(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
04 April 2024 (04.04.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/073603 A2

(51) International Patent Classification:
Not classified

(21) International Application Number:
PCT/US2023/075424

(22) International Filing Date:
28 September 2023 (28.09.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/377,472 28 September 2022 (28.09.2022) US

(71) Applicant: **ATALANTA THERAPEUTICS, INC.** [US/US]; 51 Sleeper Street, 7th Floor, Boston, MA 02210 (US).

(72) Inventors: **GALLANT-BEHM, Corrie**; 44 Mansfield Rd, Wellesley, MA 02481 (US). **KOKULAPALAN, Wimalanthan**; 43 Murray Hill Rd, Cambridge, MA 02140 (US). **ANDREONE, Benjamin**; 407 D St, Boston, MA 02210 (US). **HASSLER, Matthew**; 126 Border St, Apt 512, Boston, MA 02128 (US). **CURTIS, Daniel**; 57 Brettwood Rd, Belmont, MA 02478 (US). **DA CRUZ GODINHO, Bruno, Miguel**; 4 Fielding Rd, Reading, MA 01867 (US).

(74) Agent: **QUARTARARO, Justin, S.** et al.; Clark & Elbing LLP, 101 Federal Street, 15th Floor, Boston, MA 02210 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2024/073603 A2

(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF NEUROINFLAMMATORY DISEASES

(57) Abstract: The present disclosure provides single- or double-stranded interfering RNA molecules (e.g., siRNA) that target a SiglecS *(CD33)* 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, or tetra-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 a neuroinflammatory disease (e.g., Alzheimer's disease).

COMPOSITIONS AND METHODS FOR TREATMENT OF NEUROINFLAMMATORY DISEASES

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 "51436-040WO2_Sequence_Listing_9_20_23" and is 505,859 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 Siglec3 (*CD33*) gene. The disclosure further describes methods for silencing of *CD33* and the treatment of diseases that may benefit from the silencing of *CD33* (e.g., neuroinflammatory diseases such as Alzheimer's disease) by delivering *CD33*-targeting siRNA molecules to a target tissue of a subject in need.

Background

CD33 (Siglec3) encodes a protein that is implicated in the pathology of diseases including neuroinflammatory diseases (e.g., Alzheimer's disease). Accordingly, there is a need for therapeutics capable of selectively diminishing *CD33* activity in a manner that provides effective treatment for neuroinflammatory diseases (e.g., Alzheimer's disease) or other *CD33*-related diseases or disorders.

Summary of the Invention

The present disclosure provides compositions and methods for reduction of Siglec3 (*CD33*) expression by way of small interfering RNA (siRNA)-mediated silencing of *CD33* transcripts. The compositions and methods provide the benefit of exhibiting high selectivity toward *CD33* over other genes.

The siRNA molecules of the disclosure can be used to silence the *CD33* gene, thereby preventing the translation of the corresponding mRNA transcript and reducing Siglec3 protein expression. This reduction of Siglec3 levels thus prevents disease onset or progression, as a polymorphism in the *CD33* gene has been associated with the onset of neuroinflammatory disease (e.g., Alzheimer's disease). Siglecs are expressed in microglial cells, and microglia have been revealed to be involved in neurodegenerative disease (e.g., Alzheimer's disease) by the modulation of neuroinflammation. The siRNA molecules of the disclosure can be delivered directly to a subject in need of *CD33* 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 a *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192. 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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192. 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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192. 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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192. 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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192. 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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192. 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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192. 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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

In some embodiments, the antisense strand has at least 75% complementarity to the region within the *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192. 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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID Nos: 1-192.

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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

In some embodiments, the antisense strand has 9 or fewer nucleotide mismatches relative to a region of 21 contiguous nucleobases of the *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192, 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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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: 385-576.

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: 385-576.

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: 385-576, 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: 385-576.

In some embodiments, the antisense strand has 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: 193-384.

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: 193-384.

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: 193-384, 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: 193-384.

In some embodiments, the siRNA molecule has a sense strand having the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

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 $C\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2\text{-}D\text{-}P^2$;

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, 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 $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^2\text{-}C\text{-}P^2\text{-}C$, $(C\text{-}P^2)_3\text{-}D\text{-}P^1\text{-}C\text{-}P^1\text{-}D$, 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 (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

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-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

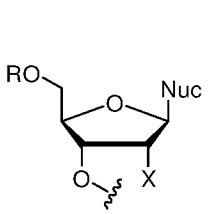
Formula S9;

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.

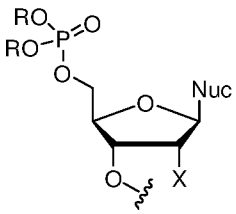
In some embodiments, the antisense strand also has a 5' phosphorus stabilizing moiety at the 5' end of the antisense strand.

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

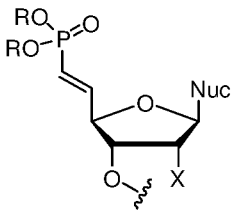
In some embodiments, each 5' phosphorus stabilizing moiety is, independently, represented by any one of Formulas IX, XX, XI, XII, XIII, XIV, XV, or XVI:



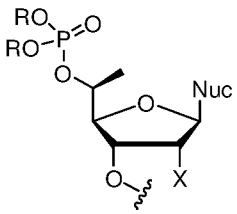
Formula IX



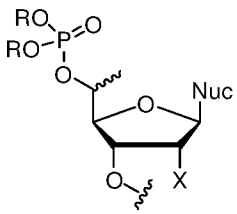
Formula X



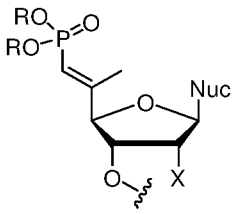
Formula XI



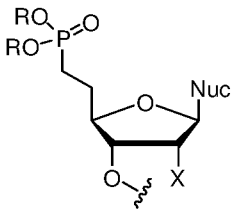
Formula XII



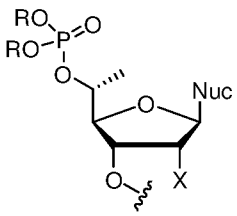
Formula XIII



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, and tocopherol.

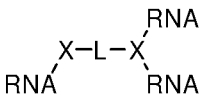
In some embodiments, the siRNA molecule is a branched siRNA molecule.

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

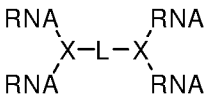
In some embodiments, the siRNA molecule is di-branched, optionally wherein the di-branched siRNA molecule is represented by any one of Formulas XVII, XVIII, or XIX:

RNA–L–RNA

Formula XVII;



Formula XVIII;

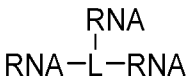


Formula XIX;

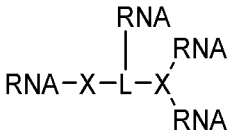
wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

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.

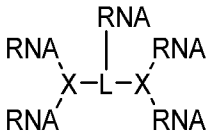
In some embodiments, the siRNA molecule is tri-branched, optionally wherein the tri-branched siRNA molecule is represented by any one of Formulas XX, XXI, XXII, or XXIII:



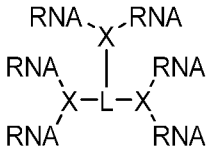
Formula XX;



Formula XXI;



Formula XXII;

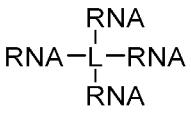


Formula XXIII;

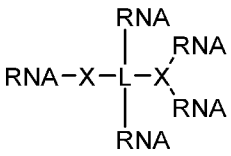
wherein each RNA is, independently, an siRNA molecule, L is a linker, and each X, independently, represents a branch point moiety.

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.

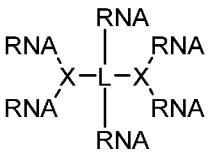
In some embodiments, the siRNA molecule is tetra-branched, optionally wherein the tetra-branched siRNA molecule is represented by any one of Formulas XXIV, XXV, XXVI, XXVII, or XXVIII:



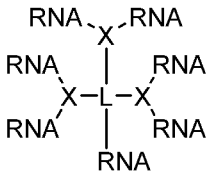
Formula XXIV;



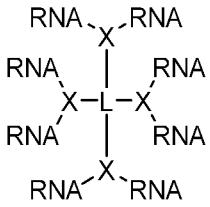
Formula XXV;



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.

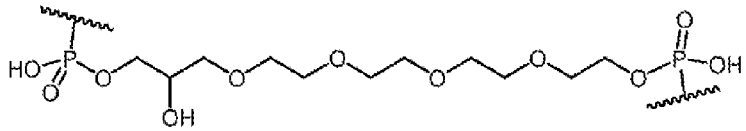
In some embodiments, the ethylene glycol oligomer is a PEG. In some embodiments, the PEG is a TrEG. In some embodiments, the PEG is a TEG.

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.

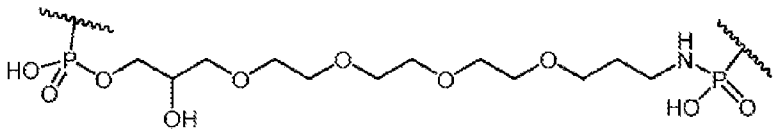
In some embodiments, the covalent bond-forming moiety is selected from the group consisting of an alkyl, ester, amide, carbamate, phosphonate, phosphate, phosphorothioate, phosphoroamidate, triazole, urea, and formacetal.

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



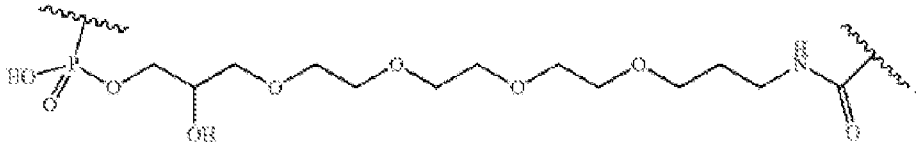
(**Formula L1**)

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



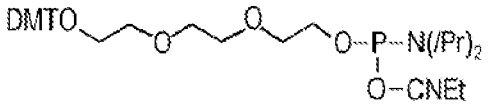
(**Formula L2**)

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



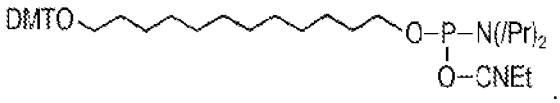
(**Formula L3**)

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



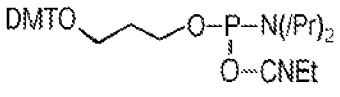
(**Formula L4**)

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



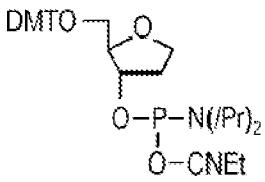
(**Formula L5**)

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



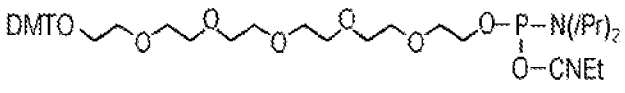
(**Formula L6**)

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



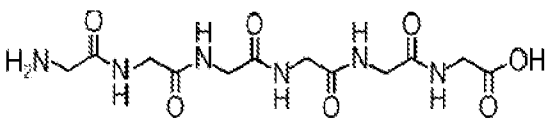
(**Formula L7**)

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

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

embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 25 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 26 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 27 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 28 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 29 nucleotides in length. In some embodiments, the antisense strand is 30 nucleotides in length and the sense strand is 30 nucleotides in length.

In 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 a neuroinflammatory disease 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 a neuroinflammatory disease 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 some embodiments of the methods described herein, the neuroinflammatory disease is Alzheimer's disease.

In another aspect, the disclosure provides a method of reducing *CD33* 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 of the methods described herein, 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 of any of the methods described herein, 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.

Brief Description of the Drawings

FIG. 1 is a graph showing an increase in phospho-SYK, a consensus marker for TREM2 activity, in iPSC-derived microglial-like cells that were administered an siRNA molecule of the disclosure with a sense strand having SEQ ID No.: 326 and an antisense strand having SEQ ID No.: 518.

FIG. 2 is a graph showing an increase in phagocytic capacity in iPSC-derived microglial-like cells that were administered an siRNA molecule of the disclosure with a sense strand having SEQ ID No.: 326 and an antisense strand having SEQ ID No.: 518, followed by treatment with pHrodo-labeled oligomeric amyloid-beta.

FIG. 3 is a graph showing reduction in the inflammatory cytokines IL1β, TNFα, and IL6 in iPSC-derived microglial-like cells that were administered an siRNA molecule of the disclosure with a sense strand having SEQ ID No.: 326 and an antisense strand having SEQ ID No.: 518, followed by treatment with LPS.

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 *CD33*, 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 neuroinflammatory disease or disorder (e.g., Alzheimer’s disease) and/or contains a gain-of-function *CD33* variant allele or a polymorphism in the *CD33* gene that is linked to disease pathology.

As used herein, the term “*CD33*” refers to the gene encoding Siglec3, including any native *CD33* gene from any source. The term encompasses “full-length,” unprocessed *CD33* as well as any form of *CD33* that results from processing in the cell. The term also encompasses naturally occurring variants of *CD33*, e.g., splice variants or allelic variants. The nucleic acid sequence of an exemplary *CD33* gene is shown in European Nucleotide Archive (ENA) Accession No. AY040541.1. The amino acid sequence of an exemplary protein encoded by an *CD33* gene is shown in UNIPROT™ Accession No. P20138.

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 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, a neuroinflammatory disease such as Alzheimer's disease. For example, clinical benefits in the context of a subject having Alzheimer's disease administered an siRNA molecule or siRNA composition of the disclosure include, without limitation, a reduction in cognitive impairment or memory loss. "Benefit" and "response" are also used interchangeably to refer to, for example, a reduction in wild type *CD33* transcripts, mutant *CD33* transcripts, variant *CD33* transcripts, splice isoforms of *CD33* transcripts, and/or overexpressed *CD33* transcripts.

Detailed Description

The present disclosure provides compositions of small interfering RNA (siRNA) molecules with sequence homology to a Siglec3 (*CD33*) 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 *CD33*, 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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192. The degree of complementarity of the antisense strand to the region of the *CD33* mRNA transcript may be sufficient for the antisense strand to anneal over the full length of the region of the *CD33* 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 *CD33* mRNA transcript.

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: 385-576, 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: 385-576.

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: 193-384, 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: 193-384.

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 *CD33* mRNA transcript that are targeted by each antisense strand.

Table 1. Nucleotide sequences for gene-specific *CD33*-targeting siRNA

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
1	CUGCUUCCUCA GACAUGCCGC	193	UCCUCAGACAUG CCGA	385	UCGGCAUGUCUGAGG AAGCAG
2	GUGACGGUACA GGAGGGUUUG	194	GGUACAGGAGGG UUUA	386	UAAACCCUCCUGUAC CGUCAC
3	GUACAGGAGGG UUUGUGCGUC	195	GGAGGGUUUGUG CGUA	387	UACGCACAAACCCUC CUGUAC
4	UACAGGAGGGU UUGUGCGUCC	196	GAGGGUUUGUGC GUCA	388	UGACGCACAAACCCU CCUGUA
5	UGCCCUGCACU UUCUUCCAUC	197	UGCACUUUCUUC CAUA	389	UAUGGAAGAAAGUGC AGGGCA
6	CCUGCACUUUC UUCCAUCCCA	198	ACUUUCUUCCAU CCCA	390	UGGGAUGGAAGAAAG UGCAGG
7	GUUCAUGGUUA CUGGUUCCGG	199	UGGUUACUGGUU CCGA	391	UCGGAACCAGUAACC AUGAAC
8	UCAUGGUUACU GGUUCCGGGA	200	GUUACUGGUUCC GGGA	392	UCCCGGAACCAGUAA CCAUGA
9	CAUGGUUACUG GUUCCGGGAA	201	UUACUGGUUCCG GGAA	393	UUCCCGGAACCAGUA ACCAUG
10	AUGGUUACUGG UUCCGGGAAG	202	UACUGGUUCCGG GAAA	394	UUUCCCGGAACCAGU AACCAU

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
11	CUGGUUCCGGG AAGGAGCCAU	203	UCCGGGAAGGAG CCAA	395	UUGGCUCCUUCCCGG AACCAG
12	UGGUUCCGGGA AGGAGCCAUU	204	CCGGGAAGGAGC CAUA	396	UAUGGCUCCUUCCCG GAACCA
13	GGUUCCGGGAA GGAGCCAUUA	205	CGGGAAGGAGCC AUUA	397	UAAUGGCUCCUUCCC GGAACC
14	CCAGGGACUCU CCAGUGGCCA	206	GACUCUCCAGUG GCCA	398	UGGCCACUGGAGAGU CCCUGG
15	CAGGGACUCUC CAGUGGCCAC	207	ACUCUCCAGUGG CCAA	399	UUGGCCACUGGAGAG UCCCUG
16	UGGCCACAAACA AGCUAGAUC	208	ACAAACAAGCUAG AUA	400	UAUCUAGCUUGUUUG UGGCCA
17	GGCCACAAACAA GCUAGAUCA	209	CAAACAAGCUAGA UCA	401	UGAUCUAGCUUGUUU GUGGCC
18	GCCACAAACAAG CUAGAUCAA	210	AAACAAGCUAGAU CAA	402	UUGAUCUAGCUUGUU UGUGGC
19	CCACAAACAAGC UAGAUCAAG	211	AACAAGCUAGAUC AAA	403	UUUGAUCUAGCUUGU UUGUGG
20	CACAAACAAGCU AGAUCAAGA	212	ACAAGCUAGAUCA AGA	404	UCUUGAUCUAGCUUG UUUGUG
21	ACAAACAAGCUA GAUCAAGAA	213	CAAGCUAGAUCAA GAA	405	UUCUUGAUCUAGCUU GUUUGU
22	CAAGCUAGAUCA AGAAGUACA	214	UAGAUCAAGAAGU ACA	406	UGUACUUCUUGAUCU AGCUUG
23	AAGCUAGAUCAA GAAGUACAG	215	AGAUCAAGAAGUA CAA	407	UUGUACUUCUUGAUC UAGCUU
24	AGCUAGAUCAA GAAGUACAGG	216	GAUCAAGAAGUAC AGA	408	UCUGUACUUCUUGAU CUAGCU
25	GCUAGAUCAAG AAGUACAGGA	217	AUCAAGAAGUACA GGA	409	UCCUGUACUUCUUGA UCUAGC
26	AUCCCAGUAGG AACAACUGCU	218	AGUAGGAACAACU GCA	410	UGCAGUUGUUCCUAC UGGGAU
27	UCCCAGUAGGA ACAACUGCUC	219	GUAGGAACAACU GCUA	411	UAGCAGUUGUUCCUA CUGGGA
28	CCCAGUAGGAA CAACUGCUCC	220	UAGGAACAACUG CUCA	412	UGAGCAGUUGUUCCU ACUGGG
29	UAAUGGUUCAU ACUUCUUUCG	221	GUUCAUACUUCU UUCA	413	UGAAAGAAGUAUGAA CCAUUA
30	AAUGGUUCAUA CUUCUUUCGG	222	UUCAUACUUCUU UCGA	414	UCGAAAGAAGUAUGA ACCAUU
31	AUGGUUCAUAC UUCUUUCGGA	223	UCAUACUUCUUU CGGA	415	UCCGAAAGAAGUAUG AACCAU
32	UGGUUCAUACU UCUUUCGGAU	224	CAUACUUCUUUC GGAA	416	UUCCGAAAGAAGUAU GAACCA
33	GGUUCAUACUU CUUUCGGAUG	225	AUACUUCUUUCG GAUA	417	UAUCCGAAAGAAGUA UGAACC
34	UUCAUACUUCU UUCGGAUGGA	226	ACUUCUUUCGGA UGGA	418	UCCAUCCGAAAGAAG UAUGAA
35	UCAUACUUCUU UCGGAUGGAG	227	CUUCUUUCGGAU GGAA	419	UUCCAUCCGAAAGAA GUAUGA
36	AGAGAGGAAGU ACCAAAUACA	228	GGAAGUACCAAAU ACA	420	UGUAUUUGGUACUUC CUCUCU

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
37	GAGAGGAAGUA CCAAAUACAG	229	GAAGUACCAAAUA CAA	421	UUGUAUUUGGUACUU CCUCUC
38	AGAGGAAGUAC CAAAUACAGU	230	AAGUACCAAAUAC AGA	422	UCUGUAUUUGGUACU UCCUCU
39	GAGGAAGUACC AAAUACAGUU	231	AGUACCAAAUACA GUA	423	UACUGUAUUUGGUAC UUCCUC
40	AGGAAGUACCAA AUACAGUUA	232	GUACCAAAUACAG UUA	424	UAACUGUAUUUGGUA CUUCCU
41	GGAAGUACCAAA UACAGUUAC	233	UACCAAAUACAGU UAA	425	UUAACUGUAUUUGGU ACUUCC
42	GAAGUACCAAAU ACAGUUACA	234	ACCAAAUACAGUU ACA	426	UGUAACUGUAUUUGG UACUUC
43	AAGUACCAAAUA CAGUUACAA	235	CCAAAUACAGUUA CAA	427	UUGUAACUGUAUUUG GUACUU
44	GUACCAAAUACA GUUACAAAU	236	AAAUACAGUUACA AAA	428	UUUUGUAACUGUAUU UGGUAC
45	UACCAAAUACAG UUACAAAUC	237	AAUACAGUUACAA AUA	429	UAUUUGUAACUGUAU UUGGUA
46	ACCAAAUACAGU UACAAAUCU	238	AUACAGUUACAAA UCA	430	UGAUUUGUAACUGUA UUUGGU
47	CCAAAUACAGUU ACAAAUCUC	239	UACAGUUACAAAU CUA	431	UAGAUUUGUAACUGU AUUUGG
48	CUCUGUGCAUG UGACAGACUU	240	UGCAUGUGACAG ACUA	432	UAGUCUGUCACAUGC ACAGAG
49	UCUGUGCAUGU GACAGACUUG	241	GCAUGUGACAGA CUUA	433	UAAGUCUGUCACAUG CACAGA
50	AUGUGACAGAC UUGACCCACA	242	ACAGACUUGACC CACA	434	UGUGGGUCAAGUCUG UCACAU
51	UGACAGACUUG ACCCACAGGC	243	GACUUGACCCAC AGGA	435	UCCUGUGGGUCAAGU CUGUCA
52	GACAGACUUGA CCCACAGGCC	244	ACUUGACCCACA GGCA	436	UGCCUGUGGGUCAAG UCUGUC
53	CCACUCCAAAAA CCUGACCUG	245	CCAAAAACCUGAC CUA	437	UAGGUCAGGUUUUUG GAGUGG
54	CACUCCAAAAAC CUGACCUGC	246	CAAAAACCUGACC UGA	438	UCAGGUCAGGUUUUU GGAGUG
55	ACUCCAAAAACC UGACCUGCU	247	AAAAACCUGACCU GCA	439	UGCAGGUCAGGUUUU UGGAGU
56	UGGGCCUGUGA GCAGGGAACA	248	CUGUGAGCAGGG AACA	440	UGUUCCCUGCUCACA GGCCCA
57	GCCUGUGAGCA GGGAACACCC	249	UGAGCAGGGAAC ACCA	441	UGGUGUUCCCUGCUC ACAGGC
58	UACUCACUCCU CGGUGCUCAU	250	ACUCCUCGGUGC UCAA	442	UUGAGCACCGAGGAG UGAGUA
59	ACUCACUCCUC GGUGCUCAUA	251	CUCCUCGGUGCU CAUA	443	UAUGAGCACCGAGGA GUGAGU
60	CUCACUCCUCG GUGCUCAUAA	252	UCCUCGGUGCUC AUAA	444	UUAUGAGCACCGAGG AGUGAG
61	UCACUCCUCGG UGCUCAUAAU	253	CCUCGGUGCUCA UAAA	445	UUUAUGAGCACCGAG GAGUGA
62	CAGGACCACGG CACCAACCUG	254	CCACGGCACCAA CCUA	446	UAGGUUGGUGCCGU GGUCCUG

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
63	UGACCUGUCAG GUGAAGUUCG	255	UGUCAGGUGAAG UUCA	447	UGAACUUCACCUGAC AGGUCA
64	GUGACUACGGA GAGAACCAUC	256	UACGGAGAGAAC CAUA	448	UAUGGUUCUCUCCGU AGUCAC
65	GACUACGGAGA GAACCAUCCA	257	CGGAGAGAACCA UCCA	449	UGGAUGGUUCUCUCC GUAGUC
66	ACUACGGAGAG AACCAUCCAG	258	GGAGAGAACCAU CCAA	450	UUGGAUGGUUCUCUC CGUAGU
67	AGAUGGCUCAG GGAAACAAGA	259	GCUCAGGGAAAC AAGA	451	UCUUGUUUCCCUGAG CCAUCU
68	AGCCCUGCUCG CUCUUUGUCU	260	UGCUCGCUCUUU GUCA	452	UGACAAAGAGCGAGC AGGGCU
69	CCCUGCUCGCU CUUUGUCUCU	261	CUCGCUCUUUGU CUCA	453	UGAGACAAAGAGCGA GCAGGG
70	UGCUCGCUCUU UGUCUCUGCC	262	GCUCUUUGUCUC UGCA	454	UGCAGAGACAAAGAG CGAGCA
71	GCUCGCUCUUU GUCUCUGCCU	263	CUCUUUGUCUCU GCCA	455	UGGCAGAGACAAAGA GCGAGC
72	UCGCUCUUUGU CUCUGCCUCA	264	CUUUGUCUCUGC CUCA	456	UGAGGCAGAGACAAA GAGCGA
73	GCUCUUUGUCU CUGCCUCAUC	265	UUGUCUCUGCCU CAUA	457	UAUGAGGCAGAGACA AAGAGC
74	CUCUUUGUCUC UGCCUCAUCU	266	UGUCUCUGCCUC AUCA	458	UGAUGAGGCAGAGAC AAAGAG
75	CUUUGUCUCUG CCUCAUCUUC	267	UCUCUGCCUCAU CUUA	459	UAAGAUGAGGCAGAG ACAAAG
76	UUUGUCUCUGC CUCAUCUUCU	268	CUCUGCCUCAUC UUCA	460	UGAAGAUGAGGCAGA GACAAA
77	CACAGGAGGAA AGCAGCCAGG	269	GAGGAAAGCAGC CAGA	461	UCUGGCUGCUUUCCU CCUGUG
78	AGGAGGAAAGC AGCCAGGACA	270	GAAAGCAGCCAG GACA	462	UGUCCUGGCUGCUUU CCUCCU
79	AAGCAGCCAGG ACAGCAGUGG	271	GCCAGGACAGCA GUGA	463	UCACUGCUGUCCUGG CUGCUU
80	CCAGGACAGCA GUGGGCAGGA	272	ACAGCAGUGGGC AGGA	464	UCCUGCCCACUGCUG UCCUGG
81	CCGAAACACCAG AAGAAGUCC	273	ACACCAGAAGAAG UCA	465	UGACUUCUUCUGGUG UUUCGG
82	CGAAACACCAGA AGAAGUCCA	274	CACCAGAAGAAGU CCA	466	UGGACUUCUUCUGGU GUUUCG
83	CACCAGAAGAAG UCCAAGUUA	275	GAAGAAGUCCAA GUUA	467	UAACUUGGACUUCUU CUGGUG
84	CCAGAAGAAGU CCAAGUUACA	276	AGAAGUCCAAGU UACA	468	UGUAACUUGGACUUC UUCUGG
85	AGAAGAAGUCCA AGUUACAUG	277	AAGUCCAAGUUAC AUA	469	UAUGUAACUUGGACU UCUUCU
86	GGAGAUGGAUG AGGAGCUGCA	278	UGGAUGAGGAGC UGCA	470	UGCAGCUCCUCAUCC AUCUCC
87	GAGAUGGAUGA GGAGCUGCAU	279	GGAUGAGGAGCU GCAA	471	UUGCAGCUCCUCAUC CAUCUC
88	UUAUGCUUCCC UCAACUUUCA	280	CUUCCCUCAACU UUCA	472	UGAAAGUUGAGGGAA GCAUAA

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
89	UGCUUCCCUCA ACUUUCAUGG	281	CCCUCAACUUUCA UGA	473	UCAUGAAAGUUGAGG GAAGCA
90	GCUUCCCUCAA CUUUCAUGGG	282	CCUCAACUUUCAU GGA	474	UCCAUGAAAGUUGAG GGAAGC
91	CUUCCCUCAAC UUUCAUGGGA	283	CUCAACUUUCAU GGGA	475	UCCCAUGAAAGUUGA GGGAAG
92	UUCCCUCAACU UUCAUGGGAU	284	UCAACUUUCAUG GGAA	476	UUCCCAUGAAAGUUG AGGGAA
93	UCCCUCAACUU UCAUGGGAUG	285	CAACUUUCAUGG GAUA	477	UAUCCCAUGAAAGUU GAGGGA
94	CCUCAACUUUCA UGGGAUGAA	286	ACUUUCAUGGGA UGAA	478	UUCAUCCCAUGAAAG UUGAGG
95	CUCAACUUUCAU GGGAUGAAU	287	CUUUCAUGGGAU GAAA	479	UUUCAUCCCAUGAAA GUUGAG
96	AACUUUCAUGG GAUGAAUCCU	288	UCAUGGGAUGAA UCCA	480	UGGAUUCAUCCCAUG AAAGUU
97	ACUUUCAUGGG AUGAAUCCUU	289	CAUGGGAUGAAU CCUA	481	UAGGAUUCAUCCCAU GAAAGU
98	CUUUCAUGGGA UGAAUCCUUC	290	AUGGGAUGAAUC CUUA	482	UAAGGAUUCAUCCCA UGAAAG
99	UUUCAUGGGAU GAAUCCUUCC	291	UGGGAUGAAUCC UUCA	483	UGAAGGAUUCAUCCC AUGAAA
100	UCCAAGGACAC CUCCACCGAA	292	GGACACCUCCAC CGAA	484	UUCGGUGGAGGUGU CCUUGGA
101	CAAGGACACCU CCACCGAAUA	293	ACACCUCCACCGA AUA	485	UAUUCGGUGGAGGU GUCCUUG
102	AGGACACCUCC ACCGAAUACU	294	ACCUCCACCGAAU ACA	486	UGUAUUCGGUGGAG GUGUCCU
103	GGACACCUCCA CCGAAUACUC	295	CCUCCACCGAAUA CUA	487	UAGUAUUCGGUGGAG GUGUCC
104	ACACCUCCACC GAAUACUCAG	296	UCCACCGAAUACU CAA	488	UUGAGUAUUCGGUGG AGGUGU
105	CACCUCCACCG AAUACUCAGA	297	CCACCGAAUACUC AGA	489	UCUGAGUAUUCGGUG GAGGUG
106	CACCGAAUACUC AGAGGUCAG	298	AAUACUCAGAGG UCAA	490	UUGACCUCUGAGUAU UCGGUG
107	AGAGGUCAGGA CCCAGUGAGG	299	UCAGGACCCAGU GAGA	491	UCUCACUGGGUCCUG ACCUCU
108	CAGGACCCAGU GAGGAACCCA	300	CCCAGUGAGGAA CCCA	492	UGGGUUCCUCACUGG GUCCUG
109	AGGACCCAGUG AGGAACCCAC	301	CCAGUGAGGAAC CCAA	493	UUGGGUUCCUCACUG GGUCCU
110	ACCCAGUGAGG AACCCACAAG	302	GUGAGGAACCCA CAAA	494	UUUGUGGGUUCCUCA CUGGGU
111	GAUCCACAUCC UCUACAGGUC	303	ACAUCCUCUACAG GUA	495	UACCUGUAGAGGAUG UGGAUC
112	UCCACAUCCUC UACAGGUCGG	304	AUCCUCUACAGG UCGA	496	UCGACCUGUAGAGGA UGUGGA
113	CCACAUCCUCUA CAGGUCGGG	305	UCCUCUACAGGU CGGA	497	UCCGACCUGUAGAGG AUGUGG
114	GGCUGAUUCUU GGAGAUUUAA	306	AUUCUUGGAGAU UUAA	498	UUAAAUCUCCAAGAA UCAGCC

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
115	GCUGAUUCUUG GAGAUUUAAC	307	UUCUUGGAGAUU UAAA	499	UUUAAAUCUCCAAGA AUCAGC
116	CUGAUUCUUGG AGAUUUAACA	308	UCUUGGAGAUUU AACA	500	UGUUAAAUCUCCAAG AAUCAG
117	GAUUCUUGGAG AUUUAACACC	309	UUGGAGAUUUAA CACA	501	UGUGUUAAAUCUCCA AGAAUC
118	UUCUUGGAGAU UUAACACCCC	310	GGAGAUUUAACA CCCA	502	UGGGUGUUAAAUCUC CAAGAA
119	CACAGGCAAUG GGUUUAUAGA	311	GCAAUGGGUUUA UAGA	503	UCUAUAAACCCAUUG CCUGUG
120	ACAGGCAAUGG GUUUAUAGAC	312	CAAUGGGUUUAU AGAA	504	UUCUAUAAACCCAUU GCCUGU
121	CAGGCAAUGGG UUUAUAGACA	313	AAUGGGUUUAUA GACA	505	UGUCUAUAAACCCAU UGCCUG
122	AGGCAAUGGGU UUAUAGACAU	314	AUGGGUUUAUAG ACAA	506	UUGUCUAUAAACCCA UUGCCU
123	GGCAAUGGGUU UAUAGACAUU	315	UGGGUUUAUAGA CAUA	507	UAUGUCUAUAAACCC AUUGCC
124	GCAAUGGGUUU AUAGACAUUA	316	GGGUUUAUAGAC AUUA	508	UAAUGUCUAUAAACC CAUUGC
125	CAAUGGGUUUA UAGACAUUAU	317	GGUUUAUAGACA UUAA	509	UUAAUGUCUAUAAAC CCAUUG
126	AAUGGGUUUAU AGACAUUAUG	318	GUUUAUAGACAU UAUA	510	UAUAAUGUCUAUAAA CCCAUU
127	AUGGGUUUAUA GACAUUAUGU	319	UUUAUAGACAUUA UGA	511	UCAUAAUGUCUAUAA ACCCAU
128	UGGGUUUAUAG ACAUUAUGUG	320	UUAUAGACAUUAU GUA	512	UACAUAAUGUCUAUA AACCCA
129	GGGUUUAUAGA CAUUAUGUGA	321	UAUAGACAUUAUG UGA	513	UCACAUAAUGUCUAU AAACCC
130	GGUUUAUAGAC AUUAUGUGAG	322	AUAGACAUUAUGU GAA	514	UUCACAUAAUGUCUA UAAACC
131	GUUUAUAGACA UUAUGUGAGU	323	UAGACAUUAUGU GAGA	515	UCUCACAUAAUGUCU AUAAAC
132	UUUAUAGACAUU AUGUGAGUU	324	AGACAUUAUGUG AGUA	516	UACUCACAUAAUGUC UAUAAA
133	UUAUAGACAUUA UGUGAGUUU	325	GACAUUAUGUGA GUUA	517	UAACUCACAUAAUGU CUAUAA
134	UAUAGACAUUAU GUGAGUUUC	326	ACAUUAUGUGAG UUUA	518	UAAACUCACAUAAUG UCUAUA
135	AUAGACAUUAUG UGAGUUUCC	327	CAUUAUGUGAGU UUCA	519	UGAAACUCACAUAAU GUCUAU
136	UAGACAUUAUG UGAGUUUCCU	328	AUUAUGUGAGUU UCCA	520	UGGAAACUCACAUAA UGUCUA
137	AGACAUUAUGU GAGUUUCCUG	329	UUAUGUGAGUUU CCUA	521	UAGGAAACUCACAUA AUGUCU
138	ACAUUAUGUGA GUUUCCUGCU	330	AUGUGAGUUUCC UGCA	522	UGCAGGAAACUCACA UAAUGU
139	CAUUAUGUGAG UUUCCUGCUA	331	UGUGAGUUUCCU GCUA	523	UAGCAGGAAACUCAC AUAAUG
140	AUUAUGUGAGU UUCCUGCUAU	332	GUGAGUUUCCUG CUAA	524	UUAGCAGGAAACUCA CAUAAU

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
141	UUAUGUGAGUU UCCUGCUAUA	333	UGAGUUUCCUGC UAUA	525	UAUAGCAGGAAACUC ACAUAA
142	UAUGUGAGUUU CCUGCUAUAU	334	GAGUUUCCUGCU AUAA	526	UUAUAGCAGGAAACU CACAUA
143	AUGUGAGUUUC CUGCUAUAUU	335	AGUUUCCUGCUA UAUA	527	UAUAUAGCAGGAAAC UCACAU
144	UGUGAGUUUCC UGCUAUAUUA	336	GUUUCCUGCUAU AUUA	528	UAAUAUAGCAGGAAA CUCACA
145	AUUAACAUCAUC UUAGACUUU	337	CAUCAUCUUAGAC UUA	529	UAAGUCUAAGAUGAU GUUAAU
146	UUAACAUCAUCU UAGACUUUG	338	AUCAUCUUAGACU UUA	530	UAAAGUCUAAGAUGA UGUUAA
147	UAACAUCAUCUU AGACUUUGC	339	UCAUCUUAGACU UUGA	531	UCAAAGUCUAAGAUG AUGUUA
148	CUUUGCAAGCA GAGAGUCGUG	340	CAAGCAGAGAGU CGUA	532	UACGACUCUCUGCUU GCAAAG
149	UUGCAAGCAGA GAGUCGUGGA	341	AGCAGAGAGUCG UGGA	533	UCCACGACUCUCUGC UUGCAA
150	UGCAAGCAGAG AGUCGUGGAA	342	GCAGAGAGUCGU GGAA	534	UUCCACGACUCUCUG CUUGCA
151	GCAAGCAGAGA GUCGUGGAAU	343	CAGAGAGUCGUG GAAA	535	UUUCCACGACUCUCU GCUUGC
152	CAAGCAGAGAG UCGUGGAAUC	344	AGAGAGUCGUGG AAUA	536	UAUUCCACGACUCUC UGCUUG
153	AGCAGAGAGUC GUGGAAUCAA	345	AGAGUCGUGGAA UCAA	537	UUGAUUCCACGACUC UCUGCU
154	GCAGAGAGUCG UGGAAUCAAA	346	GAGUCGUGGAAU CAAA	538	UUUGAUUCCACGACU CUCUGC
155	GAGAGUCGUGG AAUCAAAUCU	347	UCGUGGAAUCAA AUCA	539	UGAUUUGAUUCCACG ACUCUC
156	AGAGUCGUGGA AUCAAAUCUG	348	CGUGGAAUCAAA UCUA	540	UAGAUUUGAUUCCAC GACUCU
157	GAGUCGUGGAA UCAAAUCUGU	349	GUGGAAUCAAAU CUGA	541	UCAGAUUUGAUUCCA CGACUC
158	AGUCGUGGAAU CAAAUCUGUG	350	UGGAAUCAAAUCU GUA	542	UACAGAUUUGAUUCC ACGACU
159	GUCGUGGAAUC AAAUCUGUGC	351	GGAAUCAAAUCU GUGA	543	UCACAGAUUUGAUUC CACGAC
160	UCGUGGAAUCA AAUCUGUGCU	352	GAAUCAAAUCUGU GCA	544	UGCACAGAUUUGAUU CCACGA
161	CGUGGAAUCAA AUCUGUGCUC	353	AAUCAAAUCUGUG CUA	545	UAGCACAGAUUUGAU UCCACG
162	GUGGAAUCAAA UCUGUGCUCU	354	AUCAAAUCUGUG CUCA	546	UGAGCACAGAUUUGA UUCCAC
163	UGGAAUCAAAUC UGUGCUCUU	355	UCAAAUCUGUGC UCUA	547	UAGAGCACAGAUUUG AUUCCA
164	GGAAUCAAAUCU GUGCUCUUU	356	CAAAUCUGUGCU CUUA	548	UAAGAGCACAGAUUU GAUUCC
165	UCAAAUCUGUG CUCUUUCAUU	357	UCUGUGCUCUUU CAUA	549	UAUGAAAGAGCACAG AUUUGA
166	CAAAUCUGUGC UCUUUCAUUU	358	CUGUGCUCUUUC AUUA	550	UAAUGAAAGAGCACA GAUUUG

Gene region SEQ ID NO:	Gene Region	Sense SEQ ID NO:	Sense Sequence	Antisense SEQ ID NO:	Antisense Sequence
167	AAAUCUGUGCU CUUUCAUUUG	359	UGUGCUCUUUCA UUUA	551	UAAAUGAAAGAGCAC AGAUUU
168	UGUGCUCUUUC AUUUGCUAAG	360	UCUUUCAUUUGC UAAA	552	UUUAGCAAAUGAAAG AGCACA
169	AUGAUGUCACA CAAGCUCCUU	361	GUCACACAAGCU CCUA	553	UAGGAGCUUGUGUGA CAUCAU
170	UGAUGUCACAC AAGCUCCUUA	362	UCACACAAGCUCC UUA	554	UAAGGAGCUUGUGUG ACAUCA
171	GAUGUCACACAA GCUCCUUAA	363	CACACAAGCUCCU UAA	555	UUAAGGAGCUUGUGU GACAUC
172	AUGUCACACAAG CUCCUUAAC	364	ACACAAGCUCCUU AAA	556	UUUAAGGAGCUUGUG UGACAU
173	GUCACACAAGC UCCUUAACCU	365	ACAAGCUCCUUAA CCA	557	UGGUUAAGGAGCUUG UGUGAC
174	UCACACAAGCUC CUUAACCUU	366	CAAGCUCCUUAAC CUA	558	UAGGUUAAGGAGCUU GUGUGA
175	ACACAAGCUCCU UAACCUUCC	367	AGCUCCUUAACC UUCA	559	UGAAGGUUAAGGAGC UUGUGU
176	CACAAGCUCCU UAACCUUCCA	368	GCUCCUUAACCU UCCA	560	UGGAAGGUUAAGGAG CUUGUG
177	ACAAGCUCCUUA ACCUUCCAU	369	CUCCUUAACCUU CCAA	561	UUGGAAGGUUAAGGA GCUUGU
178	CAAGCUCCUUAA CCUUCCAUG	370	UCCUUAACCUUC CAUA	562	UAUGGAAGGUUAAGG AGCUUG
179	GCUCCUUAACC UUCCAUGUCU	371	UUAACCUUCCAU GUCA	563	UGACAUGGAAGGUUA AGGAGC
180	CUCCUUAACCU UCCAUGUCUC	372	UAACCUUCCAUG UCUA	564	UAGACAUGGAAGGUU AAGGAG
181	UCCUUAACCUU CCAUGUCUCC	373	AACCUUCCAUGU CUCA	565	UGAGACAUGGAAGGU UAAGGA
182	CCUUAACCUUC CAUGUCUCCA	374	ACCUUCCAUGUC UCCA	566	UGGAGACAUGGAAGG UUAAGG
183	CUUAACCUUCCA UGUCUCCAU	375	CCUUCCAUGUCU CCAA	567	UUGGAGACAUGGAAG GUUAAG
184	UUAACCUUCCAU GUCUCCAUU	376	CUUCCAUGUCUC CAUA	568	UAUGGAGACAUGGAA GGUUAA
185	UAACCUUCCAU GUCUCCAUUU	377	UUCCAUGUCUCC AUUA	569	UAAUGGAGACAUGGA AGGUUA
186	GUAUAAGAAGU CCUAUCUCAU	378	AGAAGUCCUAUC UCAA	570	UUGAGAUAGGACUUC UUAUAC
187	CAUAGGGAUGC UGUGAGCAUU	379	GGAUGCUGUGAG CAUA	571	UAUGCUCACAGCAUC CCUAUG
188	AUAGGGAUGCU GUGAGCAUUA	380	GAUGCUGUGAGC AUUA	572	UAAUGCUCACAGCAU CCCUAU
189	UAGGGAUGCUG UGAGCAUUAA	381	AUGCUGUGAGCA UUAA	573	UUAAUGCUCACAGCA UCCCUA
190	AGGGAUGCUGU GAGCAUUAAA	382	UGCUGUGAGCAU UAAA	574	UUUAAUGCUCACAGC AUCCCU
191	GGGAUGCUGUG AGCAUUAAAU	383	GCUGUGAGCAUU AAAA	575	UUUUAAUGCUCACAG CAUCCC
192	GAUGCUGUGAG CAUUAAAUAA	384	UGUGAGCAUUAA AUAA	576	UUAUUUAAUGCUCAC AGCAUC

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 $C\text{-}P^1\text{-}D\text{-}P^1$; 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 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-P^1-D-P^1; each B is represented by the formula C-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-P^2-C-P^2; F is represented by the formula D-P^1-C-P^1; each G is represented by the formula C-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-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

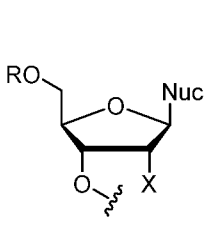
The siRNA molecules of the disclosure can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

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.

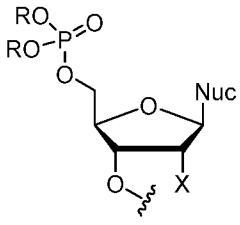
Further, it is contemplated that for any siRNA agent disclosed herein, further optimization could be achieved by systematically either adding or removing linked nucleosides to generate longer or shorter sequences. Further still, such optimized sequences can be adjusted by, e.g., the introduction of modified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

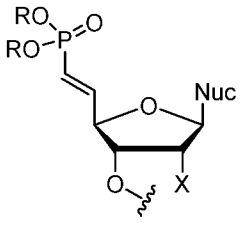
To further protect the siRNA molecules of this disclosure from degradation, a 5′-phosphorus stabilizing moiety may be employed. A 5′-phosphorus stabilizing moiety replaces the 5′-phosphate to prevent hydrolysis of the phosphate. Hydrolysis of the 5′-phosphate prevents binding to RISC, a necessary step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



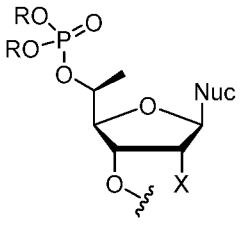
I **Formula IX**



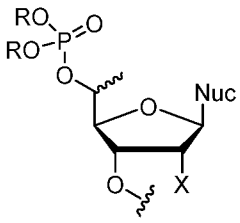
Formula X



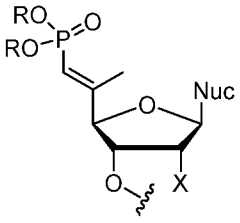
III **Formula XI**



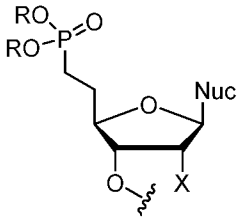
I' **Formula XII**



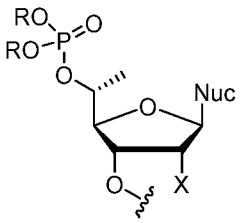
V **Formula XIII**



\ **Formula XIV**



VII **Formula XV**



VI **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.

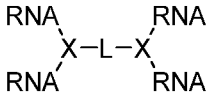
siRNA Branching

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.

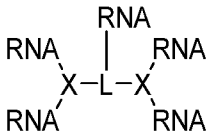
According to the present disclosure, the siRNA molecules disclosed herein may be branched siRNA molecules. The siRNA molecule may not be branched, or may be di-branched, tri-branched, or tetra-branched, connected through a linker. Each main branch may be further branched to allow for 2, 3, 4, 5, 6, 7, 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

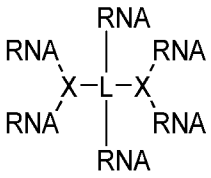
Di-branched	Tri-branched	Tetra-branched
RNA–L–RNA **Formula XVII**	RNA RNA–L–RNA **Formula XX**	RNA RNA–L–RNA RNA **Formula XXIV**
RNA X–L–X RNA RNA **Formula XVIII**	RNA RNA RNA–X–L–X RNA **Formula XXI**	RNA RNA RNA–X–L–X RNA RNA **Formula XXV**



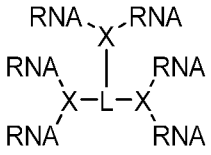
Formula XIX



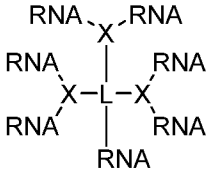
Formula XXII



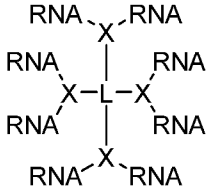
Formula XXVI



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.

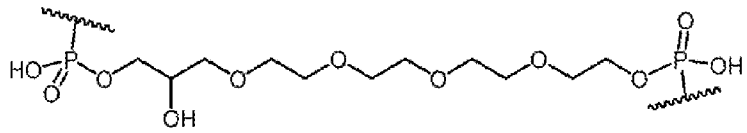
Examples of non-linear PEG linkers include branched PEGs, linear forked PEGs, or branched forked PEGs.

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.

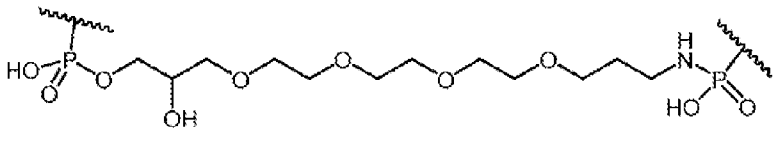
Linkers may covalently link 2, 3, 4, or 5 unique siRNA strands. The linker may covalently bind to any part of the siRNA oligomer. In some embodiments, the linker attaches to the 3′ end of nucleosides of each siRNA strand. In some embodiments, the linker attaches to the 5′ end of nucleosides of each siRNA 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:



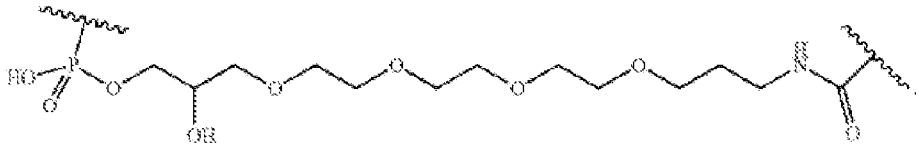
(**Formula L1**)

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



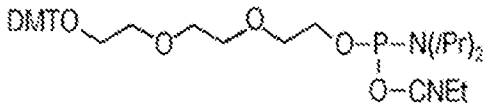
(**Formula L2**)

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



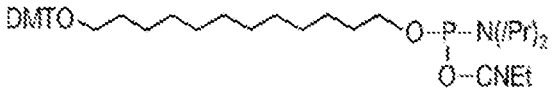
(**Formula L3**)

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



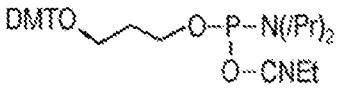
(**Formula L4**)

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



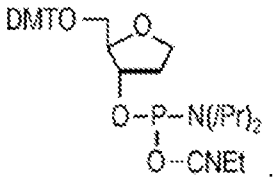
(**Formula L5**)

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



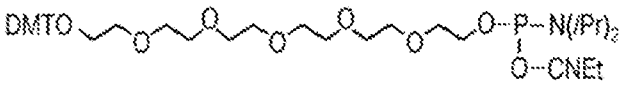
(**Formula L6**)

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



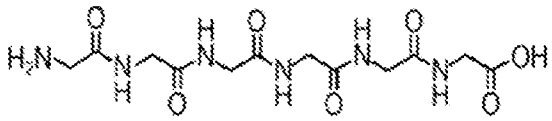
(**Formula L7**)

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 *CD33*-targeting siRNA molecules of the disclosure may be delivered to a subject, thereby treating a neuroinflammatory disease, such as Alzherimer's disease, and/or mitigating phenotypes associated with the disorders. For example, the siRNA molecules may be delivered to a subject, thereby treating Alzheimer's disease and/or mitigating Alzheimer's disease associated phenotypes (e.g., cognitive impairment or memory loss). Furthermore, the siRNA molecules of the disclosure may also be delivered to a subject having a variant of the *CD33* gene for which siRNA-mediated gene silencing of the *CD33* variant gene reduces the expression level of *CD33* transcript, thereby treating a neuroinflammatory disease (e.g., Alzheimer's disease) or other *CD33*-related diseases or disorders.

The disclosure provides methods of treating a subject by way of *CD33* 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 *CD33* transcripts, mutant *CD33* transcripts, splice isoforms of *CD33* transcripts, and/or overexpressed *CD33* 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, a neuroinflammatory disease (e.g., Alzheimer's disease) and/or any other medical risk(s) associated with gain of function mutations in the *CD33* 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 *CD33* 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. *CD33* 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 *CD33* mRNA transcript to effectuate reduced expression of the *CD33* gene.

Materials and Methods

HEK cells stably expressing CD33 (HEK-CD33) were actively transfected with *CD33* siRNA at either 20 nM or 0.2 nM concentration. After 24 hours, cells were lysed, and mRNA levels of *CD33* 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 CD33 mRNA relative to untreated control cells in the same assay (% UNT *CD33* 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 0.2 nM. The knockdown results are reported in Table 3 (20 nM) and Table 4 (0.2 nM).

Table 3. Table 4. *CD33* knockdown with siRNA molecules of the disclosure at 20 nM

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20 nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20 nM
193	385	97.09	289	481	19.87
194	386	56.16	290	482	21.50
195	387	17.79	291	483	40.06
196	388	30.38	292	484	90.33
197	389	30.22	293	485	68.30
198	390	26.27	294	486	85.10
199	391	38.12	295	487	77.04
200	392	83.95	296	488	93.99
201	393	108.88	297	489	109.84
202	394	80.99	298	490	52.97
203	395	109.11	299	491	36.44
204	396	101.53	300	492	99.23
205	397	106.45	301	493	21.25
206	398	95.86	302	494	60.87
207	399	112.31	303	495	20.11
208	400	21.93	304	496	53.44
209	401	20.41	305	497	75.72
210	402	56.31	306	498	29.33
211	403	89.94	307	499	18.56
212	404	34.80	308	500	22.64
213	405	39.18	309	501	37.52
214	406	62.29	310	502	49.79
215	407	70.27	311	503	38.37
216	408	63.44	312	504	36.25
217	409	62.31	313	505	16.95
218	410	77.45	314	506	31.35
219	411	45.31	315	507	27.23
220	412	87.02	316	508	37.08
221	413	64.60	317	509	26.06
222	414	82.02	318	510	20.53
223	415	20.15	319	511	23.59
224	416	15.55	320	512	17.12
225	417	31.10	321	513	22.39
226	418	44.33	322	514	24.80
227	419	38.87	323	515	40.12
228	420	25.05	324	516	44.87

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20 nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20 nM
229	421	41.77	325	517	22.84
230	422	66.24	326	518	12.97
231	423	58.28	327	519	20.75
232	424	62.50	328	520	22.07
233	425	75.55	329	521	16.63
234	426	49.96	330	522	17.37
235	427	49.11	331	523	20.83
236	428	33.89	332	524	14.37
237	429	67.20	333	525	14.33
238	430	34.84	334	526	23.61
239	431	60.23	335	527	24.89
240	432	91.10	336	528	23.57
241	433	36.40	337	529	36.04
242	434	42.82	338	530	83.32
243	435	100.69	339	531	25.62
244	436	36.15	340	532	17.96
245	437	29.88	341	533	22.51
246	438	84.67	342	534	23.33
247	439	54.13	343	535	19.26
248	440	89.82	344	536	18.23
249	441	73.87	345	537	33.49
250	442	100.17	346	538	25.90
251	443	111.35	347	539	35.32
252	444	101.34	348	540	19.52
253	445	62.17	349	541	18.79
254	446	88.22	350	542	16.19
255	447	56.05	351	543	26.27
256	448	63.50	352	544	35.51
257	449	60.54	353	545	24.75
258	450	69.85	354	546	18.32
259	451	71.51	355	547	16.71
260	452	52.20	356	548	20.93
261	453	42.12	357	549	21.53
262	454	41.58	358	550	25.24
263	455	23.79	359	551	19.62
264	456	22.58	360	552	20.28
265	457	72.10	361	553	55.05

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20 nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 20 nM
266	458	92.56	362	554	20.07
267	459	98.88	363	555	26.74
268	460	43.76	364	556	25.63
269	461	72.40	365	557	19.35
270	462	66.33	366	558	15.60
271	463	98.42	367	559	22.69
272	464	70.87	368	560	63.01
273	465	49.16	369	561	33.62
274	466	65.74	370	562	26.73
275	467	18.02	371	563	20.04
276	468	25.35	372	564	16.69
277	469	65.97	373	565	33.36
278	470	42.09	374	566	34.98
279	471	28.78	375	567	23.00
280	472	40.43	376	568	24.12
281	473	78.36	377	569	31.39
282	474	65.98	378	570	17.15
283	475	67.48	379	571	20.34
284	476	18.57	380	572	18.18
285	477	19.97	381	573	26.47
286	478	37.72	382	574	34.33
287	479	19.71	383	575	24.42
288	480	42.53	384	576	67.45

Table 4. *CD33* knockdown with siRNA molecules of the disclosure at 0.2 nM

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.2nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.2nM
193	385	83.64	289	481	58.54
194	386	82.52	290	482	67.4
195	387	42.55	291	483	71.75
196	388	62.31	292	484	87.03
197	389	61.08	293	485	96.23
198	390	50.54	294	486	96.92

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.2nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.2nM
199	391	84.5	295	487	95.63
200	392	97.67	296	488	92.72
201	393	94.55	297	489	94.69
202	394	91.52	298	490	91.14
203	395	97.16	299	491	88.09
204	396	98.59	300	492	86.08
205	397	76.63	301	493	71.84
206	398	89.43	302	494	94.71
207	399	85.47	303	495	42.09
208	400	73.51	304	496	60.42
209	401	40.74	305	497	83.8
210	402	71.05	306	498	44.3
211	403	71.58	307	499	46.42
212	404	49.11	308	500	35.94
213	405	60.45	309	501	84.38
214	406	74.81	310	502	87.75
215	407	75.71	311	503	66.92
216	408	82.97	312	504	50.21
217	409	75.24	313	505	43.38
218	410	85.55	314	506	51.86
219	411	81.13	315	507	62.15
220	412	80.32	316	508	75.9
221	413	63.48	317	509	58.32
222	414	85.12	318	510	47.86
223	415	35.51	319	511	65.09
224	416	43.15	320	512	40.18
225	417	74.77	321	513	54.8
226	418	70.11	322	514	95.53
227	419	69.51	323	515	64.78
228	420	53.35	324	516	59.06
229	421	67.17	325	517	37.64
230	422	76.54	326	518	30.8
231	423	76.26	327	519	35.45
232	424	68.3	328	520	41.38
233	425	67.12	329	521	31.15
234	426	56.4	330	522	55.96

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.2nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.2nM
235	427	59.96	331	523	68.31
236	428	102.17	332	524	50.39
237	429	71.46	333	525	22.47
238	430	84.89	334	526	53.81
239	431	56.84	335	527	41.01
240	432	96.55	336	528	31.9
241	433	67.06	337	529	65.78
242	434	59.36	338	530	69.52
243	435	93.69	339	531	44.64
244	436	80.84	340	532	54.13
245	437	69.87	341	533	33.99
246	438	84.47	342	534	45.53
247	439	80.11	343	535	60.75
248	440	94.16	344	536	45.64
249	441	84.74	345	537	45.49
250	442	94.36	346	538	41.28
251	443	105.13	347	539	62.19
252	444	101.3	348	540	39.95
253	445	75.15	349	541	46.49
254	446	86.68	350	542	27.28
255	447	72.9	351	543	49.12
256	448	89.53	352	544	61.81
257	449	86.22	353	545	45.47
258	450	87.46	354	546	42.57
259	451	81.68	355	547	21.28
260	452	86.51	356	548	41.08
261	453	84.72	357	549	31.63
262	454	92.59	358	550	48.96
263	455	94.86	359	551	50.82
264	456	72.34	360	552	33.96
265	457	80.33	361	553	90.85
266	458	89.43	362	554	23.17
267	459	89	363	555	46.18
268	460	80.89	364	556	48.79
269	461	97	365	557	41.29
270	462	91.45	366	558	22.44

Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.2nM	Sense SEQ ID NO:	Antisense SEQ ID NO:	Residual mRNA expression (% of untreated) at 0.2nM
271	463	98.27	367	559	40.19
272	464	97.92	368	560	78.34
273	465	74.98	369	561	79.78
274	466	78.79	370	562	34.01
275	467	71.33	371	563	33.63
276	468	54.67	372	564	23.9
277	469	77.87	373	565	61.02
278	470	96.77	374	566	70.3
279	471	89.33	375	567	68.94
280	472	52.81	376	568	33.78
281	473	87.07	377	569	50.4
282	474	84.73	378	570	53.29
283	475	87.73	379	571	47.77
284	476	40.16	380	572	48.64
285	477	77.04	381	573	68.28
286	478	68.96	382	574	81.16
287	479	46.06	383	575	80.36
288	480	87.44	384	576	82.99

Example 2. IC50 Determination of *CD33*-targeting siRNA Molecules

Objective

This Example describes the results of a series of experiments undertaken to determine the IC50 of siRNA molecules complementary to specific regions within a human *CD33* mRNA.

Materials and Methods

HEK cells stably expressing CD33 (HEK-CD33) were actively transfected with CD33 siRNA at concentrations of 1 fM to 100nM for 24 hours. Expression of CD33 mRNA was assessed at 72 hours using RT-qPCR as described above, and the IC50 of each compound was calculated.

Results

Cells were treated with an siRNA molecule of the disclosure having an antisense strand and a sense strand as shown in Table 5, below. The IC50 was calculated and the knockdown results are reported in Table 5.

Table 5. IC50 of *CD33*-Targeting siRNA Molecules

Sense SEQ ID NO:	Antisense SEQ ID NO:	IC50 (pM)	Sense SEQ ID NO:	Antisense SEQ ID NO:	IC50 (pM)
195	387	174	333	525	20.6
198	390	221	336	528	34.3
209	401	238	341	533	64
212	404	238	344	536	96.8
223	415	122	345	537	113
224	416	126	348	540	85
228	420	144	349	541	24.3
276	468	203	350	542	28.7
284	476	118	354	546	37.9
287	479	176	355	547	45.3
289	481	191	357	549	23.5
303	495	238	360	552	12.4
307	499	67.4	362	554	119
308	500	65.3	365	557	136
313	505	69.1	366	558	216
320	512	70.6	367	559	996
325	517	44.2	370	562	269
326	518	91.3	371	563	93.5
327	519	61.7	372	564	200
329	521	27.3	376	568	45.4

Example 3. Characterization of microglia after in vitro *CD33* knockdown

Objective

This Example describes the results of a series of experiments undertaken to determine the effect of CD33 knockdown on TREM2 signaling and microglial activation.

Materials and Methods

An siRNA molecule of the disclosure was synthesized as a di-branched siRNA molecule having the structure of Formula XVII. The sense strand had the sequence of SEQ ID No.: 326 and had a pattern of chemical modifications having the broad structure of Formula III and the specific structure of Formula S1. The antisense strand had the sequence of SEQ ID No.: 518 and had a pattern of chemical modifications having the broad structure of Formula II and the specific structure of formula A2. The antisense strand further included a 5' vinyl phosphonate moiety of Formula XI.

TREM2 signaling

To interrogate the effect of CD33 knockdown on TREM2 signaling, *CD33* siRNA- and mock-transfected iPSC-derived microglial-like cells were plated on poly-D lysine-coated cell culture plates in

serum-free medium. Following a two-hour treatment with pHrodo-labeled oligomeric amyloid-beta, cells were lysed and assayed for phosphorylated SYK (pSYK) levels via a commercial AlphaLISA assay. Data are reported as the pSYK AlphaLISA signal relative to the total protein in the cellular lysate. Results are shown in **Figure 1**.

Microglial phagocytic capacity

To assess the effect of CD33 knockdown on microglial phagocytic capacity, *CD33* siRNA- and mock-transfected iPSC-derived microglial-like cells were plated on poly-D lysine-coated cell culture plates in serum-free medium. Cells were treated with pHrodo-labeled oligomeric amyloid-beta for two hours and imaged live to visualize amyloid-beta phagocytosis. Data are presented as the corrected integrated intensity of each sample (AU). Results are shown in **Figure 2**.

Microglial inflammatory signal

Finally, to assess the impact of CD33 knockdown on the microglial inflammatory signal, *CD33* siRNA- and mock-transfected iPSC-derived microglial-like cells were plated on poly-D lysine-coated cell culture plates in serum-free medium. Following a twenty-four-hour treatment with LPS, cell culture supernatants were collected and assayed for cytokine levels via commercial Meso Scale Discovery (MSD) ELISA assays. Data are reported as the cytokine levels in pg/mL of cell culture supernatant. Results are shown in **Figure 3**.

Conclusion

The results in this Example demonstrate that an siRNA molecule of the disclosure, with a sense strand having SEQ ID No.: 326 and an antisense strand having SEQ ID No.: 518, successfully increased TREM2 signaling, increased microglial phagocytic capacity, and decreased microglial inflammatory signature upon challenge.

Example 4. Generating *CD33*-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 *CD33* 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 5. Optimizing *CD33*-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 6. Preparation and Administrating *CD33*-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 7. Methods for the Treatment of a Neuroinflammatory Disease Using *CD33*-targeting siRNA Molecules

A subject in need of treatment of a neuroinflammatory disease (e.g., Alzheimer's disease), is treated with a dosage of the siRNA molecule or siRNA composition of the disclosure, formulated as a salt, at frequency determined by a practitioner. A physician having ordinary skill in the art can readily determine an effective amount of the siRNA molecule for administration to a mammalian subject (e.g., a human) in need thereof. For example, a physician could start prescribing doses of one of the siRNA molecules of the disclosure at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. Alternatively, a physician may begin a treatment regimen by administering one of the siRNA molecules of the disclosure at a high dose and subsequently administer progressively lower doses until a minimum dose that produces a therapeutic effect (e.g., a reduction in expression of *CD33* mRNA or suitable biomarker or a reduction of Alzheimers disease-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 Siglec3 (*CD33*) mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192, optionally wherein the antisense strand has at least 70% complementarity to the *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192, 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 *CD33* mRNA transcript having the nucleic acid sequence of any one of SEQ ID Nos: 1-192.

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 *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

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 *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192, 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 *CD33* RNA transcript having the nucleic acid sequence of any one of SEQ ID NOs: 1-192.

13. The siRNA molecule of any one of claims 1-12, 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: 385-576.

14. The siRNA molecule of claim 13, 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: 385-576.

15. The siRNA molecule of claim 14, wherein the antisense strand has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NOs: 385-576, 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: 385-576.

16. The siRNA molecule of claim 15, wherein the antisense strand has the nucleic acid sequence of any one of SEQ ID NOs: 385-576.

17. The siRNA molecule of any one of claims 1-16, 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: 193-384.

18. The siRNA molecule of claim 17, 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: 193-384.

19. The siRNA molecule of claim 18, wherein the sense strand has a nucleic acid sequence that is at least 95% identical to the nucleic acid sequence of SEQ ID NOs: 193-384, 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: 193-384.

20. The siRNA molecule of claim 19, wherein the sense strand has the nucleic acid sequence of any one of SEQ ID NOs: 193-384.

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

22. The siRNA molecule of claim 21, 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.

23. The siRNA molecule of any one of claims 1-20, 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.

24. The siRNA molecule of claim 23, 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.

25. The siRNA molecule of any one of claims 1-24, 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.

26. The siRNA molecule of claim 25, 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.

27. The siRNA molecule of claim 25, 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.

28. The siRNA molecule of claim 25, 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.

29. The siRNA molecule of claim 25, 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.

30. The siRNA molecule of any one of claims 1-20 and 25-29, 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.

31. The siRNA molecule of claim 30, 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-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.

32. The siRNA molecule of any one of claims 1-24, 30, and 31, 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.

33. The siRNA molecule of claim 32, 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.

34. The siRNA molecule of claim 32, 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.

35. The siRNA molecule of claim 32, 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.

36. The siRNA molecule of claim 32, 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.

37. The siRNA molecule of any one of claims 1-20, 25-29 and 32-36, 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-P^1-D-P^1;
each B is represented by the formula C-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-P^2-C-P^2;
F is represented by the formula D-P^1-C-P^1;
each G is represented by the formula C-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.

38. The siRNA molecule of claim 37, 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.

39. The siRNA molecule of any one of claims 1-24, 30, 31, 37, and 38, wherein the sense strand comprises a structure represented by Formula VII, wherein Formula VII is, in the 5'-to-3' direction:

H-B_m-I_n-A'-B_o-H-C
Formula VII;

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

40. The siRNA molecule of claim 39, 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

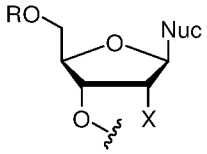
Formula S9;

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.

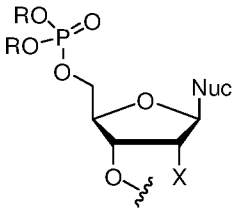
41. The siRNA molecule of any one of claims 1-40, wherein the antisense strand further comprises a 5' phosphorus stabilizing moiety at the 5' end of the antisense strand.

42. The siRNA molecule of any one of claims 1-41, wherein the sense strand further comprises a 5' phosphorus stabilizing moiety at the 5' end of the sense strand.

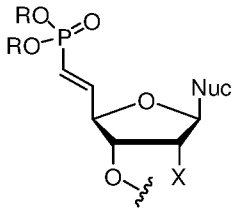
43. The siRNA molecule of claim 41 or 42, wherein each 5' phosphorus stabilizing moiety is, independently, represented by any one of Formulas IX-XVI:



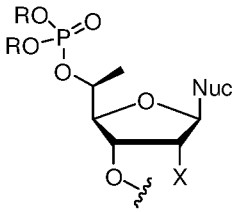
Formula IX



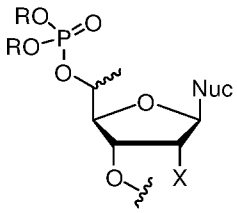
Formula X



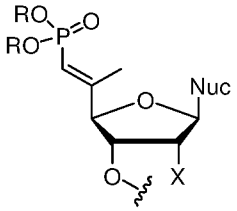
Formula XI



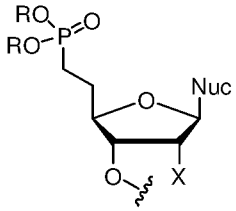
Formula XII



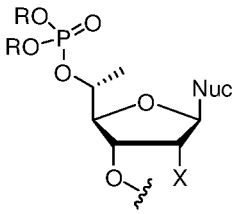
Formula XIII



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.

44. The siRNA molecule of claim 43, wherein the nucleobase is an adenine, uracil, guanine, thymine, or cytosine.

45. The siRNA molecule of any one of claims 41-44, wherein the 5' phosphorus stabilizing moiety is (E)-vinylphosphonate represented by Formula XI.

46. The siRNA molecule of any one of claims 1-45, wherein the siRNA molecule further comprises a hydrophobic moiety at the 5' or the 3' end of the siRNA molecule.

47. The siRNA molecule of claim 46, wherein the hydrophobic moiety is selected from a group consisting of cholesterol, vitamin D, or tocopherol.

48. The siRNA molecule of any one of claims 1-47, wherein the length of the sense strand is between 10 and 30 nucleotides.

49. The siRNA molecule of claim 48, wherein the length of the sense strand is between 10 and 25 nucleotides.

50. The siRNA molecule of claim 49, wherein the length of the sense strand is between 12 and 25 nucleotides.

51. The siRNA molecule of claim 50, wherein the length of the sense strand is between 12 and 20 nucleotides.

52. The siRNA molecule of claim 51, wherein the length of the sense strand is between 12 and 19 nucleotides.

53. The siRNA molecule of claim 52, wherein the length of the sense strand is 15 nucleotides.

54. The siRNA molecule of claim 52, wherein the length of the sense strand is 16 nucleotides.

55. The siRNA molecule of claim 52, wherein the length of the sense strand is 18 nucleotides.

56. The siRNA molecule of any one of claims 1-55, wherein the length of the antisense strand is between 10 and 30 nucleotides.

57. The siRNA molecule of claim 56, wherein the length of the antisense strand is between 12 and 30 nucleotides.

58. The siRNA molecule of claim 57, wherein the length of the antisense strand is between 15 and 30 nucleotides.

59. The siRNA molecule of claim 58, wherein the length of the antisense strand is between 18 and 30 nucleotides.

60. The siRNA molecule of claim 59, wherein the length of the antisense strand is between 18 and 25 nucleotides.

61. The siRNA molecule of claim 60, wherein the length of the antisense strand is between 18 and 21 nucleotides.

62. The siRNA molecule of claim 61, wherein the length of the antisense strand is 18 nucleotides.

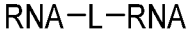
63. The siRNA molecule of claim 61, wherein the length of the antisense strand is 20 nucleotides.

64. The siRNA molecule of claim 61, wherein the length of the antisense strand is 21 nucleotides.

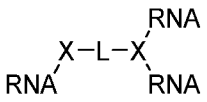
65. The siRNA molecule of any one of claims 1-64, wherein the siRNA molecule is a branched siRNA molecule.

66. The siRNA molecule of claim 65, wherein the branched siRNA molecule is di-branched, tri-branched, or tetra-branched.

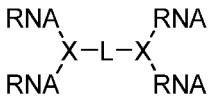
67. The siRNA molecule of claim 66, wherein the siRNA molecule is a di-branched siRNA molecule, optionally wherein the di-branched siRNA molecule is represented by any one of Formulas XVII-XIX:



Formula XVII;



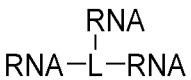
Formula XVIII;



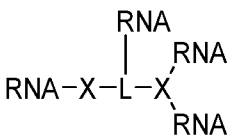
Formula XIX;

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

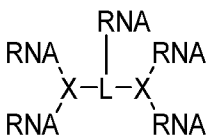
68. The siRNA molecule of claim 66, wherein the siRNA molecule is a tri-branched siRNA molecule, optionally wherein the tri-branched siRNA molecule is represented by any one of Formulas XX-XXIII:



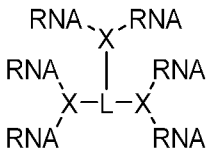
Formula XX;



Formula XXI;



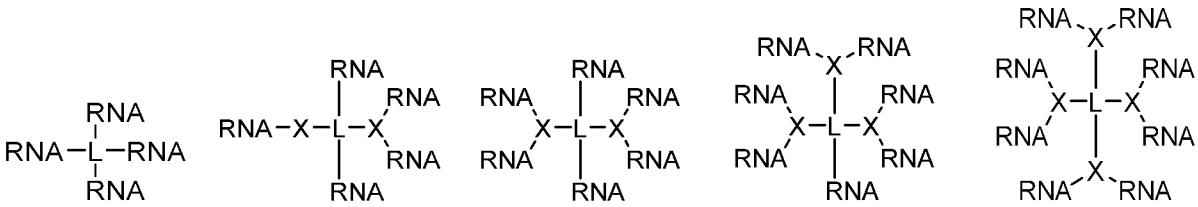
Formula XXII;



Formula XXIII;

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

69. The siRNA molecule of claim 66, 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.

70. The siRNA molecule of any one of claims 67-69, 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.

71. The siRNA molecule of claim 70, wherein the one or more contiguous subunits is 2 to 20 contiguous subunits.

72. A pharmaceutical composition comprising the siRNA molecule of any one of claims 1-71 and a pharmaceutically acceptable excipient, carrier, or diluent.

73. A method of delivering an siRNA molecule to a subject diagnosed as having a neuroinflammatory disease, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-71 or the pharmaceutical composition of claim 72 to the subject.

74. A method of treating a neuroinflammatory disease in a subject in need thereof, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-71 or the pharmaceutical composition of claim 72 to the subject.

75. The method of claim 73 or 74, wherein the neuroinflammatory disease is Alzheimer's disease.

76. A method of reducing *CD33* expression in a subject in need thereof, the method comprising administering a therapeutically effective amount of the siRNA molecule of any one of claims 1-71 or the pharmaceutical composition of claim 72 to the subject.

77. The method of any one of claims 73-76, wherein the siRNA molecule or the pharmaceutical composition is administered to the subject by way of intracerebroventricular, intrastriatal, intraparenchymal, or intrathecal injection.

78. The method of any one of claims 73-76, wherein the siRNA molecule or the pharmaceutical composition is administered to the subject by way of intravenous, intramuscular, or subcutaneous injection.

79. The method of any one of claims 73-78, wherein the subject is a human.

80. A kit comprising the siRNA molecule of any one of claims 1-71, or the pharmaceutical composition of claim 72, and a package insert, wherein the package insert instructs a user of the kit to perform the method of any one of claims 73-79.

FIG. 1

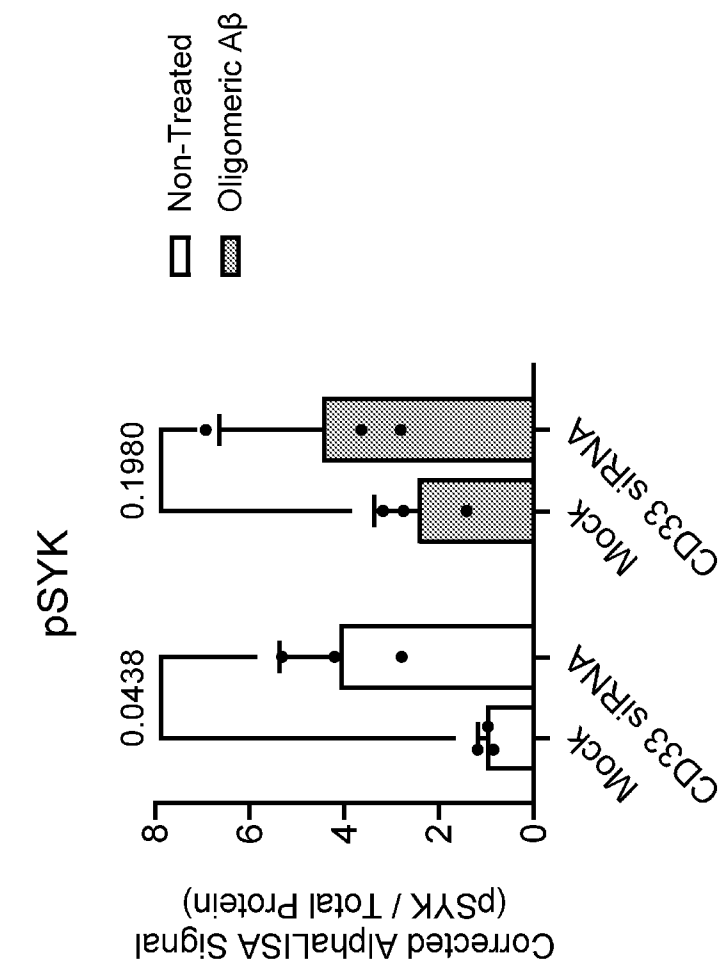

pSYK
Non-Treated
Oligomeric Aβ
0.0438
0.1980
Corrected AlphaLISA Signal
(pSYK / Total Protein)
0
2
4
6
8
Mock
CD33 siRNA
Mock
CD33 siRNA

FIG. 2

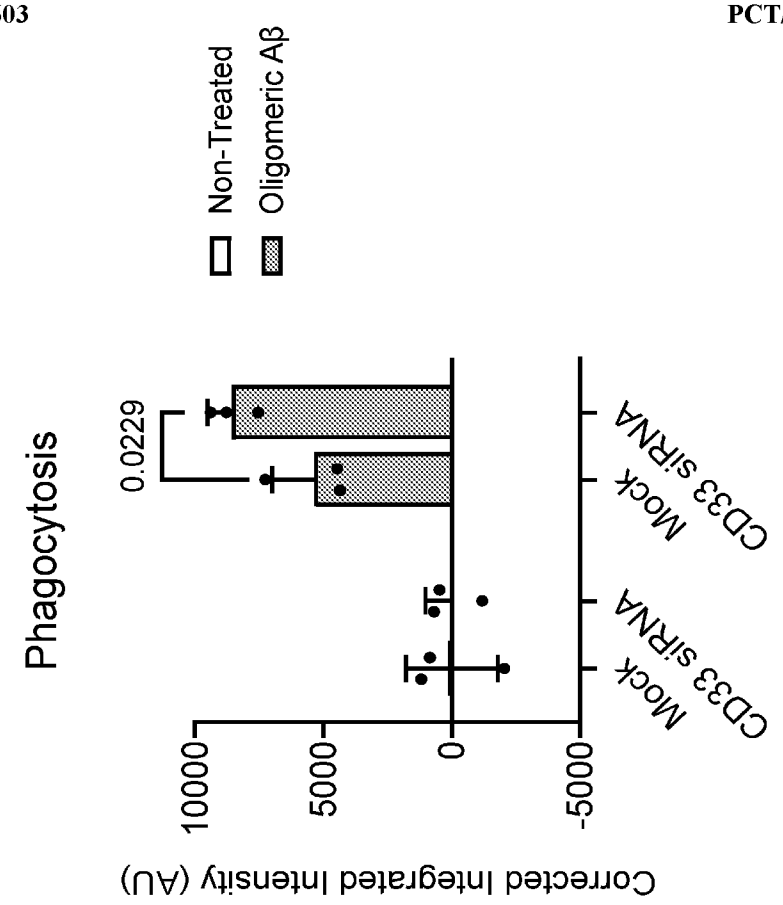
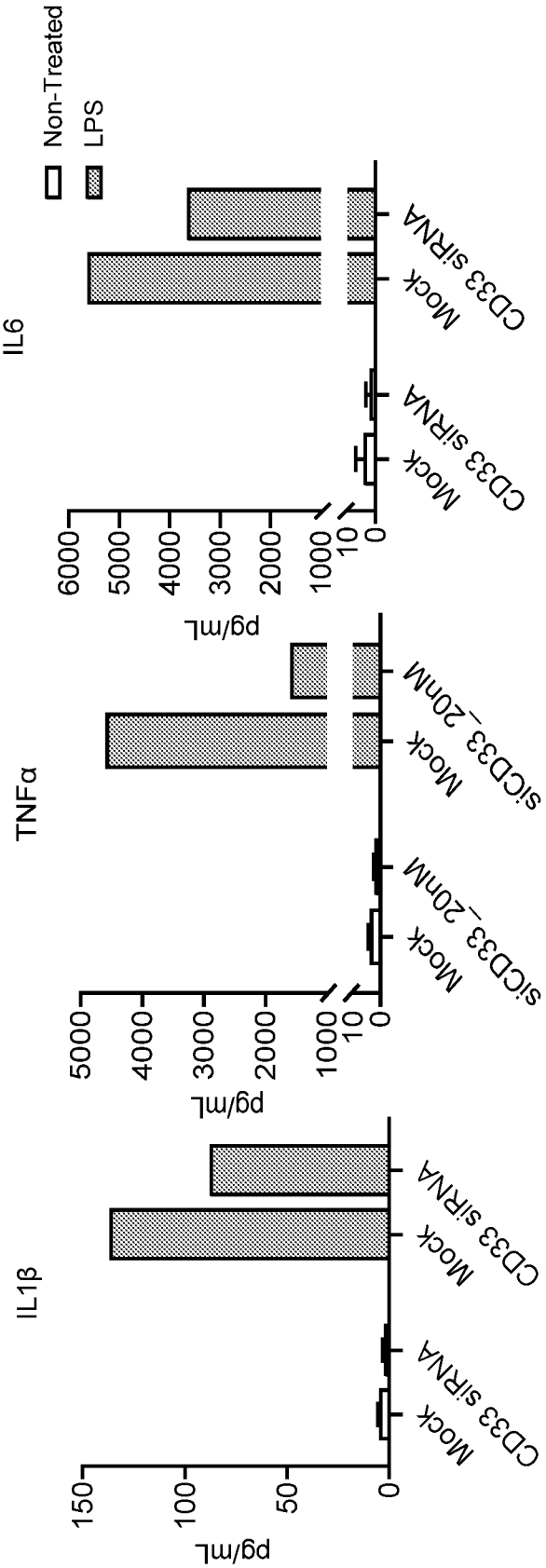

Phagocytosis
Non-Treated
Oligomeric Aβ
0.0229
Corrected Integrated Intensity (AU)
10000
5000
0
-5000
Mock
CD33 siRNA
Mock
CD33 siRNA

FIG. 3


Non-Treated
LPS
IL6
pg/mL
6000
5000
4000
3000
2000
1000
10
0
Mock
CD33 siRNA
Mock
CD33 siRNA
TNFα
pg/mL
5000
4000
3000
2000
1000
10
0
Mock
siCD33_20nM
Mock
siCD33_20nM
IL1β
pg/mL
150
100
50
0
Mock
CD33 siRNA
Mock
CD33 siRNA