACAUUUGGGC UCCAAAGACA	403	UUGGGCUCCAAA GACA	787	UGUCUUUGGAGCCCA AAUGUG
20	ACAUUUGGGCU CCAAAGACAG	404	UGGGCUCCAAAG ACAA	788	UUGUCUUUGGAGCCC AAAUGU
21	CAUUUGGGCUC CAAAGACAGA	405	GGGCUCCAAAGA CAGA	789	UCUGUCUUUGGAGCC CAAAUG
22	AGAAAUAACUUU UCUUGCCAA	406	UAACUUUUCUUG CCAA	790	UUGGCAAGAAAAGUU AUUUCU
23	AAUAACUUUUCU UGCCAACCA	407	CUUUUCUUGCCA ACCA	791	UGGUUGGCAAGAAAA GUUAUU
24	AUAACUUUUCUU GCCAACCAC	408	UUUUCUUGCCAA CCAA	792	UUGGUUGGCAAGAAA AGUUAU
25	UAACUUUUCUU GCCAACCACA	409	UUUCUUGCCAAC CACA	793	UGUGGUUGGCAAGAA AAGUUA
26	UUUUCUUGCCA ACCACACUCU	410	UUGCCAACCACAC UCA	794	UGAGUGUGGUUGGC AAGAAAA
27	UUUCUUGCCAA CCACACUCUA	411	UGCCAACCACACU CUA	795	UAGAGUGUGGUUGG CAAGAAA
28	UUCUUGCCAAC CACACUCUAA	412	GCCAACCACACUC UAA	796	UUAGAGUGUGGUUG GCAAGAA
29	UCUUGCCAACC ACACUCUAAA	413	CCAACCACACUCU AAA	797	UUUAGAGUGUGGUUG GCAAGA
30	CUUGCCAACCA CACUCUAAAU	414	CAACCACACUCUA AAA	798	UUUUAGAGUGUGGUU GGCAAG
31	UUGCCAACCACA CUCUAAAUG	415	AACCACACUCUAA AUA	799	UAUUUAGAGUGUGGU UGGCAA
32	UGCCAACCACAC UCUAAAUGG	416	ACCACACUCUAAA UGA	800	UCAUUUAGAGUGUGG UUGGCA
33	CAACCACACUCU AAAUGGAGA	417	ACACUCUAAAUGG AGA	801	UCUCCAUUUAGAGUG UGGUUG

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
34	AAUCCUUCGAAA UGCAGAGAG	418	UUCGAAAUGCAG AGAA	802	UUCUCUGCAUUUCGA AGGAUU
35	CCUUCGAAAUG CAGAGAGUGG	419	GAAAUGCAGAGA GUGA	803	UCACUCUCUGCAUUU CGAAGG
36	AGAGUGGUGCU AUAGAUGUAA	420	GGUGCUAUAGAU GUAA	804	UUACAUCUAUAGCAC CACUCU
37	GAGUGGUGCUA UAGAUGUAAA	421	GUGCUAUAGAUG UAAA	805	UUUACAUCUAUAGCA CCACUC
38	UGGUGCUAUAG AUGUAAAGUU	422	CUAUAGAUGUAAA GUA	806	UACUUUACAUCUAUA GCACCA
39	GUGCUAUAGAU GUAAAGUUUU	423	AUAGAUGUAAAGU UUA	807	UAAACUUUACAUCUA UAGCAC
40	UUUGUCUUGUC UGAAAAGGGA	424	CUUGUCUGAAAA GGGA	808	UCCCUUUUCAGACAA GACAAA
41	UCUUGUCUGAA AAGGGAGUGA	425	UCUGAAAAGGGA GUGA	809	UCACUCCCUUUUCAG ACAAGA
42	GUCUGAAAAGG GAGUGAUUAU	426	AAAAGGGAGUGA UUAA	810	UUAAUCACUCCCUUU UCAGAC
43	UCUGAAAAGGG AGUGAUUAUU	427	AAAGGGAGUGAU UAUA	811	UAUAAUCACUCCCUU UUCAGA
44	AAAAGGGAGUG AUUAUUGUUU	428	GGAGUGAUUAUU GUUA	812	UAACAAUAAUCACUC CCUUUU
45	AUCUUUGAUGG AAACUGGAAU	429	UGAUGGAAACUG GAAA	813	UUUCCAGUUUCCAUC AAAGAU
46	AUUAUACUUCCA CAGACAGAA	430	ACUUCCACAGACA GAA	814	UUCUGUCUGUGGAAG UAUAAU
47	UUAUACUUCCAC AGACAGAAC	431	CUUCCACAGACA GAAA	815	UUUCUGUCUGUGGAA GUAUAA
48	UACUUCCACAGA CAGAACUUA	432	CCACAGACAGAAC UUA	816	UAAGUUCUGUCUGUG GAAGUA
49	CACAGACAGAAC UUAGUUUCU	433	ACAGAACUUAGUU UCA	817	UGAAACUAAGUUCUG UCUGUG
50	UAGUUUCUACC UCCCACUUCA	434	UCUACCUCCCAC UUCA	818	UGAAGUGGGAGGUAG AAACUA
51	AGUUUCUACCU CCCACUUCAU	435	CUACCUCCCACU UCAA	819	UUGAAGUGGGAGGUA GAAACU
52	CACAUAUAAUCC GGAAAGGAA	436	AUAAUCCGGAAAG GAA	820	UUCCUUUCCGGAUUA UAUGUG
53	UAUAAUCCGGAA AGGAAGAAU	437	UCCGGAAAGGAA GAAA	821	UUUCUUCCUUUCCGG AUUAUA
54	AAUCCGGAAAG GAAGAAUAUG	438	GGAAAGGAAGAA UAUA	822	UAUAUUCUUCCUUUC CGGAUU
55	UCCGGAAAGGA AGAAUAUGGA	439	AAAGGAAGAAUAU GGA	823	UCCAUAUUCUUCCUU UCCGGA
56	AAGGAAGAAUAU GGAUGCAUA	440	AGAAUAUGGAUG CAUA	824	UAUGCAUCCAUAUUC UUCCUU
57	GAAGAAUAUGG AUGCAUAAGG	441	AUAUGGAUGCAU AAGA	825	UCUUAUGCAUCCAUA UUCUUC
58	AGAAUAUGGAU GCAUAAGGAA	442	AUGGAUGCAUAA GGAA	826	UUCCUUAUGCAUCCA UAUUCU
59	GAAUAUGGAUG CAUAAGGAAA	443	UGGAUGCAUAAG GAAA	827	UUUCCUUAUGCAUCC AUAUUC

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
60	AAUAUGGAUGC AUAAGGAAAG	444	GGAUGCAUAAGG AAAA	828	UUUUCCUUAUGCAUC CAUAUU
61	AUGGAUGCAUA AGGAAAGACA	445	UGCAUAAGGAAA GACA	829	UGUCUUUCCUUAUGC AUCCAU
62	UGGAUGCAUAA GGAAAGACAA	446	GCAUAAGGAAAGA CAA	830	UUGUCUUUCCUUAUG CAUCCA
63	GGAUGCAUAAG GAAAGACAAG	447	CAUAAGGAAAGAC AAA	831	UUUGUCUUUCCUUAU GCAUCC
64	AGGAAAGACAAG AAAAUGUCC	448	AGACAAGAAAAUG UCA	832	UGACAUUUUCUUGUC UUUCCU
65	AAGACAAGAAAA UGUCCAGAA	449	AAGAAAAUGUCCA GAA	833	UUCUGGACAUUUUCU UGUCUU
66	UAUCUUAGAAG GCACAGAGAG	450	UAGAAGGCACAG AGAA	834	UUCUCUGUGCCUUCU AAGAUA
67	AGAGAGAAUGG AAGAUCAGGG	451	GAAUGGAAGAUC AGGA	835	UCCUGAUCUUCCAUU CUCUCU
68	GAGAAUGGAAG AUCAGGGUCA	452	UGGAAGAUCAGG GUCA	836	UGACCCUGAUCUUCC AUUCUC
69	AGAAUGGAAGA UCAGGGUCAG	453	GGAAGAUCAGGG UCAA	837	UUGACCCUGAUCUUC CAUUCU
70	GAAUGGAAGAU CAGGGUCAGA	454	GAAGAUCAGGGU CAGA	838	UCUGACCCUGAUCUU CCAUUC
71	AAUGGAAGAUCA GGGUCAGAG	455	AAGAUCAGGGUC AGAA	839	UUCUGACCCUGAUCU UCCAUU
72	AUGGAAGAUCA GGGUCAGAGU	456	AGAUCAGGGUCA GAGA	840	UCUCUGACCCUGAUC UUCCAU
73	UGGAAGAUCAG GGUCAGAGUA	457	GAUCAGGGUCAG AGUA	841	UACUCUGACCCUGAU CUUCCA
74	GGAAGAUCAGG GUCAGAGUAU	458	AUCAGGGUCAGA GUAA	842	UUACUCUGACCCUGA UCUUCC
75	GAAGAUCAGGG UCAGAGUAUU	459	UCAGGGUCAGAG UAUA	843	UAUACUCUGACCCUG AUCUUC
76	GAUCAGGGUCA GAGUAUUAUU	460	GGGUCAGAGUAU UAUA	844	UAUAAUACUCUGACC CUGAUC
77	CAGGGUCAGAG UAUUAUUCCA	461	UCAGAGUAUUAU UCCA	845	UGGAAUAAUACUCUG ACCCUG
78	UGGAGAAGUGA UUCCUGUAAU	462	AAGUGAUUCCUG UAAA	846	UUUACAGGAAUCACU UCUCCA
79	CUGUAAUGGAA CUGCUUUCAU	463	AUGGAACUGCUU UCAA	847	UUGAAAGCAGUUCCA UUACAG
80	AUGGAACUGCU UUCAUCUAUG	464	ACUGCUUUCAUC UAUA	848	UAUAGAUGAAAGCAG UUCCAU
81	UUCAUCUAUGAA AUCACACAG	465	CUAUGAAAUCACA CAA	849	UUGUGUGAUUUCAUA GAUGAA
82	CUAUGAAAUCAC ACAGUGUUC	466	AAAUCACACAGUG UUA	850	UAACACUGUGUGAUU UCAUAG
83	UCCUGAAGAAAU AGAUAUAGC	467	AAGAAAUAGAUAU AGA	851	UCUAUAUCUAUUUCU UCAGGA
84	AAUAGAUAUAGC UGAUACAGU	468	AUAUAGCUGAUAC AGA	852	UCUGUAUCAGCUAUA UCUAUU
85	CUGAUACAGUA CUCAAUGAUG	469	ACAGUACUCAAUG AUA	853	UAUCAUUGAGUACUG UAUCAG

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
86	UGAAGGCUUUC UUCUCAAUGC	470	GCUUUCUUCUCA AUGA	854	UCAUUGAGAAGAAAG CCUUCA
87	GAAGGCUUUCU UCUCAAUGCC	471	CUUUCUUCUCAA UGCA	855	UGCAUUGAGAAGAAA GCCUUC
88	AAGGCUUUCUU CUCAAUGCCA	472	UUUCUUCUCAAU GCCA	856	UGGCAUUGAGAAGAA AGCCUU
89	CUUCUCAAUGC CAUCAGCUCA	473	CAAUGCCAUCAG CUCA	857	UGAGCUGAUGGCAUU GAGAAG
90	UUCUCAAUGCC AUCAGCUCAC	474	AAUGCCAUCAGC UCAA	858	UUGAGCUGAUGGCAU UGAGAA
91	UCUCAAUGCCA UCAGCUCACA	475	AUGCCAUCAGCU CACA	859	UGUGAGCUGAUGGCA UUGAGA
92	UCAAUGCCAUCA GCUCACACU	476	GCCAUCAGCUCA CACA	860	UGUGUGAGCUGAUG GCAUUGA
93	CAAACCUGUGG CUGUUCCGUU	477	CUGUGGCUGUUC CGUA	861	UACGGAACAGCCACA GGUUUG
94	ACCUGUGGCUG UUCCGUUGUA	478	UGGCUGUUCCGU UGUA	862	UACAACGGAACAGCC ACAGGU
95	CUGUGGCUGUU CCGUUGUAGU	479	GCUGUUCCGUUG UAGA	863	UCUACAACGGAACAG CCACAG
96	GUUGUAGUAGG UAGCAGUGCA	480	AGUAGGUAGCAG UGCA	864	UGCACUGCUACCUAC UACAAC
97	GGUAGCAGUGC AGAGAAAGUA	481	CAGUGCAGAGAA AGUA	865	UACUUUCUCUGCACU GCUACC
98	AGCAGUGCAGA GAAAGUAAAU	482	UGCAGAGAAAGU AAAA	866	UUUUACUUUCUCUGC ACUGCU
99	GCAGUGCAGAG AAAGUAAAUA	483	GCAGAGAAAGUAA AUA	867	UAUUUACUUUCUCUG CACUGC
100	GCAGAGAAAGU AAAUAAGAUA	484	GAAAGUAAAUAAG AUA	868	UAUCUUAUUUACUUU CUCUGC
101	GAUAGUCAGAA CAUUAUGCCU	485	UCAGAACAUUAUG CCA	869	UGGCAUAAUGUUCUG ACUAUC
102	UGAAGCAGAAU CAUCAUUUAA	486	CAGAAUCAUCAUU UAA	870	UUAAAUGAUGAUUCU GCUUCA
103	GAAGCAGAAUCA UCAUUUAAA	487	AGAAUCAUCAUUU AAA	871	UUUAAAUGAUGAUUC UGCUUC
104	CUGCUAAAGGA UUCAACUGGA	488	AAAGGAUUCAACU GGA	872	UCCAGUUGAAUCCUU UAGCAG
105	UUCCGGCAAGU CAUGUAUGCU	489	GCAAGUCAUGUA UGCA	873	UGCAUACAUGACUUG CCGGAA
106	CUCCAUAUCCCA CCACACACA	490	UAUCCCACCACAC ACA	874	UGUGUGUGGUGGGA UAUGGAG
107	UGCCACCCUGU CAUGAACAUA	491	CCCUGUCAUGAA CAUA	875	UAUGUUCAUGACAGG GUGGCA
108	GCCACCCUGUC AUGAACAUAU	492	CCUGUCAUGAAC AUAA	876	UUAUGUUCAUGACAG GGUGGC
109	CCCUGUCAUGA ACAUAUUUAU	493	UCAUGAACAUAUU UAA	877	UUAAAUAUGUUCAUG ACAGGG
110	UUAUAAUCAGC GUAGAUACAU	494	AUCAGCGUAGAU ACAA	878	UUGUAUCUACGCUGA UUAUAA
111	AAUCAGCGUAG AUACAUGAGA	495	GCGUAGAUACAU GAGA	879	UCUCAUGUAUCUACG CUGAUU

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
112	AUGGCUCAGGA UACGAUCAUC	496	UCAGGAUACGAU CAUA	880	UAUGAUCGUAUCCUG AGCCAU
113	CGAUCAUCUACA CUGACGAAA	497	AUCUACACUGAC GAAA	881	UUUCGUCAGUGUAGA UGAUCG
114	UGACGAAAGCU UUACUCCUGA	498	AAAGCUUUACUCC UGA	882	UCAGGAGUAAAGCUU UCGUCA
115	CUUUACUCCUG AUUUGAAUAU	499	CUCCUGAUUUGA AUAA	883	UUAUUCAAAUCAGGA GUAAAG
116	UUUCAAGAUGU CUUACACAGA	500	AGAUGUCUUACA CAGA	884	UCUGUGUAAGACAUC UUGAAA
117	AUGUCUUACACA GAGACACUC	501	UUACACAGAGACA CUA	885	UAGUGUCUCUGUGUA AGACAU
118	UGUCUUACACA GAGACACUCU	502	UACACAGAGACAC UCA	886	UGAGUGUCUCUGUGU AAGACA
119	GUCUUACACAG AGACACUCUA	503	ACACAGAGACACU CUA	887	UAGAGUGUCUCUGUG UAAGAC
120	UCUUACACAGA GACACUCUAG	504	CACAGAGACACUC UAA	888	UUAGAGUGUCUCUGU GUAAGA
121	CUUACACAGAGA CACUCUAGU	505	ACAGAGACACUCU AGA	889	UCUAGAGUGUCUCUG UGUAAG
122	UUACACAGAGAC ACUCUAGUG	506	CAGAGACACUCUA GUA	890	UACUAGAGUGUCUCU GUGUAA
123	UACACAGAGACA CUCUAGUGA	507	AGAGACACUCUA GUGA	891	UCACUAGAGUGUCUC UGUGUA
124	ACACAGAGACAC UCUAGUGAA	508	GAGACACUCUAG UGAA	892	UUCACUAGAGUGUCU CUGUGU
125	CACAGAGACACU CUAGUGAAA	509	AGACACUCUAGU GAAA	893	UUUCACUAGAGUGUC UCUGUG
126	ACAGAGACACUC UAGUGAAAG	510	GACACUCUAGUG AAAA	894	UUUUCACUAGAGUGU CUCUGU
127	CAGAAGUACUU UCCUUGCACA	511	GUACUUUCCUUG CACA	895	UGUGCAAGGAAAGUA CUUCUG
128	AGUACUUUCCU UGCACAGUUU	512	UUUCCUUGCACA GUUA	896	UAACUGUGCAAGGAA AGUACU
129	CCUUCACAGAAA AGCCUUGAC	513	ACAGAAAAGCCUU GAA	897	UUCAAGGCUUUUCUG UGAAGG
130	CACAGAAAAGCC UUGACACUA	514	AAAAGCCUUGACA CUA	898	UAGUGUCAAGGCUUU UCUGUG
131	ACAGAAAAGCCU UGACACUAA	515	AAAGCCUUGACAC UAA	899	UUAGUGUCAAGGCUU UUCUGU
132	CAGAAAAGCCUU GACACUAAU	516	AAGCCUUGACAC UAAA	900	UUUAGUGUCAAGGCU UUUCUG
133	ACGAUACGCAG AAGGGAAAAA	517	ACGCAGAAGGGA AAAA	901	UUUUUCCCUUCUGCG UAUCGU
134	CGAUACGCAGA AGGGAAAAAA	518	CGCAGAAGGGAA AAAA	902	UUUUUUCCCUUCUGC GUAUCG
135	GACCUUGAUUU AACAGCAGAG	519	UGAUUUAACAGCA GAA	903	UUCUGCUGUUAAAUC AAGGUC
136	ACCUUGAUUUAA CAGCAGAGG	520	GAUUUAACAGCA GAGA	904	UCUCUGCUGUUAAAU CAAGGU
137	CCUUGAUUUAA CAGCAGAGGG	521	AUUUAACAGCAGA GGA	905	UCCUCUGCUGUUAAA UCAAGG

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
138	CUUGAUUUAACA GCAGAGGGC	522	UUUAACAGCAGA GGGA	906	UCCCUCUGCUGUUAA AUCAAG
139	UUGAUUUAACA GCAGAGGGCG	523	UUAACAGCAGAG GGCA	907	UGCCCUCUGCUGUUA AAUCAA
140	UGAUUUAACAG CAGAGGGCGA	524	UAACAGCAGAGG GCGA	908	UCGCCCUCUGCUGUU AAAUCA
141	AUUUAACAGCAG AGGGCGAUC	525	ACAGCAGAGGGC GAUA	909	UAUCGCCCUCUGCUG UUAAAU
142	UUUAACAGCAGA GGGCGAUCU	526	CAGCAGAGGGCG AUCA	910	UGAUCGCCCUCUGCU GUUAAA
143	UAACAGCAGAG GGCGAUCUUA	527	GCAGAGGGCGAU CUUA	911	UAAGAUCGCCCUCUG CUGUUA
144	AACAGCAGAGG GCGAUCUUAA	528	CAGAGGGCGAUC UUAA	912	UUAAGAUCGCCCUCU GCUGUU
145	CAGCAGAGGGC GAUCUUAACA	529	GAGGGCGAUCUU AACA	913	UGUUAAGAUCGCCCU CUGCUG
146	AGAGGGCGAUC UUAACAUAAU	530	GCGAUCUUAACA UAAA	914	UUUAUGUUAAGAUCG CCCUCU
147	GAGGGCGAUCU UAACAUAAUA	531	CGAUCUUAACAUA AUA	915	UAUUAUGUUAAGAUC GCCCUC
148	AGGGCGAUCUU AACAUAAUAA	532	GAUCUUAACAUAA UAA	916	UUAUUAUGUUAAGAU CGCCCU
149	GGGCGAUCUUA ACAUAAUAAU	533	AUCUUAACAUAAU AAA	917	UUUAUUAUGUUAAGA UCGCCC
150	CGAUCUUAACAU AAUAAUGGC	534	UUAACAUAAUAAU GGA	918	UCCAUUAUUAUGUUA AGAUCG
151	GAUCUUAACAUA AUAAUGGCU	535	UAACAUAAUAAUG GCA	919	UGCCAUUAUUAUGUU AAGAUC
152	UCUUAACAUAAU AAUGGCUCU	536	ACAUAAUAAUGGC UCA	920	UGAGCCAUUAUUAUG UUAAGA
153	CUUAACAUAAUA AUGGCUCUG	537	CAUAAUAAUGGCU CUA	921	UAGAGCCAUUAUUAU GUUAAG
154	GGCUCUGGCUG AGAAAAUUAA	538	UGGCUGAGAAAA UUAA	922	UUAAUUUUCUCAGCC AGAGCC
155	CUCUGGCUGAG AAAAUUAAAC	539	GCUGAGAAAAUUA AAA	923	UUUUAAUUUUCUCAG CCAGAG
156	UAAACCAGGCC UACACUCUUU	540	CAGGCCUACACU CUUA	924	UAAGAGUGUAGGCCU GGUUUA
157	UGGAAGACCUU UCUACACUAG	541	GACCUUUCUACA CUAA	925	UUAGUGUAGAAAGGU CUUCCA
158	GGAAGACCUUU CUACACUAGU	542	ACCUUUCUACACU AGA	926	UCUAGUGUAGAAAGG UCUUCC
159	GAAGACCUUUC UACACUAGUG	543	CCUUUCUACACUA GUA	927	UACUAGUGUAGAAAG GUCUUC
160	AAGACCUUUCUA CACUAGUGU	544	CUUUCUACACUA GUGA	928	UCACUAGUGUAGAAA GGUCUU
161	UGCAAGAACGA GAUGUUCUAA	545	GAACGAGAUGUU CUAA	929	UUAGAACAUCUCGUU CUUGCA
162	GUGUAACUUAA UAAGCCUAUU	546	ACUUAAUAAGCCU AUA	930	UAUAGGCUUAUUAAG UUACAC
163	UAACUUAAUAAG CCUAUUCCA	547	UAAUAAGCCUAUU CCA	931	UGGAAUAGGCUUAUU AAGUUA

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
164	UAAUAAGCCUAUUCCAUCACA	548	AGCCUAUUCCAUCACA	932	UGUGAUGGAAUAGGCUUAUUA
165	CAGCAAUUGCAGUUAAGUAAG	549	AUUGCAGUUAAGUAAA	933	UUUACUUAACUGCAAUUGCUG
166	UGCAGUUAAGUAAGUUACACU	550	UUAAGUAAGUUACACA	934	UGUGUAACUUACUUAACUGCA
167	GUAAGUUACACUACAGUUCUC	551	UUACACUACAGUUCUA	935	UAGAACUGUAGUGUAACUUAC
168	CAGGUGCAUCAUUACAUUGGG	552	GCAUCAUUACAUUGGA	936	UCCAAUGUAAUGAUGCACCUG
169	UGCUUUUGGGAUACAGACCUA	553	UUGGGAUACAGACCUA	937	UAGGUCUGUAUCCCAAAAGCA
170	GCUUUUGGGAUACAGACCUAU	554	UGGGAUACAGACCUAA	938	UUAGGUCUGUAUCCCAAAAGC
171	UUUUGGGAUACAGACCUAUGU	555	GGAUACAGACCUAUGA	939	UCAUAGGUCUGUAUCCCAAAA
172	UUGGGAUACAGACCUAUGUUU	556	AUACAGACCUAUGUUA	940	UAACAUAGGUCUGUAUCCCAA
173	UGGGAUACAGACCUAUGUUUA	557	UACAGACCUAUGUUUA	941	UAAACAUAGGUCUGUAUCCCA
174	UACAGACCUAUGUUUACAAUA	558	ACCUAUGUUUACAAUA	942	UAUUGUAAACAUAGGUCUGUA
175	CAGACCUAUGUUUACAAUAUA	559	CUAUGUUUACAAUAUA	943	UAUAUUGUAAACAUAGGUCUG
176	AUACAUGAUUCAUGGUUUACA	560	UGAUUCAUGGUUUACA	944	UGUAAACCAUGAAUCAUGUAU
177	GUUGACUUUCUAUCUUUUGGC	561	CUUUCUAUCUUUUGGA	945	UCCAAAAGAUAGAAAGUCAAC
178	AUUCCACAGAAAGAAAGUGAG	562	ACAGAAAGAAAGUGAA	946	UUCACUUUCUUUCUGUGGAAU
179	UGAUCAAGCAGAUGUUUAAUU	563	AAGCAGAUGUUUAAUA	947	UAUUAAACAUCUGCUUGAUCA
180	CAAGCAGAUGUUUAAUUGGAA	564	AGAUGUUUAAUUGGAA	948	UUCCAAUUAAACAUCUGCUUG
181	CCCUGGGAUUCAGUCUGUAGA	565	GGAUUCAGUCUGUAGA	949	UCUACAGACUGAAUCCCAGGG
182	UUCAGUCUGUAGAAAUGUCUA	566	UCUGUAGAAAUGUCUA	950	UAGACAUUUCUACAGACUGAA
183	UCCUGGUGAACCACAGUUAGG	567	GUGAACCACAGUUAGA	951	UCUAACUGUGGUUCACCAGGA
184	UAUGUAGUUGAGCUCUGUAAA	568	AGUUGAGCUCUGUAAA	952	UUUACAGAGCUCAACUACAUA
185	AUGUAGUUGAGCUCUGUAAAA	569	GUUGAGCUCUGUAAAA	953	UUUUACAGAGCUCAACUACAU
186	GUAGUUGAGCUCUGUAAAAGG	570	UGAGCUCUGUAAAAGA	954	UCUUUUACAGAGCUCAACUAC
187	AGUUGAGCUCUGUAAAAGGAA	571	AGCUCUGUAAAAGGAA	955	UUCCUUUUACAGAGCUCAACU
188	GUUGAGCUCUGUAAAAGGAAA	572	GCUCUGUAAAAGGAAA	956	UUUCCUUUUACAGAGCUCAAC
189	UUGAGCCAAAUUGAAAUGUGC	573	CCAAAUUGAAAUGUGA	957	UCACAUUUCAAUUUGGCUCAA

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
190	UGUGCACCUCC UGUGCCUUUU	574	ACCUCCUGUGCC UUUA	958	UAAAGGCACAGGAGG UGCACA
191	UUCAGAUUUCA CUGGUCAGUC	575	AUUUCACUGGUC AGUA	959	UACUGACCAGUGAAA UCUGAA
192	UCUUACCAUGU ACCUGCUUUG	576	CCAUGUACCUGC UUUA	960	UAAAGCAGGUACAUG GUAAGA
193	GUCCCGCUAGG AAAGAGAGGU	577	GCUAGGAAAGAG AGGA	961	UCCUCUCUUUCCUAG CGGGAC
194	GUCAAACAGCG ACAAGUUCCG	578	ACAGCGACAAGU UCCA	962	UGGAACUUGUCGCUG UUUGAC
195	UCAAACAGCGAC AAGUUCCGC	579	CAGCGACAAGUU CCGA	963	UCGGAACUUGUCGCU GUUUGA
196	CAGCGACAAGU UCCGCCCACG	580	ACAAGUUCCGCC CACA	964	UGUGGGCGGAACUU GUCGCUG
197	CGACAAGUUCC GCCCACGUAA	581	AGUUCCGCCCAC GUAA	965	UUACGUGGGCGGAAC UUGUCG
198	GACAAGUUCCG CCCACGUAAA	582	GUUCCGCCCACG UAAA	966	UUUACGUGGGCGGAA CUUGUC
199	AAGUUCCGCCC ACGUAAAAGA	583	CCGCCCACGUAA AAGA	967	UCUUUUACGUGGGCG GAACUU
200	UUCCGCCCACG UAAAAGAUGA	584	CCCACGUAAAAGA UGA	968	UCAUCUUUUACGUGG GCGGAA
201	UCCGCCCACGU AAAAGAUGAC	585	CCACGUAAAAGAU GAA	969	UUCAUCUUUUACGUG GGCGGA
202	CCGCCCACGUA AAAGAUGACG	586	CACGUAAAAGAUG ACA	970	UGUCAUCUUUUACGU GGGCGG
203	CGCCCACGUAA AAGAUGACGC	587	ACGUAAAAGAUGA CGA	971	UCGUCAUCUUUUACG UGGGCG
204	CCACGUAAAAGA UGACGCUUG	588	UAAAAGAUGACGC UUA	972	UAAGCGUCAUCUUUU ACGUGG
205	GUGUGUCAGCC GUCCCUGCUG	589	UCAGCCGUCCCU GCUA	973	UAGCAGGGACGGCUG ACACAC
206	UGCUGCCCGGU UGCUUCUCUU	590	CCCGGUUGCUUC UCUA	974	UAGAGAAGCAACCGG GCAGCA
207	GUCUAGCAAGA GCAGGUGUGG	591	GCAAGAGCAGGU GUGA	975	UCACACCUGCUCUUG CUAGAC
208	CAAGAGCAGGU GUGGGUUUAG	592	GCAGGUGUGGGU UUAA	976	UUAAACCCACACCUG CUCUUG
209	AGGUGUGGGUU UAGGAGGUGU	593	UGGGUUUAGGAG GUGA	977	UCACCUCCUAAACCC ACACCU
210	GGUGUGGGUUU AGGAGGUGUG	594	GGGUUUAGGAGG UGUA	978	UACACCUCCUAAACC CACACC
211	GUGUGGGUUUA GGAGGUGUGU	595	GGUUUAGGAGGU GUGA	979	UCACACCUCCUAAAC CCACAC
212	GUGGGUUUAGG AGGUGUGUGU	596	UUUAGGAGGUGU GUGA	980	UCACACACCUCCUAA ACCCAC
213	UGGGUUUAGGA GGUGUGUGUU	597	UUAGGAGGUGUG UGUA	981	UACACACACCUCCUA AACCCA
214	GGGUUUAGGAG GUGUGUGUUU	598	UAGGAGGUGUGU GUUA	982	UAACACACACCUCCU AAACCC
215	GUUUAGGAGGU GUGUGUUUUU	599	GGAGGUGUGUGU UUUA	983	UAAAACACACACCUC CUAAAC

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
216	UUUAGGAGGUG UGUGUUUUUG	600	GAGGUGUGUGUU UUUA	984	UAAAAACACACACCU CCUAAA
217	UUAGGAGGUGU GUGUUUUUGU	601	AGGUGUGUGUUU UUGA	985	UCAAAAACACACACC UCCUAA
218	GUUUUUGUUUU UCCCACCCUC	602	UGUUUUUCCCAC CCUA	986	UAGGGUGGGAAAAAC AAAAAC
219	UCGCUGAGGGU GAACAAGAAA	603	GAGGGUGAACAA GAAA	987	UUUCUUGUUCACCCU CAGCGA
220	CGCUGAGGGUG AACAAGAAAA	604	AGGGUGAACAAG AAAA	988	UUUUCUUGUUCACCC UCAGCG
221	GAGGGUGAACA AGAAAAGACC	605	UGAACAAGAAAAG ACA	989	UGUCUUUUCUUGUUC ACCCUC
222	GGUGAACAAGA AAAGACCUGA	606	ACAAGAAAAGACC UGA	990	UCAGGUCUUUUCUUG UUCACC
223	GUGAACAAGAAA AGACCUGAU	607	CAAGAAAAGACCU GAA	991	UUCAGGUCUUUUCUU GUUCAC
224	UGAACAAGAAAA GACCUGAUA	608	AAGAAAAGACCUG AUA	992	UAUCAGGUCUUUUCU UGUUCA
225	AACAAGAAAAGA CCUGAUAAA	609	GAAAAGACCUGAU AAA	993	UUUAUCAGGUCUUUU CUUGUU
226	AGACCUGAUAAA GAUUAACCA	610	UGAUAAAGAUUAA CCA	994	UGGUUAAUCUUUAUC AGGUCU
227	GACCUGAUAAA GAUUAACCAG	611	GAUAAAGAUUAAC CAA	995	UUGGUUAAUCUUUAU CAGGUC
228	ACCUGAUAAAGA UUAACCAGA	612	AUAAAGAUUAACC AGA	996	UCUGGUUAAUCUUUA UCAGGU
229	CUGAUAAAGAUU AACCAGAAG	613	AAAGAUUAACCAG AAA	997	UUUCUGGUUAAUCUU UAUCAG
230	UGAUAAAGAUUA ACCAGAAGA	614	AAGAUUAACCAGA AGA	998	UCUUCUGGUUAAUCU UUAUCA
231	GAUUAACCAGAA GAAAACAAG	615	ACCAGAAGAAAAC AAA	999	UUUGUUUUCUUCUGG UUAAUC
232	AUUAACCAGAAG AAAACAAGG	616	CCAGAAGAAAACA AGA	1000	UCUUGUUUUCUUCUG GUUAAU
233	UUAACCAGAAGA AAACAAGGA	617	CAGAAGAAAACAA GGA	1001	UCCUUGUUUUCUUCU GGUUAA
234	ACAAGGAGGGA AACAACCGCA	618	GAGGGAAACAAC CGCA	1002	UGCGGUUGUUUCCCU CCUUGU
235	CAAGGAGGGAA ACAACCGCAG	619	AGGGAAACAACC GCAA	1003	UUGCGGUUGUUUCCC UCCUUG
236	GGAGGGAAACA ACCGCAGCCU	620	GAAACAACCGCAG CCA	1004	UGGCUGCGGUUGUU UCCCUCC
237	GGGAAACAACC GCAGCCUGUA	621	ACAACCGCAGCC UGUA	1005	UACAGGCUGCGGUUG UUUCCC
238	CAGGAGUCGCG CGCUAGGGGC	622	GUCGCGCGCUAG GGGA	1006	UCCCCUAGCGCGCGA CUCCUG
239	GGGGCCGGGGC CGGGGCCGGG	623	CGGGGCCGGGGC CGGA	1007	UCCGGCCCCGGCCCC GGCCCC
240	GGGCCGGGGCC GGGGCCGGGG	624	GGGGCCGGGGCC GGGA	1008	UCCCGGCCCCGGCCC CGGCCC
241	GGCCGGGGCCG GGGCCGGGGC	625	GGGCCGGGGCCG GGGA	1009	UCCCCGGCCCCGGCC CCGGCC

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
242	GCCGGGGCCGG GGCCGGGGCC	626	GGCCGGGGCCGG GGCA	1010	UGCCCCGGCCCCGG CCCCGGC
243	CCGGGGCCGGG GCCGGGGCCG	627	GCCGGGGCCGGG GCCA	1011	UGGCCCCGGCCCCG GCCCCGG
244	CGGGGCCGGGG CCGGGGCCGG	628	CCGGGGCCGGGG CCGA	1012	UCGGCCCCGGCCCC GGCCCCG
245	CUCAGAGCUCG ACGCAUUUUU	629	AGCUCGACGCAU UUUA	1013	UAAAAUGCGUCGAGC UCUGAG
246	UCAGAGCUCGA CGCAUUUUUA	630	GCUCGACGCAUU UUUA	1014	UAAAAAUGCGUCGAG CUCUGA
247	UCGACGCAUUU UUACUUUCCC	631	GCAUUUUUACUU UCCA	1015	UGGAAAGUAAAAAUG CGUCGA
248	GACGCAUUUUU ACUUUCCCUC	632	AUUUUUACUUUC CCUA	1016	UAGGGAAAGUAAAAA UGCGUC
249	UUCCCUCUCAU UUCUCUGACC	633	UCUCAUUUCUCU GACA	1017	UGUCAGAGAAAUGAG AGGGAA
250	CCUCUCAUUUC UCUGACCGAA	634	CAUUUCUCUGAC CGAA	1018	UUCGGUCAGAGAAAU GAGAGG
251	CUCUCAUUUCU CUGACCGAAG	635	AUUUCUCUGACC GAAA	1019	UUUCGGUCAGAGAAA UGAGAG
252	UUCUCUGACCG AAGCUGGGUG	636	UGACCGAAGCUG GGUA	1020	UACCCAGCUUCGGUC AGAGAA
253	CUUUCGCCUCU AGCGACUGGU	637	GCCUCUAGCGAC UGGA	1021	UCCAGUCGCUAGAGG CGAAAG
254	UUUCGCCUCUA GCGACUGGUG	638	CCUCUAGCGACU GGUA	1022	UACCAGUCGCUAGAG GCGAAA
255	UCGCCUCUAGC GACUGGUGGA	639	UCUAGCGACUGG UGGA	1023	UCCACCAGUCGCUAG AGGCGA
256	CGCCUCUAGCG ACUGGUGGAA	640	CUAGCGACUGGU GGAA	1024	UUCCACCAGUCGCUA GAGGCG
257	UUCCCGCCCUC AGUACCCGAG	641	GCCCUCAGUACC CGAA	1025	UUCGGGUACUGAGG GCGGGAA
258	GGAGACGCCUG CACAAUUUCA	642	CGCCUGCACAAU UUCA	1026	UGAAAUUGUGCAGGC GUCUCC
259	CGCCUGCACAA UUUCAGCCCA	643	GCACAAUUUCAG CCCA	1027	UGGGCUGAAAUUGUG CAGGCG
260	UCUAGAGAGUG GUGAUGACUU	644	AGAGUGGUGAUG ACUA	1028	UAGUCAUCACCACUC UCUAGA
261	UAGAGAGUGGU GAUGACUUGC	645	AGUGGUGAUGAC UUGA	1029	UCAAGUCAUCACCAC UCUCUA
262	GGUGAUGACUU GCAUAUGAGG	646	UGACUUGCAUAU GAGA	1030	UCUCAUAUGCAAGUC AUCACC
263	UGAUGACUUGC AUAUGAGGGC	647	ACUUGCAUAUGA GGGA	1031	UCCCUCAUAUGCAAG UCAUCA
264	GAUGACUUGCA UAUGAGGGCA	648	CUUGCAUAUGAG GGCA	1032	UGCCCUCAUAUGCAA GUCAUC
265	CUUGCAUAUGA GGGCAGCAAU	649	AUAUGAGGGCAG CAAA	1033	UUUGCUGCCCUCAUA UGCAAG
266	AUAUGAGGGCA GCAAUGCAAG	650	AGGGCAGCAAUG CAAA	1034	UUUGCAUUGCUGCCC UCAUAU
267	UGAGGGCAGCA AUGCAAGUCG	651	GCAGCAAUGCAA GUCA	1035	UGACUUGCAUUGCUG CCCUCA

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
268	GGCAGCAAUGC AAGUCGGUGU	652	CAAUGCAAGUCG GUGA	1036	UCACCGACUUGCAUU GCUGCC
269	GUGGGACAUGA CCUGGUUGCU	653	ACAUGACCUGGU UGCA	1037	UGCAACCAGGUCAUG UCCCAC
270	CCUGGUUGCUU CACAGCUCCG	654	UUGCUUCACAGC UCCA	1038	UGGAGCUGUGAAGCA ACCAGG
271	UGGUUGCUUCA CAGCUCCGAG	655	GCUUCACAGCUC CGAA	1039	UUCGGAGCUGUGAAG CAACCA
272	UUGCUUCACAG CUCCGAGAUG	656	UCACAGCUCCGA GAUA	1040	UAUCUCGGAGCUGUG AAGCAA
273	UCACAGCUCCG AGAUGACACA	657	GCUCCGAGAUGA CACA	1041	UGUGUCAUCUCGGAG CUGUGA
274	CUCCGAGAUGA CACAGACUUG	658	AGAUGACACAGAC UUA	1042	UAAGUCUGUGUCAUC UCGGAG
275	UCCGAGAUGAC ACAGACUUGC	659	GAUGACACAGAC UUGA	1043	UCAAGUCUGUGUCAU CUCGGA
276	CCGAGAUGACA CAGACUUGCU	660	AUGACACAGACUU GCA	1044	UGCAAGUCUGUGUCA UCUCGG
277	AGAUGACACAGA CUUGCUUAA	661	ACACAGACUUGC UUAA	1045	UUAAGCAAGUCUGUG UCAUCU
278	AUGACACAGACU UGCUUAAAG	662	ACAGACUUGCUU AAAA	1046	UUUUAAGCAAGUCUG UGUCAU
279	CACAGACUUGC UUAAAGGAAG	663	ACUUGCUUAAAG GAAA	1047	UUUCCUUUAAGCAAG UCUGUG
280	CUUGCUUAAAG GAAGUGACUA	664	UUAAAGGAAGUG ACUA	1048	UAGUCACUUCCUUUA AGCAAG
281	UGCUUAAAGGA AGUGACUAUU	665	AAAGGAAGUGAC UAUA	1049	UAUAGUCACUUCCUU UAAGCA
282	UUAAAGGAAGU GACUAUUGUG	666	GGAAGUGACUAU UGUA	1050	UACAAUAGUCACUUC CUUUAA
283	UAAAGGAAGUG ACUAUUGUGA	667	GAAGUGACUAUU GUGA	1051	UCACAAUAGUCACUU CCUUUA
284	GAAGUGACUAU UGUGACUUGG	668	GACUAUUGUGAC UUGA	1052	UCAAGUCACAAUAGU CACUUC
285	GUGACUAUUGU GACUUGGGCA	669	UAUUGUGACUUG GGCA	1053	UGCCCAAGUCACAAU AGUCAC
286	GACUAUUGUGA CUUGGGCAUC	670	UUGUGACUUGGG CAUA	1054	UAUGCCCAAGUCACA AUAGUC
287	UUGUGACUUGG GCAUCACUUG	671	ACUUGGGCAUCA CUUA	1055	UAAGUGAUGCCCAAG UCACAA
288	UGUGACUUGGG CAUCACUUGA	672	CUUGGGCAUCAC UUGA	1056	UCAAGUGAUGCCCAA GUCACA
289	GUGACUUGGGC AUCACUUGAC	673	UUGGGCAUCACU UGAA	1057	UUCAAGUGAUGCCCA AGUCAC
290	UGACUUGGGCA UCACUUGACU	674	UGGGCAUCACUU GACA	1058	UGUCAAGUGAUGCCC AAGUCA
291	CUUGGGCAUCA CUUGACUGAU	675	GCAUCACUUGAC UGAA	1059	UUCAGUCAAGUGAUG CCCAAG
292	UUGGGCAUCAC UUGACUGAUG	676	CAUCACUUGACU GAUA	1060	UAUCAGUCAAGUGAU GCCCAA
293	UCACUUGACUG AUGGUAAUCA	677	UGACUGAUGGUA AUCA	1061	UGAUUACCAUCAGUC AAGUGA

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
294	ACUUGACUGAU GGUAAUCAGU	678	ACUGAUGGUAAU CAGA	1062	UCUGAUUACCAUCAG UCAAGU
295	UUGACUGAUGG UAAUCAGUUG	679	UGAUGGUAAUCA GUUA	1063	UAACUGAUUACCAUC AGUCAA
296	UGACUGAUGGU AAUCAGUUGU	680	GAUGGUAAUCAG UUGA	1064	UCAACUGAUUACCAU CAGUCA
297	GACUGAUGGUA AUCAGUUGUC	681	AUGGUAAUCAGU UGUA	1065	UACAACUGAUUACCA UCAGUC
298	UGAUGGUAAUC AGUUGUCUAA	682	GUAAUCAGUUGU CUAA	1066	UUAGACAACUGAUUA CCAUCA
299	GAUGGUAAUCA GUUGUCUAAA	683	UAAUCAGUUGUC UAAA	1067	UUUAGACAACUGAUU ACCAUC
300	GGUAAUCAGUU GUCUAAAGAA	684	UCAGUUGUCUAA AGAA	1068	UUCUUUAGACAACUG AUUACC
301	AUCAGUUGUCU AAAGAAGUGC	685	UUGUCUAAAGAA GUGA	1069	UCACUUCUUUAGACA ACUGAU
302	UCAGUUGUCUA AAGAAGUGCA	686	UGUCUAAAGAAG UGCA	1070	UGCACUUCUUUAGAC AACUGA
303	GUUGUCUAAAG AAGUGCACAG	687	CUAAAGAAGUGCA CAA	1071	UUGUGCACUUCUUUA GACAAC
304	UUGUCUAAAGAA GUGCACAGA	688	UAAAGAAGUGCAC AGA	1072	UCUGUGCACUUCUUU AGACAA
305	UGUCUAAAGAA GUGCACAGAU	689	AAAGAAGUGCACA GAA	1073	UUCUGUGCACUUCUU UAGACA
306	GUCUAAAGAAG UGCACAGAUU	690	AAGAAGUGCACA GAUA	1074	UAUCUGUGCACUUCU UUAGAC
307	UCUAAAGAAGU GCACAGAUUA	691	AGAAGUGCACAG AUUA	1075	UAAUCUGUGCACUUC UUUAGA
308	CUAAAGAAGUG CACAGAUUAC	692	GAAGUGCACAGA UUAA	1076	UUAAUCUGUGCACUU CUUUAG
309	UAAAGAAGUGCA CAGAUUACA	693	AAGUGCACAGAU UACA	1077	UGUAAUCUGUGCACU UCUUUA
310	AGUGCACAGAU UACAUGUCCG	694	ACAGAUUACAUGU CCA	1078	UGGACAUGUAAUCUG UGCACU
311	UGCACAGAUUA CAUGUCCGUG	695	AGAUUACAUGUC CGUA	1079	UACGGACAUGUAAUC UGUGCA
312	AGAUUACAUGU CCGUGUGCUC	696	ACAUGUCCGUGU GCUA	1080	UAGCACACGGACAUG UAAUCU
313	CGUGUGCUCAU UGGGUCUAUC	697	GCUCAUUGGGUC UAUA	1081	UAUAGACCCAAUGAG CACACG
314	UGUGCUCAUUG GGUCUAUCUG	698	UCAUUGGGUCUA UCUA	1082	UAGAUAGACCCAAUG AGCACA
315	UCAUUGGGUCU AUCUGGCCGC	699	GGGUCUAUCUGG CCGA	1083	UCGGCCAGAUAGACC CAAUGA
316	GUCUAUCUGGC CGCGUUGAAC	700	UCUGGCCGCGUU GAAA	1084	UUUCAACGCGGCCAG AUAGAC
317	CCGCGUUGAAC ACCACCAGGC	701	UUGAACACCACCA GGA	1085	UCCUGGUGGUGUUCA ACGCGG
318	GUUGAACACCA CCAGGCUUUG	702	ACACCACCAGGC UUUA	1086	UAAAGCCUGGUGGUG UUCAAC
319	UGAACACCACCA GGCUUUGUA	703	ACCACCAGGCUU UGUA	1087	UACAAAGCCUGGUGG UGUUCA

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
320	GAACACCACCAG GCUUUGUAU	704	CCACCAGGCUUU GUAA	1088	UUACAAAGCCUGGUG GUGUUC
321	UUUGUAUUCAG AAACAGGAGG	705	AUUCAGAAACAGG AGA	1089	UCUCCUGUUUCUGAA UACAAA
322	CAGAAACAGGA GGGAGGUCCU	706	ACAGGAGGGAGG UCCA	1090	UGGACCUCCCUCCUG UUUCUG
323	GAGGGAGGUCC UGCACUUUCC	707	AGGUCCUGCACU UUCA	1091	UGAAAGUGCAGGACC UCCCUC
324	GGUGGCCCUUU CAGAUGCAAU	708	CCCUUUCAGAUG CAAA	1092	UUUGCAUCUGAAAGG GCCACC
325	GUGGCCCUUUC AGAUGCAAUC	709	CCUUUCAGAUGC AAUA	1093	UAUUGCAUCUGAAAG GGCCAC
326	UGGCCCUUUCA GAUGCAAUCG	710	CUUUCAGAUGCA AUCA	1094	UGAUUGCAUCUGAAA GGGCCA
327	CCCUUUCAGAU GCAAUCGAGA	711	UCAGAUGCAAUC GAGA	1095	UCUCGAUUGCAUCUG AAAGGG
328	UUUCAGAUGCA AUCGAGAUUG	712	GAUGCAAUCGAG AUUA	1096	UAAUCUCGAUUGCAU CUGAAA
329	CAGAUGCAAUC GAGAUUGUUA	713	GCAAUCGAGAUU GUUA	1097	UAACAAUCUCGAUUG CAUCUG
330	AAUCGAGAUUG UUAGGCUCUG	714	AGAUUGUUAGGC UCUA	1098	UAGAGCCUAACAAUC UCGAUU
331	AUCGAGAUUGU UAGGCUCUGG	715	GAUUGUUAGGCU CUGA	1099	UCAGAGCCUAACAAU CUCGAU
332	UCGAGAUUGUU AGGCUCUGGG	716	AUUGUUAGGCUC UGGA	1100	UCCAGAGCCUAACAA UCUCGA
333	CGAGAUUGUUA GGCUCUGGGA	717	UUGUUAGGCUCU GGGA	1101	UCCCAGAGCCUAACA AUCUCG
334	GAGAUUGUUAG GCUCUGGGAG	718	UGUUAGGCUCUG GGAA	1102	UUCCCAGAGCCUAAC AAUCUC
335	UUGUUAGGCUC UGGGAGAGUA	719	AGGCUCUGGGAG AGUA	1103	UACUCUCCCAGAGCC UAACAA
336	GUUAGGCUCUG GGAGAGUAGU	720	GCUCUGGGAGAG UAGA	1104	UCUACUCUCCCAGAG CCUAAC
337	UUAGGCUCUGG GAGAGUAGUU	721	CUCUGGGAGAGU AGUA	1105	UACUACUCUCCCAGA GCCUAA
338	UAGGCUCUGGG AGAGUAGUUG	722	UCUGGGAGAGUA GUUA	1106	UAACUACUCUCCCAG AGCCUA
339	GGCUCUGGGAG AGUAGUUGCC	723	UGGGAGAGUAGU UGCA	1107	UGCAACUACUCUCCC AGAGCC
340	CUGGGAGAGUA GUUGCCUGGU	724	AGAGUAGUUGCC UGGA	1108	UCCAGGCAACUACUC UCCCAG
341	GGGAGAGUAGU UGCCUGGUUG	725	AGUAGUUGCCUG GUUA	1109	UAACCAGGCAACUAC UCUCCC
342	GAGAGUAGUUG CCUGGUUGUG	726	UAGUUGCCUGGU UGUA	1110	UACAACCAGGCAACU ACUCUC
343	UUGCCUGGUUG UGGCAGUUGG	727	UGGUUGUGGCAG UUGA	1111	UCAACUGCCACAACC AGGCAA
344	UGCCUGGUUGU GGCAGUUGGU	728	GGUUGUGGCAGU UGGA	1112	UCCAACUGCCACAAC CAGGCA
345	GCCUGGUUGUG GCAGUUGGUA	729	GUUGUGGCAGUU GGUA	1113	UACCAACUGCCACAA CCAGGC

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
346	CCUGGUUGUGG CAGUUGGUAA	730	UUGUGGCAGUUG GUAA	1114	UUACCAACUGCCACA ACCAGG
347	CUGGUUGUGGC AGUUGGUAAA	731	UGUGGCAGUUGG UAAA	1115	UUUACCAACUGCCAC AACCAG
348	UGGUUGUGGCA GUUGGUAAAU	732	GUGGCAGUUGGU AAAA	1116	UUUUACCAACUGCCA CAACCA
349	UUGUGGCAGUU GGUAAAUUUC	733	GCAGUUGGUAAA UUUA	1117	UAAAUUUACCAACUG CCACAA
350	UGUGGCAGUUG GUAAAUUUCU	734	CAGUUGGUAAAU UUCA	1118	UGAAAUUUACCAACU GCCACA
351	GUGGCAGUUGG UAAAUUUCUA	735	AGUUGGUAAAUU UCUA	1119	UAGAAAUUUACCAAC UGCCAC
352	UGGCAGUUGGU AAAUUUCUAU	736	GUUGGUAAAUUU CUAA	1120	UUAGAAAUUUACCAA CUGCCA
353	GGCAGUUGGUA AAUUUCUAUU	737	UUGGUAAAUUUC UAUA	1121	UAUAGAAAUUUACCA ACUGCC
354	GCAGUUGGUAA AUUUCUAUUC	738	UGGUAAAUUUCU AUUA	1122	UAAUAGAAAUUUACC AACUGC
355	CAGUUGGUAAA UUUCUAUUCA	739	GGUAAAUUUCUA UUCA	1123	UGAAUAGAAAUUUAC CAACUG
356	UCUAUUCAAACA GUUGCCAUG	740	UCAAACAGUUGC CAUA	1124	UAUGGCAACUGUUUG AAUAGA
357	UAUUCAAACAGU UGCCAUGCA	741	AAACAGUUGCCAU GCA	1125	UGCAUGGCAACUGUU UGAAUA
358	AUUCAAACAGUU GCCAUGCAC	742	AACAGUUGCCAU GCAA	1126	UUGCAUGGCAACUGU UUGAAU
359	UUCAAACAGUU GCCAUGCACC	743	ACAGUUGCCAUG CACA	1127	UGUGCAUGGCAACUG UUUGAA
360	CAGUUGCCAUG CACCAGUUGU	744	GCCAUGCACCAG UUGA	1128	UCAACUGGUGCAUGG CAACUG
361	AGUUGCCAUGC ACCAGUUGUU	745	CCAUGCACCAGU UGUA	1129	UACAACUGGUGCAUG GCAACU
362	UUGCCAUGCAC CAGUUGUUCA	746	AUGCACCAGUUG UUCA	1130	UGAACAACUGGUGCA UGGCAA
363	UGCCAUGCACC AGUUGUUCAC	747	UGCACCAGUUGU UCAA	1131	UUGAACAACUGGUGC AUGGCA
364	GCCAUGCACCA GUUGUUCACA	748	GCACCAGUUGUU CACA	1132	UGUGAACAACUGGUG CAUGGC
365	AUGCACCAGUU GUUCACAACA	749	CCAGUUGUUCAC AACA	1133	UGUUGUGAACAACUG GUGCAU
366	GUUGUUCACAA CAAGGGUACG	750	UCACAACAAGGG UACA	1134	UGUACCCUUGUUGUG AACAAC
367	UUCACAACAAGG GUACGUAAU	751	AACAAGGGUACG UAAA	1135	UUUACGUACCCUUGU UGUGAA
368	UCACAACAAGG GUACGUAAUC	752	ACAAGGGUACGU AAUA	1136	UAUUACGUACCCUUG UUGUGA
369	CACAACAAGGG UACGUAAUCU	753	CAAGGGUACGUA AUCA	1137	UGAUUACGUACCCUU GUUGUG
370	CAAGGGUACGU AAUCUGUCUG	754	GUACGUAAUCUG UCUA	1138	UAGACAGAUUACGUA CCCUUG
371	AGGGUACGUAA UCUGUCUGGC	755	ACGUAAUCUGUC UGGA	1139	UCCAGACAGAUUACG UACCCU

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
372	GUACGUAAUCU GUCUGGCAUU	756	UAAUCUGUCUGG CAUA	1140	UAUGCCAGACAGAUU ACGUAC
373	UACGUAAUCUG UCUGGCAUUA	757	AAUCUGUCUGGC AUUA	1141	UAAUGCCAGACAGAU UACGUA
374	CGUAAUCUGUC UGGCAUUACU	758	UCUGUCUGGCAU UACA	1142	UGUAAUGCCAGACAG AUUACG
375	AUCUGUCUGGC AUUACUUCUA	759	UCUGGCAUUACU UCUA	1143	UAGAAGUAAUGCCAG ACAGAU
376	CUGUCUGGCAU UACUUCUACU	760	UGGCAUUACUUC UACA	1144	UGUAGAAGUAAUGCC AGACAG
377	UCUGGCAUUAC UUCUACUUUU	761	CAUUACUUCUACU UUA	1145	UAAAGUAGAAGUAAU GCCAGA
378	CUGGCAUUACU UCUACUUUUG	762	AUUACUUCUACUU UUA	1146	UAAAAGUAGAAGUAA UGCCAG
379	UGGCAUUACUU CUACUUUUGU	763	UUACUUCUACUU UUGA	1147	UCAAAAGUAGAAGUA AUGCCA
380	UCUACUUUUGU ACAAAGGAUC	764	UUUUGUACAAAG GAUA	1148	UAUCCUUUGUACAAA AGUAGA
381	CUACUUUUGUA CAAAGGAUCA	765	UUUGUACAAAGG AUCA	1149	UGAUCCUUUGUACAA AAGUAG
382	UACUUUUGUAC AAAGGAUCAA	766	UUGUACAAAGGA UCAA	1150	UUGAUCCUUUGUACA AAAGUA
383	ACUUUUGUACAA AGGAUCAAA	767	UGUACAAAGGAU CAAA	1151	UUUGAUCCUUUGUAC AAAAGU
384	CUUUUGUACAAA GGAUCAAAA	768	GUACAAAGGAUCA AAA	1152	UUUUGAUCCUUUGUA CAAAAG

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 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-

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 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, 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 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 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 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, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, 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-$CH_2CH_2OCH_3$, also known as 2′-O-(2-methoxyethyl) or 2′-MOE). In some embodiments, the modification includes 2′-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2′-DMAOE, and 2′-dimethylaminoethoxyethoxy (also known in the art as 2′-O-dimethylamino-ethoxy-ethyl or 2′-DMAEOE), i.e., 2′-O-$CH_2OCH_2N(CH_3)_2$. Other potential sugar substituent groups include, e.g., aminopropoxy (-$OCH_2CH_2CH_2NH_2$), allyl (-CH_2-CH=CH_2), -O-allyl (-O-CH_2-CH=CH_2) 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.

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 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 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 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 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 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), and 6,7,8,9-tetrafluoro-l,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 moiety attached to the rigid 1,3-diazaphenoxazine-2-one scaffold (Lin et al., *Am. Chem. Soc.,* 120:8531-2, 1998).

Internucleoside Linkage Modifications

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 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 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 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 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 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 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; 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. 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 CH_2 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.

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

$$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C'\text{-}P^1)_k\text{-}C'$$

Formula I;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$; each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$; B is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$; 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 P^1 is a phosphorothioate internucleoside linkage; each P^2 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.

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

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 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:

$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C\text{-}P^1)_k\text{-}C'$

Formula II;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$; each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$; B is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$; 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 P^1 is a phosphorothioate internucleoside linkage; each P^2 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.

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:

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

$E\text{-}(A')_m\text{-}F$

Formula III;

wherein E is represented by the formula $(C\text{-}P^1)_2$; F is represented by the formula $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, or $(C\text{-}P^2)_3\text{-}D\text{-}P^2\text{-}C\text{-}P^2\text{-}D$; A', C, D, P^1, and P^2 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 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

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.

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;

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:

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.

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;

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:

$$A\text{-}(A')_j\text{-}C\text{-}P^2\text{-}B\text{-}(C\text{-}P^1)_k\text{-}C'$$

Formula IV;

wherein A is represented by the formula C-P^1-D-P^1; each A' is represented by the formula C-P^2-D-P^2; B is represented by the formula D-P^1-C-P^1-D-P^1; 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 P^1 is a phosphorothioate internucleoside linkage; each P^2 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 to7 (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 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:

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-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.

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:

$$E\text{-}(A')_m\text{-}C\text{-}P^2\text{-}F$$

Formula V;

wherein E is represented by the formula $(C\text{-}P^1)_2$; F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, or $D\text{-}P^2\text{-}C\text{-}P^2\text{-}D$; A', C, D, P^1, and P^2 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.

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:

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.

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:

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.

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:

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.

In some embodiments of the disclosure, the sense strand includes a structure represented by Formula S8, wherein Formula S8 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-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.

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:

$A\text{-}B_j\text{-}E\text{-}B_k\text{-}E\text{-}F\text{-}G_l\text{-}D\text{-}P^1\text{-}C'$

Formula VI;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$; each B is represented by the formula $C\text{-}P^2$; 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 E is represented by the formula $D\text{-}P^2\text{-}C\text{-}P^2$; F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1$; each G is represented by the formula $C\text{-}P^1$; each P^1 is a phosphorothioate internucleoside linkage; each P^2 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 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:

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

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 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:

$H\text{-}B_m\text{-}I_n\text{-}A'\text{-}B_o\text{-}H\text{-}C$

Formula VII;

wherein A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$; each H is represented by the formula $(C\text{-}P^1)_2$; each I is represented by the formula $(D\text{-}P^2)$; B, C, D, P^1, and P^2 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); 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:

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

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.

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

$$Z\text{-}((A\text{-}P\text{-})_n(B\text{-}P\text{-})_m)_q;$$

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 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

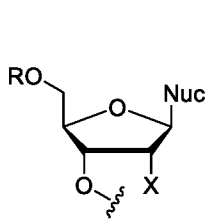
The siRNA molecules of the disclosure can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

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.

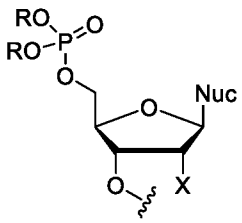
Further, it is contemplated that for any siRNA agent disclosed herein, further optimization could be achieved by systematically either adding or removing linked nucleosides to generate longer or shorter sequences. Further still, such optimized sequences can be adjusted by, e.g., the introduction of modified 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 (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).

5′ Phosphorus Stabilizing Moieties

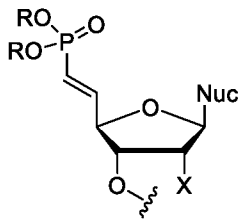
To further protect the siRNA molecules of this disclosure from degradation, a 5′-phosphorus stabilizing moiety may be employed. A 5′-phosphorus stabilizing moiety replaces the 5′-phosphate to prevent hydrolysis of the phosphate. Hydrolysis of the 5′-phosphate prevents binding to RISC, a necessary step 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.



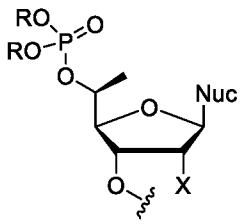
Formula IX



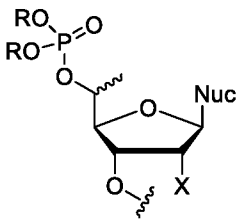
Formula X



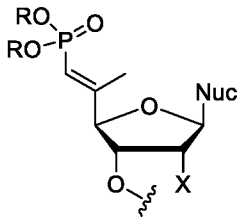
Formula XI



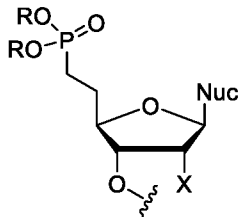
Formula XII



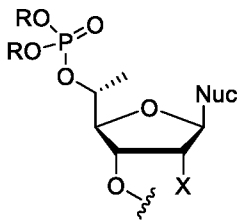
Formula XIII



Formula XIV



Formula XV



Formula XVI

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-substitued phosphates as in Formula XII, XIII, and XVI, methylenephosphonates as in Formula XV, or vinyl 5′-vinylphsophonate as a 5′-phosphorus stabilizing moiety as demonstrated in Formula XI.

Hydrophobic Moieties

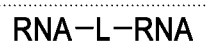
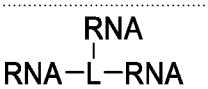
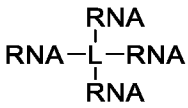
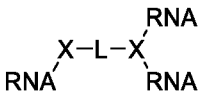
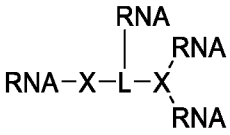
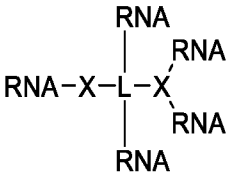
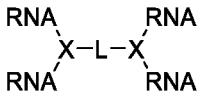
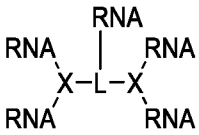
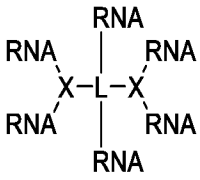
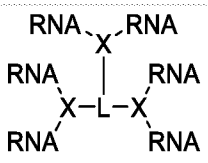
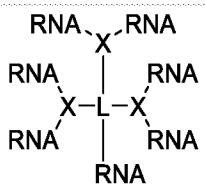
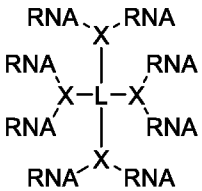
The present disclosure further provides siRNA molecules having one or more hydrophobic moieties attached thereto. The hydrophobic moiety may be covalently attached to the 5' end or the 3' end of the siRNA molecules of the disclosure. Non-limiting examples of hydrophobic moieties suitable for use with 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.

Table 2. Branched siRNA structures

Di-branched	Tri-branched	Tetra-branched
 **Formula XVII**	 **Formula XX**	 **Formula XXIV**
 **Formula XVIII**	 **Formula XXI**	 **Formula XXV**
 **Formula XIX**	 **Formula XXII**	 **Formula XXVI**
	 **Formula XXIII**	 **Formula XXVII**
		 **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).

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 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 (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 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.

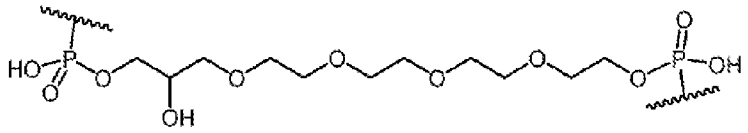
PEG linkers of various weights may be used with the disclosed compositions and methods. For example, the PEG linker may have a weight that is between 5 and 500 Daltons. In some embodiments, a PEG linker having a weight that is between 500 and 1,000 Dalton may be used. In some embodiments, a PEG linker 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. 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 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 siRNA strand (e.g., sense or antisense strand) by way of a covalent bond-forming moiety. In some

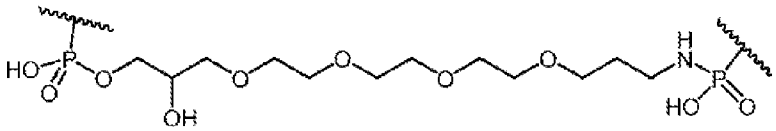
embodiments, the covalent-bond-forming moiety is selected from the group consisting of an alkyl, ester, amide, carbonate, carbamate, triazole, urea, formacetal, phosphonate, phosphate, and phosphate derivative (e.g., phosphorothioate, phosphoramidate, etc.).

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



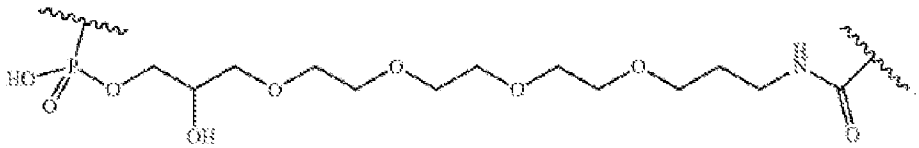
(**Formula L1**)

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



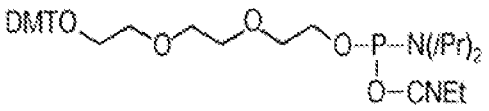
(**Formula L2**)

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



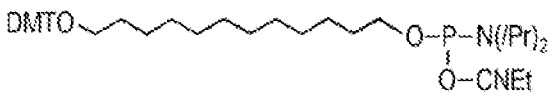
(**Formula L3**)

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



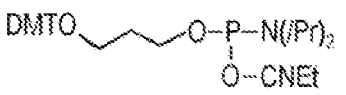
(**Formula L4**)

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



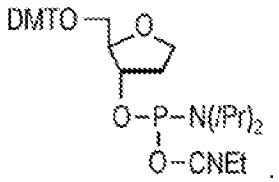
(**Formula L5**)

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



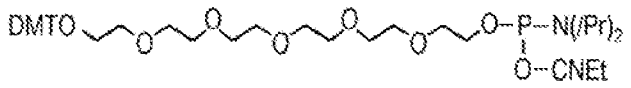
(**Formula L6**)

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



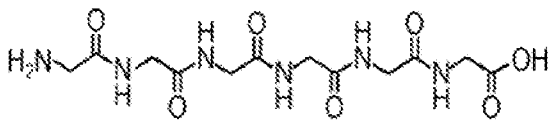
(**Formula L7**)

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



(**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. 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. (edrs.), *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 *C9orf72*-targeting siRNA molecules of the disclosure may be delivered to a subject, thereby treating amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and/or mitigating phenotypes associated with the disorders. For example, the siRNA molecules may be delivered to a subject, thereby treating ALS and/or mitigating ALS disease associated phenotypes (e.g., involuntary movements, muscle cramps, weakness, loss of motor control). Alternatively, the *C9orf72*-targeting siRNA molecules of the disclosure may be delivered to a subject, thereby treating FTD and/or mitigating FTD-associated phenotypes (e.g., memory problems, behavioral problems, and language problems). Furthermore, the siRNA molecules of the disclosure may also be delivered to a subject having a variant of the *C9orf72* gene for which siRNA-mediated gene silencing of the *C9orf72* variant gene reduces the expression level of *C9orf72*transcript, thereby treating ALS, FTD, or other *C9orf72*-related diseases or disorders.

The disclosure provides methods of treating a subject by way of *C9orf72* 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 *C9orf72* transcripts, mutant *C9orf72* transcripts, splice isoforms of *C9orf72* transcripts, and/or overexpressed *C9orf72* 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, ALS, FTD, and/or any other medical risk(s) associated with repeat expansions or a gain of function mutations in the *C9orf72* 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 *C9orf72* gene. Subjects may be adult or pediatric humans, with or without comorbid diseases.

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.

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, intrastriatally, intraparenchymally, or by intra-cisterna magna injection by catheterization.

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.

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.

Intrastriatal 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.

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.

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.

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.

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. *C9orf72* Knockdown

Objective

This Example describes the results of a series of experiments undertaken to investigate the ability of siRNA molecules complementary to specific regions within a human *C9orf72* mRNA transcript to effectuate reduced expression of the *C9orf72* gene.

Materials and Methods

SK-Mel-28 cells were actively transfected with *C9orf72* siRNA at either 20 nM or 0.5 nM concentration. After 72 hours, cells were lysed, and mRNA levels of *C9orf72* and a housekeeping gene (*ATP5b*) were assessed via reverse transcription-quantitative polymerase chain reaction (RT-qPCR), using standard reagents and Applied Biosystems TaqMan Assays. Results are presented as the percent residual *C9orf72* mRNA relative to untreated control cells in the same assay (% untreated *C9orf72* mRNA).

Results

Cells were treated with an siRNA molecule of the disclosure having an antisense strand and a sense strand as shown in Tables 3 and 4, below. The knockdown efficiency of the siRNA molecule was measured as the residual mRNA expression as a percent of untreated at 20 nM and 0.5 nM. The knockdown results are reported in Table 3 (20 nM) and Table 4 (0.5 nM).

Table 3. *C9orf72* knockdown with siRNA molecules of the disclosure at 20 nM

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20nM
385	769	74.00	481	865	52.36
386	770	85.78	482	866	71.01
387	771	79.70	483	867	44.30
388	772	77.28	484	868	49.39
389	773	54.30	485	869	46.47
390	774	64.79	486	870	57.40
391	775	58.64	487	871	49.00
392	776	54.69	488	872	46.76
393	777	55.58	489	873	57.33
394	778	51.96	490	874	80.26
395	779	58.93	491	875	32.25
396	780	44.61	492	876	68.91
397	781	86.51	493	877	50.10
398	782	102.55	494	878	92.45
399	783	112.99	495	879	78.79
400	784	91.22	496	880	74.28
401	785	89.90	497	881	77.65
402	786	83.64	498	882	74.29
403	787	61.39	499	883	71.13

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20nM
404	788	65.81	500	884	49.89
405	789	103.09	501	885	63.02
406	790	54.51	502	886	81.52
407	791	52.45	503	887	46.23
408	792	50.84	504	888	67.99
409	793	69.80	505	889	57.11
410	794	96.64	506	890	68.80
411	795	119.22	507	891	63.82
412	796	114.33	508	892	53.86
413	797	90.95	509	893	58.30
414	798	107.05	510	894	64.76
415	799	91.26	511	895	46.48
416	800	94.56	512	896	47.97
417	801	75.07	513	897	64.87
418	802	99.10	514	898	51.53
419	803	74.43	515	899	36.03
420	804	53.74	516	900	70.51
421	805	47.34	517	901	79.20
422	806	58.39	518	902	90.79
423	807	65.98	519	903	57.01
424	808	77.90	520	904	75.99
425	809	71.81	521	905	78.42
426	810	61.33	522	906	98.79
427	811	48.92	523	907	72.07
428	812	58.04	524	908	92.75
429	813	49.80	525	909	59.36
430	814	68.66	526	910	58.82
431	815	34.03	527	911	34.47
432	816	39.74	528	912	84.04
433	817	49.56	529	913	92.25
434	818	62.23	530	914	55.10
435	819	87.09	531	915	52.36
436	820	72.18	532	916	60.91
437	821	82.11	533	917	62.38
438	822	62.77	534	918	70.29
439	823	81.36	535	919	79.64

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20nM
440	824	63.83	536	920	63.22
441	825	66.81	537	921	93.23
442	826	74.00	538	922	83.98
443	827	36.80	539	923	74.11
444	828	58.77	540	924	90.48
445	829	74.49	541	925	64.63
446	830	53.66	542	926	61.97
447	831	74.80	543	927	60.43
448	832	79.27	544	928	66.78
449	833	74.76	545	929	93.84
450	834	76.84	546	930	45.91
451	835	60.15	547	931	49.41
452	836	79.33	548	932	78.51
453	837	63.84	549	933	60.34
454	838	66.99	550	934	55.75
455	839	69.32	551	935	59.67
456	840	55.81	552	936	60.02
457	841	73.89	553	937	46.00
458	842	80.64	554	938	67.79
459	843	57.18	555	939	73.43
460	844	59.46	556	940	61.62
461	845	47.81	557	941	61.09
462	846	34.66	558	942	53.05
463	847	60.85	559	943	47.16
464	848	53.90	560	944	67.65
465	849	74.73	561	945	52.99
466	850	54.02	562	946	57.49
467	851	51.19	563	947	92.57
468	852	45.04	564	948	93.27
469	853	43.66	565	949	75.14
470	854	58.92	566	950	39.93
471	855	60.19	567	951	61.67
472	856	75.60	568	952	37.64
473	857	59.15	569	953	52.46
474	858	50.57	570	954	63.78
475	859	84.36	571	955	67.30

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20nM
476	860	59.34	572	956	58.16
477	861	65.52	573	957	55.93
478	862	56.57	574	958	66.79
479	863	41.69	575	959	62.89
480	864	63.84	576	960	79.90

Table 4. *C9orf72* knockdown with siRNA molecules of the disclosure at 0.5 nM

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.5nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.5nM
385	769	84.18	481	865	52.13
386	770	97.05	482	866	82.47
387	771	81.22	483	867	62.75
388	772	97.76	484	868	86.17
389	773	79.78	485	869	50.62
390	774	51.41	486	870	69.02
391	775	79.03	487	871	55.35
392	776	58.50	488	872	61.01
393	777	61.25	489	873	83.95
394	778	65.12	490	874	95.17
395	779	86.17	491	875	77.58
396	780	84.40	492	876	86.29
397	781	94.73	493	877	67.01
398	782	92.59	494	878	80.27
399	783	92.94	495	879	111.22
400	784	99.21	496	880	85.77
401	785	92.58	497	881	87.74
402	786	92.91	498	882	58.48
403	787	93.13	499	883	94.27
404	788	93.43	500	884	85.55
405	789	99.32	501	885	97.34
406	790	87.63	502	886	107.06

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.5nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.5nM
407	791	101.10	503	887	104.85
408	792	98.80	504	888	86.50
409	793	74.44	505	889	78.24
410	794	98.27	506	890	111.98
411	795	101.08	507	891	117.07
412	796	100.91	508	892	96.64
413	797	93.69	509	893	80.66
414	798	102.04	510	894	95.83
415	799	95.92	511	895	80.29
416	800	95.63	512	896	72.07
417	801	99.42	513	897	99.75
418	802	105.08	514	898	89.84
419	803	90.83	515	899	90.49
420	804	85.58	516	900	89.41
421	805	69.67	517	901	134.33
422	806	66.74	518	902	126.60
423	807	84.63	519	903	109.67
424	808	71.51	520	904	134.21
425	809	84.20	521	905	99.58
426	810	79.29	522	906	83.35
427	811	71.81	523	907	102.65
428	812	66.27	524	908	99.01
429	813	88.06	525	909	75.79
430	814	104.23	526	910	98.36
431	815	73.08	527	911	96.92
432	816	66.69	528	912	89.97
433	817	72.79	529	913	126.77
434	818	81.19	530	914	94.63
435	819	98.19	531	915	79.73
436	820	101.08	532	916	64.65
437	821	95.62	533	917	107.40
438	822	86.32	534	918	89.47
439	823	96.12	535	919	95.48
440	824	85.32	536	920	100.38
441	825	94.05	537	921	110.71
442	826	93.73	538	922	108.97

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.5nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.5nM
443	827	82.68	539	923	82.87
444	828	88.17	540	924	84.50
445	829	83.81	541	925	80.19
446	830	84.38	542	926	124.01
447	831	94.46	543	927	80.66
448	832	88.26	544	928	97.55
449	833	87.97	545	929	94.59
450	834	84.91	546	930	49.41
451	835	88.73	547	931	76.37
452	836	93.46	548	932	99.19
453	837	96.57	549	933	66.92
454	838	96.64	550	934	76.66
455	839	87.89	551	935	90.08
456	840	85.89	552	936	89.27
457	841	77.76	553	937	44.62
458	842	95.41	554	938	114.96
459	843	69.57	555	939	106.06
460	844	71.21	556	940	85.79
461	845	64.29	557	941	60.33
462	846	70.72	558	942	75.62
463	847	94.36	559	943	70.66
464	848	81.27	560	944	69.97
465	849	84.43	561	945	96.74
466	850	94.31	562	946	81.67
467	851	91.42	563	947	92.52
468	852	94.65	564	948	109.02
469	853	58.63	565	949	59.35
470	854	68.50	566	950	58.60
471	855	69.36	567	951	71.91
472	856	89.47	568	952	76.83
473	857	88.74	569	953	58.74
474	858	78.16	570	954	50.59
475	859	70.96	571	955	67.64
476	860	94.34	572	956	49.70
477	861	78.84	573	957	52.16
478	862	93.03	574	958	68.24

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.5nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.5nM
479	863	91.53	575	959	95.02
480	864	95.85	576	960	105.03

Example 2. Generating *C9orf72*-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 *C9orf72* 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 3. Optimizing *C9orf72*-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-$CH_2CH_2OCH_3$, also known as 2′-O-(2-methoxyethyl) or 2′-MOE), 2′-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ 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-$CH_2OCH_2N(CH_3)_2$. 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 (-$OCH_2CH_2CH_2NH_2$), allyl (-CH_2-CH=CH_2), -O-allyl (-O-CH_2-CH=CH_2) 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 4. Preparation and Administrating *C9orf72*-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, intrastriatally, intraparenchymally, or intra-cisterna 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 5. Methods for the Treatment of Amyotrophic Lateral Sclerosis or Frontotemporal Dementia Using *C9orf72*-targeting siRNA Molecules

A subject in need of treatment of amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD), 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 *C9orf72* mRNA or suitable biomarker or a reduction of ALS- or FTD-associated phenotypes) 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, intrastriatally, 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 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, 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 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, intrastriatally, intraparenchymally, intravenously, intramuscularly,

subcutaneously, or by intra-cisterna magna injection via catheterization) and condition at a rate 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 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 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 sense strand having complementarity to the antisense strand, wherein the antisense strand has complementarity sufficient to hybridize to a region within a chromosome 9 open reading frame 72 (*C9orf72*) mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384.

2. The siRNA molecule of claim 1, wherein the antisense strand has at least 70% complementarity to a region of 19, 20, 21, or more contiguous nucleobases within the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384, optionally wherein the antisense strand has at least 70% complementarity to the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384.

3. The siRNA molecule of claim 2, wherein the antisense strand has at least 75% complementarity to a region of 21 contiguous nucleobases within the *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384, 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 *C9orf72* mRNA transcript having the nucleic acid sequence of any one of SEQ ID Nos: 1-384.

4. The siRNA molecule of any one of claims 1-3, 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 *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384.

5. The siRNA molecule of claim 4, 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 *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384.

6. The siRNA molecule of claim 5, 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 *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384.

7. The siRNA molecule of claim 6, 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 *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384.

8. The siRNA molecule of claim 7, 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 *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384.

9. The siRNA molecule of claim 8, wherein the antisense strand comprises from 18 to 25 contiguous nucleotides that are fully complementary to a contiguous polynucleotide segment of equal length within the region of the *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384.

10. The siRNA molecule of any one of claims 9, 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 *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384.

11. The siRNA molecule of claim 10, 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 *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384.

12. The siRNA molecule of any one of claims 1-11, wherein the antisense strand comprises 9 or fewer nucleotide mismatches relative to a region of 21 contiguous nucleobases of the *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384, 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 *C9orf72* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-384.

13. The siRNA molecule of any one of claims 1-12, wherein the region of the *C9orf72* RNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

14. The siRNA molecule of any one of claims 1-12, wherein the region of the *C9orf72* RNA transcript has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

15. The siRNA molecule of any one of claims 1-14, 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: 769-1152.

16. The siRNA molecule of claim 15, 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: 769-1152.

17. The siRNA molecule of claim 16, wherein the antisense strand has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NOs: 769-1152, 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: 769-1152.

18. The siRNA molecule of claim 17, wherein the antisense strand has the nucleic acid sequence of any one of SEQ ID NOs: 769-1152.

19. The siRNA molecule of any one of claims 15-18, wherein the nucleic acid sequence is any one of SEQ ID NOs: 769-960.

20. The siRNA molecule of any one of claims 15-18, wherein the nucleic acid sequence is any one of SEQ ID NOs: 961-1152.

21. The siRNA molecule of any one of claims 1-20, 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: 385-768.

22. The siRNA molecule of claim 21, 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: 385-768.

23. The siRNA molecule of claim 22, wherein the sense strand has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NOs: 385-768, 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: 385-768.

24. The siRNA molecule of claim 23, wherein the sense strand has the nucleic acid sequence of any one of SEQ ID NOs: 385-768.

25. The siRNA molecule of any one of claims 21-24, wherein the nucleic acid sequence is any one of SEQ ID NOs: 385-576.

26. The siRNA molecule of any one of claims 21-24, wherein the nucleic acid sequence is any one of SEQ ID NOs: 577-768.

27. The siRNA molecule of any one of claims 1-26, wherein the antisense strand comprises a structure represented by Formula I, wherein Formula I is, in the 5'-to-3' direction:

$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C'\text{-}P^1)_k\text{-}C'$

Formula I;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
B is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$;
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 P^1 is a phosphorothioate internucleoside linkage;
each P^2 is a phosphodiester internucleoside linkage;
j is an integer from 1 to 7; and
k is an integer from 1 to 7.

28. The siRNA molecule of claim 27, wherein the antisense strand comprises 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-O-A-O-B-S-A-S-A-S-A-S-B-S-A

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.

29. The siRNA molecule of any one of claims 1-26, wherein the antisense strand comprises a structure represented by Formula II, wherein Formula II is, in the 5'-to-3' direction:

$A\text{-}B\text{-}(A')_j\text{-}C\text{-}P^2\text{-}D\text{-}P^1\text{-}(C\text{-}P^1)_k\text{-}C'$

Formula II;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
B is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$;
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 P^1 is a phosphorothioate internucleoside linkage;
each P^2 is a phosphodiester internucleoside linkage;
j is an integer from 1 to 7; and
k is an integer from 1 to 7.

30. The siRNA molecule of claim 29, wherein the antisense strand comprises a structure represented by Formula A2, wherein Formula A2 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-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.

31. The siRNA molecule of any one of claims 1-30, wherein the sense strand comprises a structure represented by Formula III, wherein Formula III is, in the 5'-to-3' direction:

$E\text{-}(A')_m\text{-}F$
Formula III;

wherein E is represented by the formula $(C\text{-}P^1)_2$;
F is represented by the formula $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, or $(C\text{-}P^2)_3\text{-}D\text{-}P^2\text{-}C\text{-}P^2\text{-}D$;
A', C, D, P^1, and P^2 are as defined in Formula II; and
m is an integer from 1 to 7.

32. The siRNA molecule of claim 31, wherein the sense strand comprises 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
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.

33. The siRNA molecule of claim 31, wherein the sense strand comprises 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;

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.

34. The siRNA molecule of claim 31, wherein the sense strand comprises a structure represented by Formula S3, wherein Formula S3 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-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.

35. The siRNA molecule of claim 31, wherein the sense strand comprises 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;

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.

36. The siRNA molecule of any one of claims 1-26 and 31-35, wherein the antisense strand comprises a structure represented by Formula IV, wherein Formula IV is, in the 5'-to-3' direction:

$A\text{-}(A')_j\text{-}C\text{-}P^2\text{-}B\text{-}(C\text{-}P^1)_k\text{-}C'$

Formula IV;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
B is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1\text{-}D\text{-}P^1$;
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 P^1 is a phosphorothioate internucleoside linkage;
each P^2 is a phosphodiester internucleoside linkage;
j is an integer from 1 to 7; and
k is an integer from 1 to 7.

37. The siRNA molecule of claim 36, 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-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.

38. The siRNA molecule of any one of claims 1-30, 36, and 37, wherein the sense strand comprises a structure represented by Formula V, wherein Formula V is, in the 5'-to-3' direction:

$$E\text{-}(A')_m\text{-}C\text{-}P^2\text{-}F$$

Formula V;

wherein E is represented by the formula $(C\text{-}P^1)_2$;
F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, or $D\text{-}P^2\text{-}C\text{-}P^2\text{-}D$;
A', C, D, P^1 and P^2 are as defined in Formula IV; and
m is an integer from 1 to 7.

39. The siRNA molecule of claim 38, 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.

40. The siRNA molecule of claim 38, wherein the sense strand comprises a structure represented by Formula S6, wherein Formula S6 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-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.

41. The siRNA molecule of claim 38, wherein the sense strand comprises 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.

42. The siRNA molecule of claim 38, wherein the sense strand comprises a structure represented by Formula S8, wherein Formula S8 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-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.

43. The siRNA molecule of any one of claims 1-26, 31-35 and 38-42, wherein the antisense strand comprises a structure represented by Formula VI, wherein Formula VI is, in the 5'-to-3' direction:

$A\text{-}B_j\text{-}E\text{-}B_k\text{-}E\text{-}F\text{-}G_l\text{-}D\text{-}P^1\text{-}C'$

Formula VI;

wherein A is represented by the formula $C\text{-}P^1\text{-}D\text{-}P^1$;
each B is represented by the formula $C\text{-}P^2$;
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 E is represented by the formula $D\text{-}P^2\text{-}C\text{-}P^2$;
F is represented by the formula $D\text{-}P^1\text{-}C\text{-}P^1$;
each G is represented by the formula $C\text{-}P^1$;
each P^1 is a phosphorothioate internucleoside linkage;
each P^2 is 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.

44. The siRNA molecule of claim 43, wherein the antisense strand comprises a structure represented by Formula A4, wherein Formula A4 is, in the 5'-to-3' direction:

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

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.

45. The siRNA molecule of any one of claims 1-30, 36, 37, 43, and 44, wherein the sense strand comprises a structure represented by Formula VII, wherein Formula VII is, in the 5'-to-3' direction:

$$H\text{-}B_m\text{-}I_n\text{-}A'\text{-}B_o\text{-}H\text{-}C$$

Formula VII;

wherein A' is represented by the formula $C\text{-}P^2\text{-}D\text{-}P^2$;
each H is represented by the formula $(C\text{-}P^1)_2$;
each I is represented by the formula $(D\text{-}P^2)$;
B, C, D, P^1 and P^2 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.

46. The siRNA molecule of claim 45, wherein the sense strand comprises 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

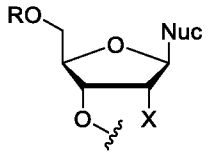
Formula S9;

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.

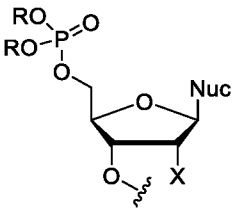
47. The siRNA molecule of any one of claims 1-46, wherein the antisense strand further comprises a 5' phosphorus stabilizing moiety at the 5' end of the antisense strand.

48. The siRNA molecule of any one of claims 1-47, wherein the sense strand further comprises a 5' phosphorus stabilizing moiety at the 5' end of the sense strand.

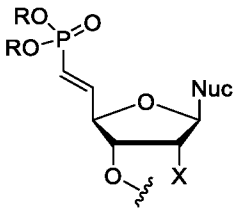
49. The siRNA molecule of claim 47 or 48, wherein each 5' phosphorus stabilizing moiety is, independently, represented by any one of Formulas IX-XVI:



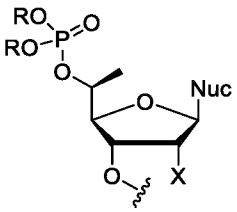
Formula IX



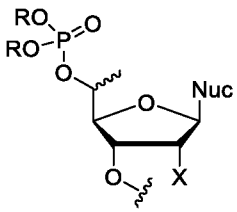
Formula X



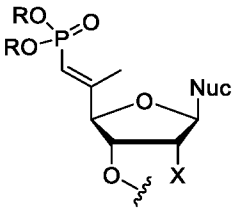
Formula XI



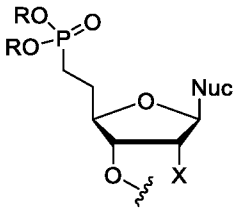
Formula XII



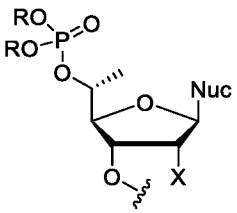
Formula XIII



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.

50. The siRNA molecule of claim 49, wherein the nucleobase is an adenine, uracil, guanine, thymine, or cytosine.

51. The siRNA molecule of any one of claims 47-50, wherein the 5' phosphorus stabilizing moiety is (E)-vinylphosphonate represented by Formula XI.

52. The siRNA molecule of any one of claims 1-51, wherein the siRNA molecule further comprises a hydrophobic moiety at the 5' or the 3' end of the siRNA molecule.

53. The siRNA molecule of claim 52, wherein the hydrophobic moiety is selected from a group consisting of cholesterol, vitamin D, or tocopherol.

54. The siRNA molecule of any one of claims 1-53, wherein the length of the sense strand is between 10 and 30 nucleotides.

55. The siRNA molecule of claim 54, wherein the length of the sense strand is between 10 and 25 nucleotides.

56. The siRNA molecule of claim 55, wherein the length of the sense strand is between 12 and 25 nucleotides.

57. The siRNA molecule of claim 56, wherein the length of the sense strand is between 12 and 20 nucleotides.

58. The siRNA molecule of claim 57, wherein the length of the sense strand is between 12 and 19 nucleotides.

59. The siRNA molecule of claim 58, wherein the length of the sense strand is 15 nucleotides.

60. The siRNA molecule of claim 58, wherein the length of the sense strand is 16 nucleotides.

61. The siRNA molecule of claim 58, wherein the length of the sense strand is 18 nucleotides.

62. The siRNA molecule of any one of claims 1-61, wherein the length of the antisense strand is between 10 and 30 nucleotides.

63. The siRNA molecule of claim 62, wherein the length of the antisense strand is between 12 and 30 nucleotides.

64. The siRNA molecule of claim 63, wherein the length of the antisense strand is between 15 and 30 nucleotides.

65. The siRNA molecule of claim 64, wherein the length of the antisense strand is between 18 and 30 nucleotides.

66. The siRNA molecule of claim 65, wherein the length of the antisense strand is between 18 and 25 nucleotides.

67. The siRNA molecule of claim 66, wherein the length of the antisense strand is between 18 and 21 nucleotides.

68. The siRNA molecule of claim 67, wherein the length of the antisense strand is 18 nucleotides.

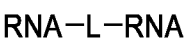
69. The siRNA molecule of claim 67, wherein the length of the antisense strand is 20 nucleotides.

70. The siRNA molecule of claim 67, wherein the length of the antisense strand is 21 nucleotides.

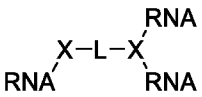
71. The siRNA molecule of any one of claims 1-70, wherein the siRNA molecule is a branched siRNA molecule.

72. The siRNA molecule of claim 71, wherein the branched siRNA molecule is di-branched, tri-branched, or tetra-branched.

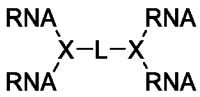
73. The siRNA molecule of claim 72, wherein the siRNA molecule is a di-branched siRNA molecule, optionally wherein the di-branched siRNA molecule is represented by any one of Formulas XVII-XIX:



Formula XVII;



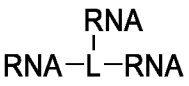
Formula XVIII;



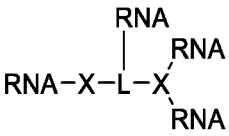
Formula XIX;

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

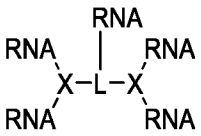
74. The siRNA molecule of claim 72, wherein the siRNA molecule is a tri-branched siRNA molecule, optionally wherein the tri-branched siRNA molecule is represented by any one of Formulas XX-XXIII:



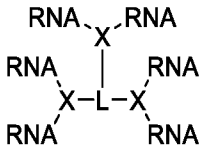
Formula XX;



Formula XXI;



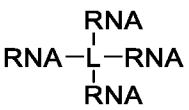
Formula XXII;



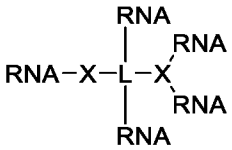
Formula XXIII;

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

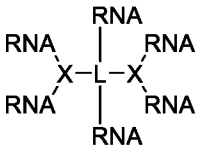
75. The siRNA molecule of claim 72, wherein the siRNA molecule is a tetra-branched siRNA molecule, optionally wherein the tetra-branched siRNA molecule is represented by any one of Formulas XXIV-XXVIII:



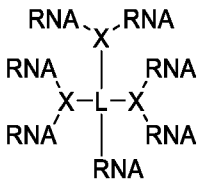
Formula XXIV;



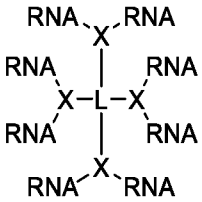
Formula XXV;



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.

76. The siRNA molecule of any one of claims 73-75, 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.

77. The siRNA molecule of claim 76, wherein the one or more contiguous subunits is 2 to 20 contiguous subunits.

78. A pharmaceutical composition comprising the siRNA molecule of any one of claims 1-77 and a pharmaceutically acceptable excipient, carrier, or diluent.

79. A method of delivering an siRNA molecule to a subject diagnosed as amyotrophic lateral sclerosis (ALS), the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-77 or the pharmaceutical composition of claim 78 to the subject.

80. A method of delivering an siRNA molecule to a subject diagnosed as frontotemporal dementia (FTD), the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-77 or the pharmaceutical composition of claim 78 to the subject.

81. A method of treating ALS in a subject in need thereof, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-77 or the pharmaceutical composition of claim 78 to the subject.

82. A method of treating FTD in a subject in need thereof, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-77 or the pharmaceutical composition of claim 78 to the subject.

83. A method of reducing *C9orf72* expression in a subject in need thereof, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-77 or the pharmaceutical composition of claim 78 to the subject.

84. The method of any one of claims 79-83, wherein the siRNA molecule or the pharmaceutical composition is administered to the subject by way of intracerebroventricular, intrastriatal, intraparenchymal, or intrathecal injection.

85. The method of any one of claims 79-83, wherein the siRNA molecule or the pharmaceutical composition is administered to the subject by way of intravenous, intramuscular, or subcutaneous injection.

86. The method of any one of claims 79-85, wherein the subject is a human.

87. A kit comprising the siRNA molecule of any one of claims 1-77, or the pharmaceutical composition of claim 78, and a package insert, wherein the package insert instructs a user of the kit to perform the method of any one of claims 79-86.